A NOVEL PROCESS FOR THE PRODUCTION OF CASTOR OIL BY DIRECT SOLVENT EXTRACTION

by

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A thesis submitted to the University of Aston in Birmingham for the degree of Doctor of Philosophy

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January 1982

SUMMARY

Ismail Hassan Hussein Ph.D. 1982

A Novel Process for the Production of Castor Oil by Direct Solvent Extraction

This research was undertaken to develop a process for the direct solvent extraction of castor oil seeds. A literature survey confirmed the desirability of establishing such a process with emphasis on the decortication, size reduction, detoxification-deallergenization, and solvent extraction operations.

A novel process was developed for the dehulling of castor seeds which consists of pressurizing the beans and then suddenly releasing the pressure to vacuum. The degree of dehulling varied according to the pressure applied and the size of the beans. Some of the batches were difficult-to-hull, and this phenomenon was investigated using the scanning electron microscope and by thickness and compressive strength measurements. The other variables studied to lesser degrees included residence time, moisture content, and temperature. The method was successfully extended to cocoa beans, and (with modifications) to peanuts. The possibility of continuous operation was looked into, and a mechanism was suggested to explain how the method works.

The work on toxins and allergens included an extensive literature survey on the properties of these substances and the methods developed for their deactivation. Part of the work involved setting up an assay method for measuring their concentration in the beans and cake, but technical difficulties prevented the completion of this aspect of the project. An appraisal of the existing deactivation methods was made in the course of searching for new ones.

A new method of reducing the size of oilseeds was introduced in this research; it involved freezing the beans in cardice and milling them in a coffee grinder. The method was found to be quick, efficient, and reliable. An application of the freezing technique was successful in dehulling soybeans and de-skinning peanut kernels.

The literature on the solvent extraction of oilseeds, especially castor, was reviewed. The survey covered processes, equipment, solvents, and mechanism of leaching. Three solvents were experimentally investigated: cyclohexane, ethanol, and acetone. Extraction with liquid ammonia and liquid butane was not effective under the conditions studied.

Based on the results of the research, a process has been suggested for the direct solvent extraction of castor seeds; the various sections of the process have been analysed, and the factors affecting the economics of the process were discussed.

Key Words Castor seeds, Decortication, Size Reduction, Solvent Extraction (Leaching), Detoxification-Deallergenization

Dedicated to my parents, my wife and my children.

Acknowledgements

The author wishes to thank Professor G.V. Jeffreys for accepting this research proposal and agreeing to supervise the work; for providing facilities for carrying out the research, and for providing partial financial support. His help, support, and encouragement were vital to the success of this project.

Thanks are also due to Dr. C.J. Mumford for his interest and advice, especially on safety.

Sincere appreciation and gratitude are felt for the help received from Dr. J.J. Rimmer, Biological Sciences Department, Aston University, for devoting much of his time for advice on the analysis of the toxins and allergens.

The author also wishes to thank his parents in the Sudan and his wife and family for tolerating the circumstances brought about by the burden of the work. He also wishes to thank Mr. A.M. Hussein for his help, advice, and interest.

The help received from the following, especially in arranging financial assistance, is gratefully appreciated: Dr. M. Safar, D. M. Walid, Dr. E. Osman, and Mr. A. Almeer. Special thanks from the author are due to his dear friend Abbas Fathulrahman for his sincere feelings and continuous help.

The author also wishes to thank:

The technical staff of the Chemical Engineering Department for their help, especially Mr. N. Roberts and Mr. P Murray.

Mrs. J. Roberts and the staff in the stores for coping with some of the unusual orders required for this research.

Mr. Steve Southwick, Biol. Sci. Dept., for helping with the Transmission Electron Microscope, and to Mr. R.G. Howell, Met. Dept., for helping with the Scanning Electron Microscope.

The Library staff, especially inter-library loans, for providing the references and articles necessary for this work.

Miles Research Laboratories for raising the antisera against ricin and the castor allergens.

Prof. R.R.A. Coombs, Cambridge University, for advice on allergen analysis, and Mr. D. Cracknell, Simon-Rosedowns Ltd., for providing information about processing the castor beans.

Prof. P.E. Barker, Chem. Eng. Dept., for his interest, Prof. T. Mulvey, Physics Dept., for valuable discussions, and Dr. D.J. Arrowsmith for helping with the SEM.

Mr. J. Holloway for his assistance with photography.

The author also wishes to thank Dr. A.M. Salih and Mr. M. Farid for their help and encouragement.

The typing of the thesis was patiently undertaken by Mrs. J. Wood, whom the author wishes to thank. Also, the author thanks Mrs. J.E. Davis who accepted to help with the typing.

Finally, the author wishes to thank all those who

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Chapter One

GENERAL

1.1 THE CASTOR-OIL PLANT

1.1.1 Introduction

Castor oil is one of the most important industrial vegetable oils. It is derived from the seeds of the castor-oil plant (Ricinus Communis, L.) which is a member of the Euphorbiacae (or Spurge) family (1). Many Euphorbiacae yield oils or fats that are unique among vegetable oils and fats, and the properties of castor oil bear a good evidence to this.

The castor-oil plant is believed to have originated from Africa and Asia. A large number of varieties exist, and as a result different types of this plant are encountered. Thus, there are dwarf as well as giant varieties which may be up to 12 feet high. The colours of stems and leaves also vary from one variety to another. Other differences are known to exist even among plants grown within small geographical regions. Although this plant started as a wild tropical species, demand for the oil caused an expansion in the cultivated areas, and extensive work was carried out on breeding new varieties for warm temperate climates (2,3). In the tropics the castor-oil plant grows as a perennial and its height may reach 30 to 40 feet, but in the temperature zones it behaves like an annual (2). For commercial production, short varieties

are desirable as they can be easily harvested by machines.

Although the plant is mainly grown as a source of castor-oil seeds, use was reported for the leaves and the stem (4). In Asia, the leaves are used to feed silk-worms, cows, and buffaloes. The stems serve as a source of fuel and in the production of cardboard, wallboard, and similar materials. Watt (5) reports various uses for the leaves, roots and stem.

1.1.2 Varieties and Breeding

The high degree of variability which exists among castor plants makes this crop an interesting model for genetical and breeding work. Since castor oil is the main product from the plant, the primary objective is to get a high oil content per seed as well as a high yield of seeds per acre. Factors which contribute to the ease of harvest and the reduction of loss of seeds equally concern the breeder. To achieve the former goal, he controls the plant height, and to realize the latter he avoids dehiscent varieties that shatter and release their seeds once they are ripe. The desirable characteristics aimed for in a breeding program are usually related to other botanical features of the plant in question.

An interesting point about the breeding of castor oil plants is the elimination, or reduction to very low levels, of the toxic and allergenic compounds present in the seed. From a survey of the published literature it appears that this proposal has not been looked into hitherto. The advances made in plant breeding in the last two decades

permitted the manipulation of oilseeds to obtain better products either by eliminating harmful compoents or rearranging the fatty acid composition. In some cases it is desirable to achieve the two goals simultaneously. Examples will be given from work on cottonseeds, sunflower and safflower seeds, and rapeseeds.

Traditional rapeseed (Brassica Napus) varieties contain high percentages (20-55%) of erucic acid (C-22:1) and of glucosinolates (4.3-6.4%). The objection to the presence of erucic acid in diet is that it may be physiologically harmful (6), while that to the glucosinolates, which are made of sulphur-containing sugars, is because they cause enlargement of the thyroid gland by preventing iodine uptake, in addition to other toxic effects (7). The discovery of the Bronowski varieity of rapeseed with a glucosinolate content of 0.94% (8) enabled the breeding of "double low" rapeseed (low erucic acid and low glucosinolate) (7) as well as "zero-zero" rapeseed.

Breeding trials on sunflower and safflower seeds (9) resulted in new varieties with new fatty acid distribution and changes in the percentages of oil and protein. In the case of cottonseeds, the genetic manipulations were directed to eliminate gossypol , a toxic polyphenolic compound, and the glandless varieties evolved are reported to be free of the toxin (10,11).

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The genetic modifications suggested for the castor seeds would benefit from the concepts and techniques applied to the other oilseeds, with the necessary adjustments to suit the nature of the castor seeds and the objectives of the breeding program. The toxic protein of the castor seeds, ricin, was found in 21 varieties of the plant (12), and the allergen found in the seeds has been proved to be the storage protein of the seed (13) Hence a variety with the lowest possible level of ricin is required for a successful start of a breeding program. The physiological function of the allergen may also prove to be an obstacle, but it is worthwhile investigating this proposition as its success could have marked influence on the castor oil industry. It is also hoped that the success of this breeding program would not affect the directly related properties of the seed especially its resistance to pests and disease.

1.2 CASTOR SEEDS

1.2.1 Characteristics

Castor seeds, which in Arabic have the name kharwá, exhibit considerable variations in size and colour. Thus there are seeds smaller than coffee beans, while there are others the size of a broad bean. The colours are bright and include red, white, green, black and chocolate. The coloured seed coat, which is smooth and glossy, is mottled, and in this respect the castor seeds seem to be unique among the common oilseeds. The usual shape of the seeds is oval but there are some which are

almost round. Each seed has a carnucle at in its tip. Fig. (1.1) shows seeds of various sizes and colours, and a drawing for a section of a capsule is shown in Fig. (1.2).

Before being harvested, the seeds are contained in a thorny outer shell. Each shell would usually contain three seeds. Removing this outer shell is shown as shelling, and this should be distinguished from decortication which is the term describing the combined operation of removing the seed coat (or husk) and separating the husks from the kernels. Dehusking exposes the kernel which is soft and always white in colour. The husks are abrasive and contain very little oil, ca. 1% by weight. The kernel contains the oil and protein of the seed. Being a dicotyledon, the kernel of the castor seed can be separated into two halves. Between the seed coat and the kernel there is a very thin white coat which is released into tiny fragments during processing.

The castor seed is classified as a high oil-content seed, the oil content being 35-57% of the seed weight (3). The soft kernels would yield oil if they were squeezed between the fingers. These two considerations - the softness of the seeds and their high oil-content - have their bearing on the handling and processing techniques. The composition of the whole seed, the kernel and the husk is given in table (1.1).

1.2.2 Ultrastructure

The ultrastructure of the castor seed is so fine that an electron microscope must be used to reveal its details.

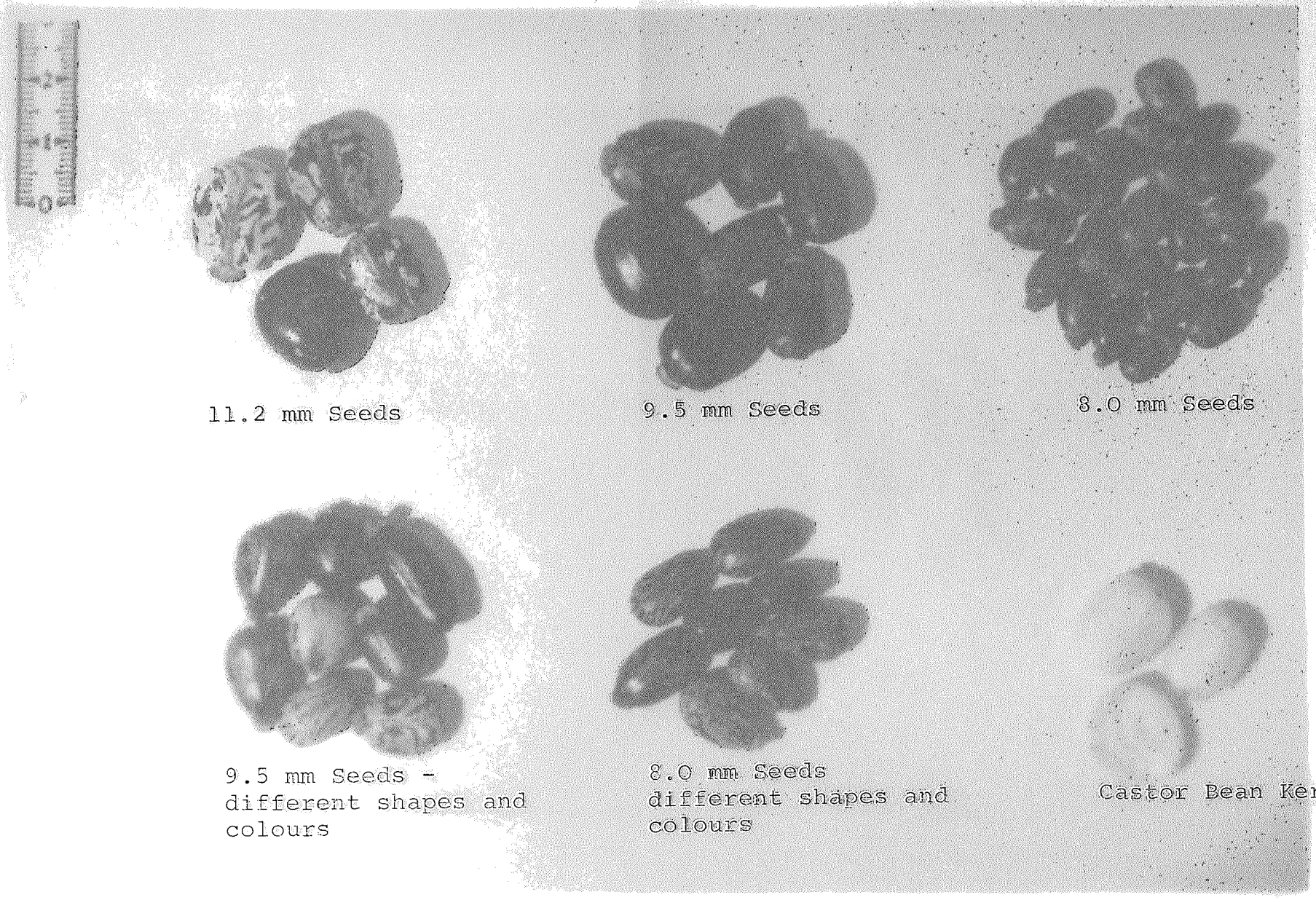


Fig. 1.1 Various Colours and Sizes of Castor Seeds

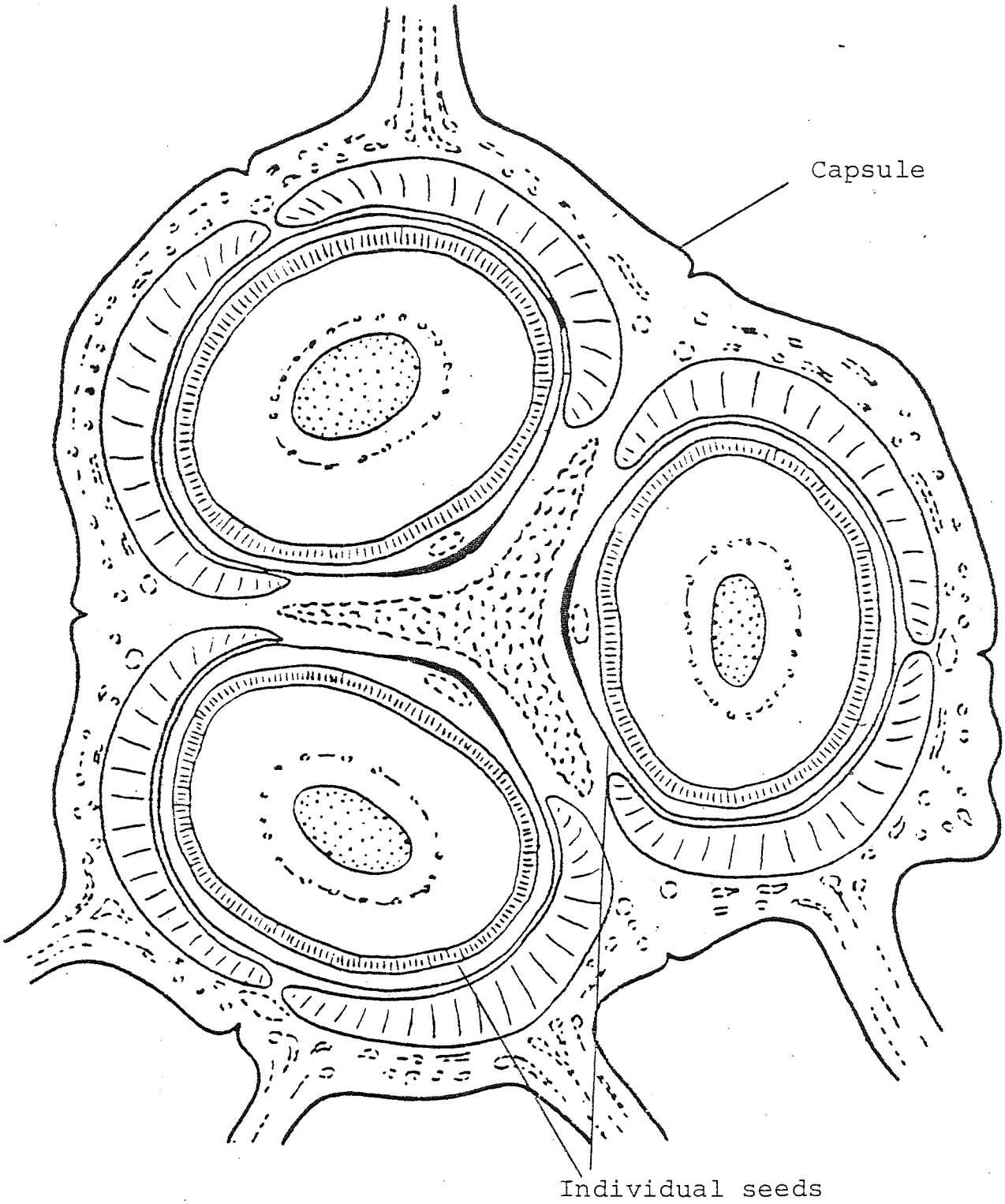


Fig. 1.2 A Cross section of a Castor Capsule showing the Three Seeds Inside it.

TABLE 1.1

Percentage Composition of Castor Seed (243)

The first published electron micrographs for castor seeds were those by Ory et al (14). Figure (5.1) shows electron micrographs for dormant castor seeds studied for this project. The individual cells are packed with oil bodies (spherosomes) about 2-4 μm in diameter and among them are dispersed the protein bodies whose average diameter is 10 μm. Cells are separated from each other by cell walls about 0.5 μm thick. These cells are morphologically similar to those of important oilseeds such as cotton (15), peanuts (16), Soybeans (17) and jojoba seeds (18). A notable example of departure from this pattern is that of rapeseeds (19).

The protein bodies of castor seeds are divided into matrix proteins and crystalloid proteins. The former are water soluble, while the latter are insoluble in water but dissolve in dilute salt solutions (13,20). More importantly, the crystalloids contain the storage proteins of the seeds (13).

1.2.3 The Deleterious Components of the Seed

The castor seed is inedible and poisonous, and the dust from the seed cake can cause severe allergenic reactions in sensitized individuals. The agents held responsible for these effects are known as ricin and the castor seed allergen. In addition, castor seeds contain a mildly toxic alkaloid, ricinine. Traces of chlorogenic acid have also been detected in castor cake.

Ricin, a protein, is a very lethal poison (12). The castor seed allergen is a glycoprotein which is not toxic

but can kill by anaphylaxis if a previously sensitized individual or animal is exposed to a sufficiently high dose. Ingestion of the seed may be fatal depending on the amount taken and the manner in which it was consumed. Thus if the seeds are swallowed whole, they would cause less harm than if they are chewed. In the Sudan, certain uses of the seed require swallowing the whole seed with the carnucle(Sec.5.2.2)intact (21). Sensitive individuals can develop skin rashes and other symptoms of allergy on eating the castor seeds, but an inevitable consequence of ingesting the seeds is diahorrea.

Nicotinic acid is the precursor of ricinine (22). This alkaloid is reported to affect the growth of animals fed on untreated castor meal. Chlorogenic acid, a phenolic compound detected in castor cake, has antinutritional properties (23). The castor seed also contains a lipase which hydrolyses triglycerides and other esters, and if the favourable conditions for its activity are met, it can seriously affect the oil content of the seeds (24). The presence of all these components in castor seeds calls for extra care when castor oil is extracted. The deactivation of the hazardous material is necessary to increase the value of the process by products and to make them safe for handling and utilization.

1.3 CASTOR OIL

1.3.1 Oils and Fats

Castor oil has been known to Man since antiquity. Thus the papyri of ancient Egypt speak about the monopoly of Ptolemy (259 B.C.) for castor oil among others (25).

Weiss (4) also elaborates on the history of castor oil in ancient civilizations. Although the pattern of its usage may have considerably changed, this oil still holds an important position among industrial oils, and new applications continue to appear.

Oils and fats are triglycerides, i.e. esters of fatty acids and glycerol. Fatty acids are monocarboxylic acids with an even number of carbon atoms in their chains. The long-chain acids are the ones usually found in naturally occurring lipids. Since the glycerol part is common to all triglycerides, it would be logical to attribute the different properties of oils and fats to the characteristics of the individual fatty acids. The term "oil" denotes triglycerides which are liquid at room temperature, while those which are solid at room temperature are termed "fats". This distinction between oils and fats, however, is not a sharp one.

The common oilseeds yield oils which are composed of essentially the same fatty acids although the percentages may differ significantly. These acids are mostly saturated, but mono-unsaturated, diunsaturated, and tri-unsaturated acids also exist. The important saturated acids include Lauric (C12), myristic (C14), palmitic (C16), stearic (C18), arachidic (C20), and behenic (C22) acids. Of the mono-unsaturated acids, Cleic acid (C18:1) and erucic acid (C22:1) are important. Lincleic acid (C18:2) represents the di-unsaturated acids, and in the tri-unsaturated acids Linolenic acid (C18:3) deserves a mention. Table (1.2)

Table 1.2 Main Vegetable Oil Categories

Principal Fatty Acid	Oil
Lauric	Coconut
	Palm Kernel
	Babassu
Palmitic	Palm
Oleic	Olive
	Peanut
Linoleic (Medium)	Soybean
	Cottonseed
	Sesame
	Corn
Linoleic (High)	Sunflower
	Safflower
Erucic	Rapeseed

gives the major fatty acids found in the common oils and fats.

Castor oil differs from the common vegetable oils in two respects; the first is that this oil has a very high percentage of ricinoleic acid (90%), and thus approaches being a pure triglyceride of this acid, which is a hydroxy fatty acid:12-hydroxy-9-Octadecenoic acid. The second point of difference is that castor oil is practically the only vegetable oil of commercial importance to have a hydroxy acid in such high percentages. The unique chemistry exhibited by castor oil among vegetable oils is due to the presence, in such high proportions, of this fatty acid. Thus the versatility of castor oil is due to its ability to react at the hydroxyl group in addition to reacting at the double bond and the ester linkage.

1.3.2 Biosynthesis in the Plant

The biosynthesis of triglycerides in plant cells is a complicated process and the account given here must only be an outline of the processes involved. The first step in triglyceride biosynthesis is the formation of fatty acids. Certain enzymes are needed to initiate this stage of the synthesis. The first acid to be formed is palmitic (16:0) acid, and an elongation process converts it to stearic acid (18:0) which is desaturated aerobically to form aleic acid (18:1). The oxidation of the oleic acid produces ricinoleic acid in castor seeds. The fatty acids are then esterified with glycerol to form the triglycerides (26). In castor seeds the oil content

sixty days after pollination is over 50% (27). The fatty acid composition of castor oil is presented in Table (1.3. The oil is located in the oil bodies in the cells of the seed, and these glyceride reserves become sites of intensive metabolic activity converting fat to sucrose when the seed germinates (28).

1.3.3 Physical and Chemical Properties. Uses

Castor oil provides a good example of how the properties of oils and fats are affected by their constituent fatty acids. It is the only major vegetable oil that approaches being a pure triglyceride since ricinoleic acid makes up about 90% of its fatty acid. As a result of the presence of this hydroxy acid in castor oil its physical and chemical properties show marked variation from the other vegetable oils. Fig. 1.3 shows the structure of the castor-oil molecule.

The viscosity of castor oil is strikingly different from the common vegetable oils. This is clearly shown in Fig (1.4) which gives the viscosities of castor oil and other oils at various temperatures. When castor oil is compared with oils of the same iodine value, a higher specific gravity is noted for castor oil. Its density was found to vary linearly from 0.972 at $0^{o}C$ to 0.870 at $154^{o}C$ (4). The density of a number of vegetable oils in hexane and other solvents were determined and are tabulated based on the general equation (29)

TABLE 1.3

The Fatty Acid Composition of Castor Oil

Fatty Acid	Percentage
Ricinoleic acid	89.5
Linoleic acid	4.2
Oleic acid	3.0
Stearic acid	1.0
Palmitic acid	1.0
Dihydroxystearic acid	0.7
Eicosanoic acid	0.3
Linolenic acid	0.3

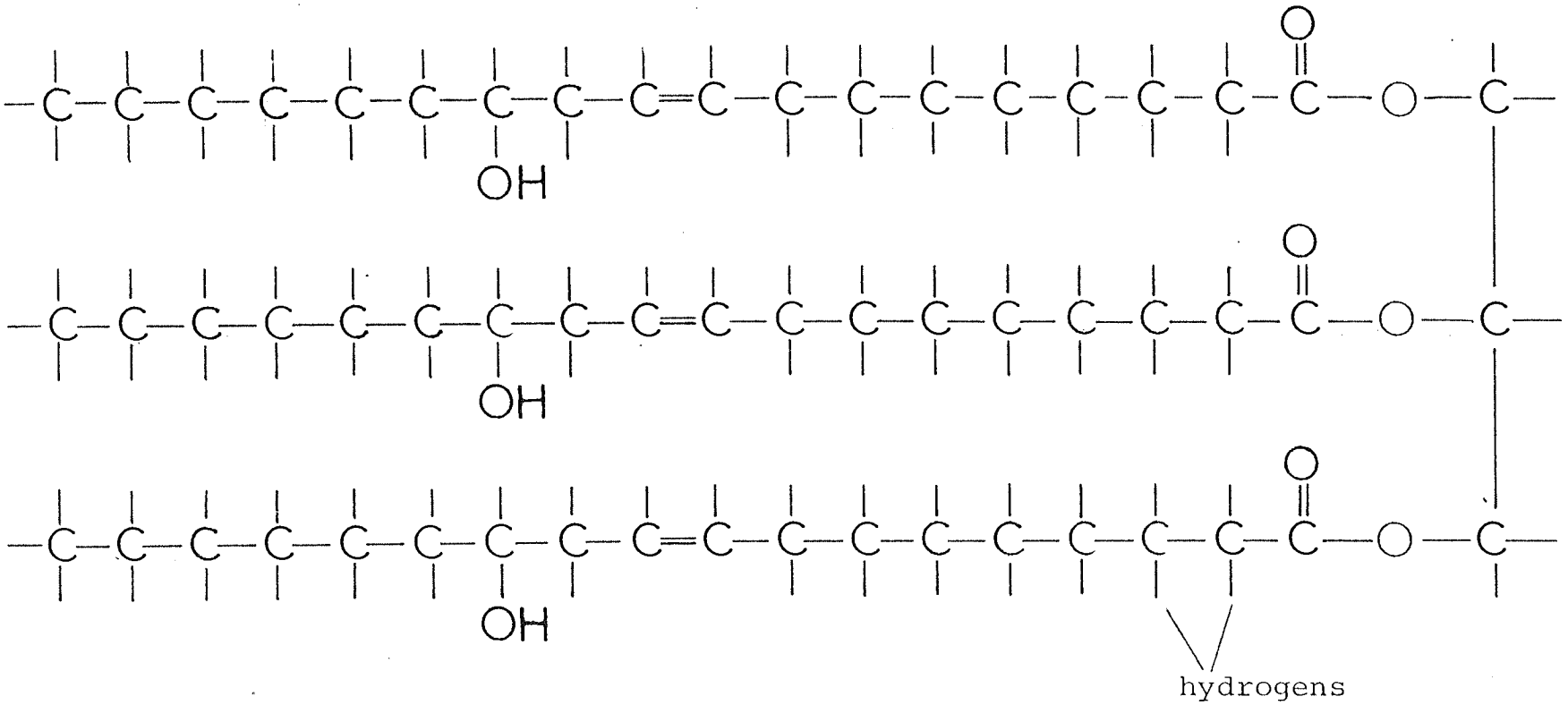


Fig. 1.3 The structure of the Castor Oil Molecule

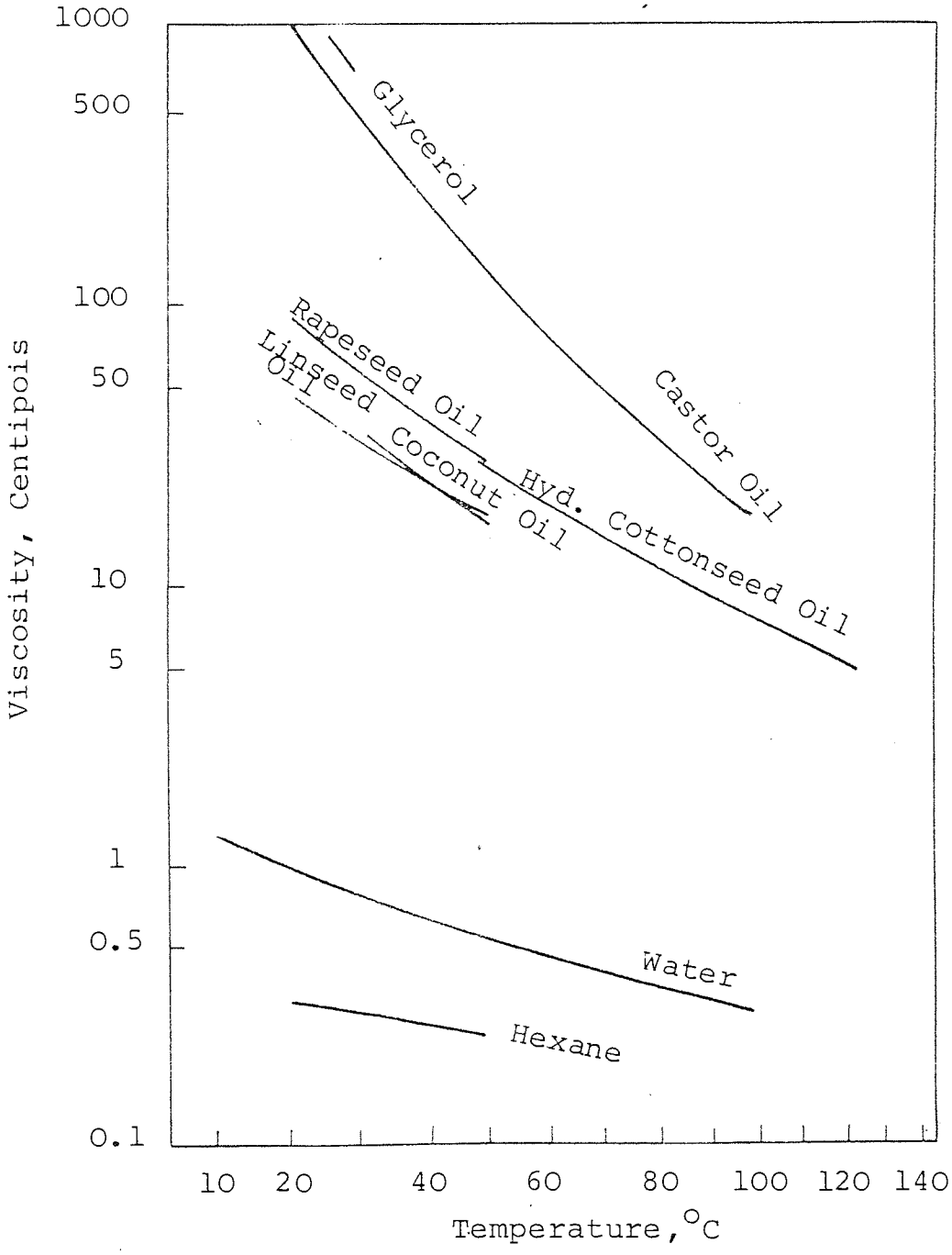


Fig 1.4 The Viscosity of Castor Oil Compared to other Substances

$$\frac{1}{D} = a-(a-b+4k)x+4kx^2$$

where D = density of oil-solvent mixture

a = reciprocal of density of ∉ the solvent

b = reciprocal of density of the oil

x = weight fraction of oil in mixture

k = constant depending only on the solvent and temperature

For castor oil, the constant k was found to be markedly different from that for other vegetable oils when dissolved in butanone (29). The surface tension of castor oil is higher than other vegetable oils at the same temperature. Table (1.4) gives surface tension values for castor oil compared to coconut and cottonseed oils.

The thermal properties of castor oil are rather scarce in the literature. The reported values were determined in the 1940's or earlier. The specific heat of castor oil (iodine value 83.0) is given in Table (1.5) and on comparison with other vegetable oils the latter exhibit lower specific heats at the same temperature (30). The liquid heat capacity of castor oil was presented in the form:

$$Cp = 0.4543-1.747 \times 10^{-3} T-9.916 \times 10^{-6}T^2+2.313 \times 10^{-8}T^3 \quad (31)$$

where Cp is in cal deg^{-1} mol^{-1} and T is in degrees Kelvin (300-500K). Calculated values of the ideal gas heat capacity, ideal gas enthalpy of formation, and ideal gas

Table 1.4

The surface tension of castor oil compared to other oils

	(dyne/centimeter) Oil		
Temperature (^{o}C)	Cottonseed	Castor	Coconut
20	35.4	39.0	33.4
80	31.3	35.2	28.4
130	27.5	33.0	24.0

Table 1.5

The specific heat of castor oil (Iodine Value 83.0)

Temperature		
^{o}C	^{o}F	Specific Heat
29.9	95.8	0.495
79.8	176.7	0.539
120.9	249.6	0.565
172.4	342.3	0.588
209.8	409.6	0.603
219.7	427.5	0.595
250.7	483.3	0.633
271.2	520.2	0.657

Gibbs energy of formation for ricinoleic acid at various temperatures are given in Table (1.6). The vapour pressure data for ricinoleic acid are not available; only two points are reported, 227°C at 10 mm and 250°C at 15 mm Hg pressure (31). However, vapour pressure data for methyl and ethyl ricinoleates are reported (32). When castor oil is cooled to 0°C it remains bright, but it congeals to a yellowish mass if cooled to -18°C (4). The melting point of ricinoleic acid is reported as 5.5°C (30). The flash point of castor oil is 230°C and the ignition temperature is 449°C (4).

Oils and fats are generally poor conductors of electricity, especially when they are dry. They possess low dielectric constants and the value reported for castor oil of 3.96 (30) is only slightly higher than the average figure. The dielectric constant of ricinoleic acid (cis) at 100°C is 3.547 and the constant at 100°C for (trans) ricinoleic acid is 3.775 (33).

The chemical properties of castor oil are also markedly influenced by its content of ricinoleic acid. Thus the acetyl (or hydroxyl) value of castor oil is much higher than other vegetable oils. At ordinary temperatures castor oil is incompletely miscible with straight-chain hydrocarbons, contrary to the common vegetable oils which are freely miscible with this class of solvents in all proportions. On the other hand, ordinary vegetable oils dissolve in alcohol at high temperature and under

Table 1.6 Some Calculated Thermodynamic Properties of Ricinoleic Acid

Temperature (K)	ΔG_f^o Kcal mol^{-1}	ΔH_f^o Kcal mol^{-1}	C_p^o Cal $deg^{-1} mol^{-1}$
298.15	-74	-195	106
300.0	-74	-195	105
400.0	-31	-198	130
500.0	+13	-202	151
600.0	+55	-205	168
700.0	+100	-208	182
800.0	+145	-210	193
900.0	+189	-213	200
1000.0	+234	-212	205

C_p^o = Ideal gas heat capacity

ΔH_f^o = Ideal Gas Enthalpy of Formation

ΔG_f^o = Ideal Gas Gibbs Energy of Formation

pressure (34), but castor oil is completely soluble in alcohol at ambient temperature. Generally, castor oil dissolves in polar organic solvents. The properties of castor oil are given in Table (1) of appendix (4). The pharmaceutical-grade oil has the specifications shown in Tables (2) and (3) of appendix (4). At present two grades of castor oil are recognized, grade one and grade three, the former being the medicinal-grade oil, while the latter is the oil used in industrial applications.

The chemical reactions which castor oil undergoes take place at the hydroxyl group or the ester linkage or the olefinic double bond. Thus the oil is very versatile and this explains its commercial importance. In addition, the properties of the oil, e.g. viscosity, water repellence, clear appearance, etc. are conferred upon its products. The most important of these reactions are dehydration, pyrolysis, hydrogenation, and sulphonation (31,35,36).

The dehydration of castor oil takes place at the hydroxyl group and is carried out catalytically to give dehydrated castor oil (DCO) which is utilized in the manufacture of protective coatings, varnishes, and alkyds. It is noted for its non yellowing performance and its remarkable colour retention. The products made from DCO are desired because of their high speed drying, flexibility, excellent chemical resistance, adhesion, gloss, and water proofness. DCO is considered a drying oil with viscosities 40-50 poise at 25^{o}C compared to 5-7 poise for ordinary

castor oil at the same temperature.

The reactant in the pyrolysis reaction can be castor oil or its methyl esters, depending on the product desired. When the castor oil methyl esters are pyrolysed they produce the raw material for the manufacture of nylon 11, a name arising from the number of carbon atoms in its polyamide. Nylon 11 is reported to be chemically resistant and better in its stability than nylon 6, nylon 6,6, and nylon 6,10. Hence it enters into numerous applications where its unique properties are required. If the pyrolysis is applied to castor oil the products are heptaldehyde and undecylenic acid, the former entering into the preparation of synthetic flavours, while the latter is employed primarily for its fungicidal and bactericidal properties. The pyrolysis reaction is usually carried out at 450-600^{o}C and atmospheric pressure. At about 425^{o}C the decomposition of the glyceryl portion of castor oil causes the evolution of acrolein, a highly poisonous material. Hence, special precautions must be taken in engineering the reaction and it may be advisable to pyrolyse ricinoleic acid rather than castor oil to avoid this problem (31).

The hydrogenation of castor oil is a high-temperature catalytic reaction directed towards the double bond of ricinoleic acid. The product is a wax with a high melting point (86-88^{o}C) which is utilized in other properiety waxes, polishes, cosmetics, and paper coatings. Partial hydro-genation is possible, thus permitting the control of the

hardness, flexibility, melting point, and iodine value of the product according to the specifications required.

The reaction of sulphuric acid with castor oil attacks the hydroxyl group of rincinoleic acid and is thus a true sulphation reaction, unlike the other vegetable oils in which the acid reacts at the double bond. The sulphation of castor oil is carried out at 25-30°C for several hours followed by neutralization with sodium hydroxide (4) or sodium carbonate (37). The product is known as Turkey red oil and finds application mainly in the textile industry. Higher degrees of sulphation were achieved by using sulphur trioxide in place of sulphuric acid, but higher temperatures are also needed in this case.

The alkali fusion of castor oil yields sebacic acid, an important intermediate in plasticiser and polymer manufacture, and 2-octanol (38) at 250°C, and different products are obtained if the reaction is run at about 200°C (31). The oxidation of castor oil with air or oxygen at 80°-130°C produces "blown oil" which finds use in plasticisers, artificial leathers, and adhesive mixtures (4). Castor oil derivatives are finding increased application in the food industry. Hundreds of other applications are made possible by changing the functionality of this unique vegetable oil.

Vegetable oils have been suggested as substitutes for diesel fuel, and considerable interest has recently been expressed in developing this idea (39). The attendant problems mainly arise from the differences between the

properties of diesel fuel and the suggested vegetable oils, but a more serious problem socially and politically is the balance between using the oils as food or fuel. Here the value of castor oil can be seen since its inedibility means that its use as fuel would not affect any food program, but unfortunately it has not been extensively studied for this purpose. The oils primarily tested were soybean oil, sunflower oil, and rapeseed oil, mainly because they were the leading vegetable oils in the countries which pioneered this type of research (40). Another reason for lack of experimentation on castor oil as fuel may be its high viscosity, but this could be reduced by suitable additives. There is no doubt that the hazards associated with the castor seed are a major deterrent to its expansion and hence the use of castor oil as fuel.

At present the trend is for a partial substitution of diesel fuel with vegetable oils, and the problems encountered at this stage are gumming and smoking. This led a number of researchers to suggest that success in using vegetable oils as fuel lies in finding the chemical treatment which will produce the correct type of fuel. Others maintain that since the existing diesel engines were designed for burning diesel fuel, then that design must be modified if vegetable oils were to provide the power for running tractors and similar machines.

The only reference to castor oil as fuel (41) concludes that there was little difference in maximum power output with well-refined castor oil and well-refined soybean

oil. It was also concluded that vegetable oils give reasonably good specific fuel economy in a diesel engine. However, the greater the percentage of vegetable oil in the blend, the poorer the specific fuel economy when compared with the specific consumption obtained with diesel fuel.

This section would not be complete without reference to the medicinal use of castor oil. The cathartic properties of the oil seems to result from the saponification it undergoes in the alkaline portion of the intestines. Although several cases of allergy towards castor seeds or castor cake are reported (42) as well as hundreds of cases of intoxication due to ricin (12), only one case of allergy is reported after the oral administration of castor oil (43). However, recently the issue of allergy towards castor oil has come to light following reports of adverse reactions to two anaesthetic agents, althesin (44) and propanidid (45), both of which are solubilized in polyoxyethylated castor oil which was implicated as the causative agent of the allergenic eruptions in patients. Lehrer et al (46) were able to detect the presence of castor allergens in castor wax products made from castor oil. These reports do not mention any tests with pure castor oil or pure polyoxyethylated castor oil to determine if the allergy was associated with either of them. Other vegetable oils, like tung oil and cottonseed oil, cause allergy to those who contact or ingest them. In the USA, 2-6% of the 10% suffering from some allergy are sensitized by cottonseed

oil. The majority of those known to react to cottonseeds were also allergic to cottonseed oil (47).

1.4 CASTOR CAKE

1.4.1 Introduction

Castor cake is the residue left after removing the oil from castor seeds. It is also called castor meal, castor pomace, or poonac. Finding new outlets for the pomace is essential if expansion in castor seed production is to take place, but these new markets require that the pomace be made safe from its hazardous components. Since proteins are the most valuable constituents of castor meal, the rest of this section would look briefly into their nature, properties and usage.

There is mounting concern in the world over the expected protein shortage from animal sources and the increase in world population. Efforts are therefore being made to utilize all possible protein sources, especially oilseeds. Although this applies primarily to edible oilseeds, there is no reason why castor seed should not provide good protein if it is properly treated. The amino acid composition of the castor cake is presented in Table (1.7).

1.4.2 Proteins in the Pomace

Proteins are polymers of amino acids linked by peptide bonds. Some amino acids are termed "essential" because they are needed for growth but cannot be synthesized by

the animal's organs. In some cases, essential amino acids can be synthesized by the animal but in insufficient amounts. Hence it is necessary to know the deficient amino acids in an oilseed meal in order to make the necessary supplementation. A "reference protein" was suggested by FAO as a standard for evaluating the nutritional value of protein feedstocks (48). A list of its contents is given in Table (1.7).

The protein content of castor pomace maybe as high as 40% (49) (Protein = NX 6.25), and its quality is also high (50). The percentage of protein in the castor meal is high compared to the other oilseed meals. That the pomace can be a rich protein source is appreciated on knowing that the deficiency in some essential amino acids is known even in those oilseeds whose proteins enter into human nutrition, e.g. soybeans. Thus, the limiting amino acids in soy meal are the sulphur-containing acids methionine and cystine (51). The coconut protein is deficient in lysine, methionine, and threonine (52). In safflower protein the limiting amino acids are lysine and isoleucine. The first limiting amino acid in castor pomace is lysine and the second is tryptophan (53). This deficiency does not preclude the application of the pomace as a fertilizer because plants take proteins in the form of nitrogen. In fact, Kulkarni rates castor pomace highly and justifies using it as a fertilizer for processing the following qualities: (24)

(i) It is rich in nitrogen (6.4%), phosphoric acid (2.5%) and potash (1%)

Table 1.7

The Amino Acid Composition of Castor Cake*

Amino Acid	Kodras 1947 (229)	Mottola 1971 (97)	Ambekar 1957 (243)	A.Sattar (50)	Weiss 1971 (4)	Vilhjalmosdottin 1971 (53)	FAO Reference Protein (48)
Arginine	11.0	10.91	16.0	5.50	10.02	8.61	-
Aspartic Acid	4.6	9.46	4.6	3.09	-	9.67	-
Glutamic Acid	18.0	18.30	18.0	16.25	-	18.87	-
Histidine	2.5	2.08	2.5	3.40	1.68	1.25	-
Isoleucine	5.3	4.87	) 12.5	8.10	4.65	4.68	4.2
Leucine	7.2	6.27	)	7.40	5.65	6.42	4.8
Lysine	3.1	2.96	3.1	3.64	0.30	2.68	4.2
Methionine	1.5	1.75	1.5	1.30	1.46	1.51	2.2
Phenylalanine	4.2	3.87	4.2	4.06	4.67	4.02	2.8
Proline	3.9	3.43	3.9	2.26	-	3.74	-
Threonine	3.6	3.47	3.6	5.11	3.24	3.44	2.8
Tyrosine	3.2	2.22	3.2	-	-	2.82	-
Valine	6.6	5.86	6.6	8.85	5.40	5.44	4.2

Table 1.7 Continued

Amino Acid	Kodras 1947 (229)	Mottola 1971 (97)	Ambekar 1957 (243)	A.Sattar (50)	Weiss 1971 (4)	Vilhjalmosdottin 1971 (53)	FAO Reference Protein (48)
Tryptophan	0.62	-	0.8	3.86	1.11	0.31	1.4
½ Cystine	-	2.12	-	-	-	0.68	-
Glycine	-	4.25	-	-	-	4.31	-
Alanine	-	4.34	-	0.97	-	4.26	-
Serine	-	5.61	-	-	-	5.44	-
Hydroxyproline	-		-	-	1	0.28	-
Ornithine	-	0.07	-	-	-	-	-
Ammonia	-	2.24	-	-	-	-	-
Other N Cpds.	-	-	19.5	-	-	-	-

* g/16 g N i.e. g/100g protein

(ii) It rates as high as organic fertilizers on the basis of nitrogen nitrified

(iii) It contains many secondary nutrients

(iv) It forms humus

(v) It decomposes slowly in the soil, thus assuring a steady supply of nutrients,

(vi) It retains moisture well.

In feeding experiments, castor proteins supplemented by the required amino acids gave results similar, and sometimes superior to known vegetable proteins (48).

Mo st oilseeds contain one or more substances which can be held responsible for some unpleasant effects associated with their products. These effects range from bitter taste and flavour reversion to toxicity and allergicity. Thus the bitter principle of sunflower seeds has been attributed to the presence of a glucoside (48). There are compounds in the soybean that are responsible for the reversion of its oil's flavour on storage (51,54). The soybean also contains a number of trypsin inhibitors (51) which reduce the nutritive value of its proteins. Antinutritional agents are also found in rape, mustard, and crambe seeds (8). Appreciable amounts of chlorogenic acid, caffeic and quinic acids exist in the kernels of sunflower seeds (48). These phenolic compounds interfere with the availability of certain amino acids in the protein of the meal in question (23).

Toxins and allergens are also found in oilseeds, as is the case of a number of natural products. Thus cottonseeds are famous for their content of gossypoll, a polyphenolic toxic compound (10). A Cyanogenetic glucoside has been identified as the toxic principle in flaxseed (55). Mahua seeds are known to contain a toxic glucoside (55). The tung fruit is toxic and its oil causes allergy and gastrointestinal problems (5). Allergens are found in cottonseeds and in kapok seeds (56). Undesirable effects are associated with alkaloids in seeds such as the argemone oilseed (57) and cyano glucosides in the meal producced from jojoba seeds (58). Abrin, robin and Crotin are deadly poisons belonging to the family of phytotoxins (59) and they bear a close resemblance to ricin, the castor bean toxin. This list is not intended to be exhaustive, but it serves to illustrate that the presence in castor seeds of a phytotoxin, an alkaloid, and allergens is not unique. What is unique, however, is the potency of the castor bean toxin and allergen, a potency that makes these compounds some of the most dangerous hazards known in natural products.

1.5 VEGETABLE OILS IN THE SUDAN

1.5.1 The Vegetable Oil Industry

The Sudan is an agricultural country where oilseeds contribute to a substantial percentage of the national economy. The seeds grown on a commercial basis are those yielding edible oils, and the major crops include cottonseeds, peanuts and sesame. Cotton is grown for

its fibre and the cottonseeds are a by-product of its processing. Most of the Sudanese cotton is grown under irrigation in the Gezira Scheme, Suki, Khashmal Girba, and Rahad. Rain-grown cotton is confined to small areas in the Blue Nile Province and Southern Kordofan Province. Two main types of cotton are grown and hence two types of cottonseeds are produced. The Egyptian long-staple variety is grown under irrigation and yields black cotton-seeds containing about 20% oil. The other type, the American short and medium staple cotton, is rainfed and irrigated - in that order. The oil content of the white fuzzy seed from this type is around 15%. Sesame is exclusively produced in rainfed areas, mechanized production is in the Kasala and Blue Nile Provinces, but small holdings are dominant in Western Sudan. Groundnuts are grown under rainfall in small holdings in Western Sudan, and in small irrigated holdings in Middle and Eastern areas. Significant changes in the information given do happen due to problems or to changes in policy.

These seeds are processed in factories belonging to both the public and private sectors. The public sector tends to concentrate on processing cottonseeds, and may at times refine crude groundnuts oil. The private owners process cottonseeds, groundnuts, and sesame. Oil palms and coconuts grow in Southern Sudan and are processed on site. Potentially important seeds include soybeans, sunflower, and safflower. Of the non-edible oils, castor oil is the only one of commercial importance.

The edible oils are used for food, but the low grades go into soap manufacture. The cake produced is mainly consumed as animal feed and some of it is exported. Recently, solvent extraction units were installed to defat the cake which may have as high as 7% oil content. Screw presses are the dominant equipment in the industry, and the machines are imported from the United Kingdom and Europe. In rural areas presses driven by camels are still popular but their efficiency may be low.

1.5.2 Research

Research into the vegetable oils in the Sudan is mainly carried out in the Food Research Centre in Shambat, which was founded with help from the United Nations. The Ministry of Industry established the Industrial Research Institute with a view to helping the Industry by giving advice and making available the services of expert technical staff. Most of the work of the Institute in the vegetable oil industry was directed towards cottonseeds, although there has been attempts at working with other oilseeds, especially groundnuts.

Research on the castor seeds has almost been exclusively confined to its agricultural aspects. This was initiated in the 1950's in the Gezira and Tozi research stations, but was transferred to the Hudeiba station in 1961 (3). The main topics studied were agronomic aspects, variety trials, hybrid production, and association between the yield components. Some of the varieties investigated are shown in Table (1.8).

Table 1.8

Some of the Castor Seed Varieties Investigated in the Sudan

Varieties	Origin and description
32/1	A selection made at Hudeiba. Stem colour is green; inflorescence is interspersed.
53/1	A selection made at Hudeiba. Stem and petiole colour is green; inflorescence is interspersed.
P98	A selection from variety Cimarron made at Hudeiba. It is comparable to Cimarron but somewhat vigorous and late maturing.
Barker 296	An introduction from U.S.A. It is a dwarf variety with red bloomy stems and petioles.
Lynn	An introduction from U.S.A. The variety is dwarf with light green bloomy stems and petioles.
29/1	A selection made at Hudeiba. The colour of stems and petioles is green; inflorescence is interspersed.

Table 1.8 continued

Varieties	Origin and description
N 145-4 (Monoecious)	Introduced from U.S.A. Stems and petioles with green colour. Very early flowering.
60/1	A selection made at Hudeiba. Stems and petioles are green with bloom.
Hale	An introduction from U.S.A. Dwarf internoded variety. Stems and petiols are green with bloom.
Cimarron	An introduction from U.S.A. Leaves are relatively small. Stems and petioles are greenish red with bloom.
2/1	A selection made at Hudeiba. A normal-internoded strain. The stem colour is green.
22/1	A selection made at Hudeiba. Stems and petioles have light green colour.

Table 1.3 continued

Varieties	Origin and description
358/1-6	A selection made at Hudeiba. The variety is dwarf with some normal off-types. Stems and petioles have red colour with bloom.
1118	An introduction from the United Kingdom. Stem and petiole colour is red with bloom. The variety is very early flowering and the capsules are spineless.
Baker 292	An introduction from U.S.A. The variety is dwarf. Stem and petiole colour is red with bloom.
PO/6	A selection made at Hudeiba. It is vigorous, variable, poor branching and late maturing. Stem and petiole colour is mostly green.

Table 1.8 continued

Varieties	Origin and description
PO/11	A selection made at Hudeiba. It is very tall and poor branching. Many off-types are present. Flowering and maturity are comparatively very late. Stem and petiole colour is green.

1.5.3 The Castor Oil Industry

Although wild varieties of the castor seed plant are known throughout the Sudan, no organized production was started prior to 1958. Regular production was commenced in the 1959/60 season in the Gash Delta in Eastern Sudan. By 1971/72 the area grown was over 30,000 feddans (1 feddan = 1.038 acres). The main problems facing castor production in the Gash Delta are the fluctuations of the Gash river floods and the infestation of weeds. The commercial variety grown is 'Pacific 6', a hybrid produced locally from parents introduced from the United States. Presented in Tables 1.9 and 1.10 are the production figures and area estimates. The figures for the Gash Delta area are included in the data given for Kasala Province.

The castor seeds produced in the Gash Delta were pressed in a factory near the area of production. Numerous problems have been due to the allergenic reactions manifested by some of the local population. Production of the oil was halted and the seeds were grown wholly for export. The stems of the castor plant were also utilized for making cardboard in a factory in Aroma in Eastern Sudan, but it also had to close for technical difficulties.

1.6 THE PROBLEM INVESTIGATED. SCOPE

The large-scale production of castor oil is carried out by mechanical expression, or prepressing followed by solvent extraction, but both routes pose operational problems. The direct solvent extraction of the castor seed is not commercially practiced due to the difficulty of preparing the seeds for

extraction, in addition to its high oil-content. The unique properties of the oil, especially its exceptionally high viscosity, add to the problems experienced in dehulling the seed and reducing its size. Moreover, the presence of potent toxins and allergens in the seed constitutes a major hazard that must be carefully controlled.

This study was initiated to develop a process for the direct solvent extraction of castor seeds with emphasis on the following areas: dehulling, size reduction, solvent extraction, and the detoxification-deallergenization of castor cake. The technical feasibility of the direct extraction process has already been demonstrated on a bench scale (88), but the problems in the preparation section and in detoxifying the cake were not completely resolved.

Although this is a Chemical Engineering thesis, a study of the biochemistry of the castor toxins and allergens had to be made, and is reported in Chapter four; the reasons that necessitated this study are given in section 5.6 However, only the most relevant aspects of the subject were included, and a glossary was compiled to help in understanding the contents of that chapter. Also, because of the various areas covered in this research, the references were listed in full to enable the reader to have an idea about the contents of the work referred to.

Table 1.9

Estimate of Area, production, and average yield of Castor Seeds in the Sudan, by Provinces: 1961/62 - 1969/70 (60)

Ka		23
TO		23
		69-70
Nor		333
Blu		
Kas		511

Table 1.10

Estimate of area, production, and average yield for Castor in Sudan (61)

1970-1976

Chapter Two

METHODS OF PRODUCTION OF VEGETABLE OILS

2.1 INTRODUCTION

Oil-bearing sources in the vegetable kingdom fall into two classes according to the type of fruit in question. Thus we have the oil-bearing nuts from trees, e.g. coconut, oil palm, olive, and tung, which yield fruits and nuts. The other class is that known as the annual plants and is represented by cottonseeds, castor, and peanuts, and the fruits obtained from them are called nuts, seeds, and beans. This division does not involve the use of the oil in terms of its being edible or not. Most of the discussion which follows refers to the second category since its members are more or less similar. The most obvious difference between these two classes is that the sources in the first category yield oil from the flesh and kernel, whereas the oil from the annual type is almost exclusively obtained from the kernel.

Oilseeds have variable sizes and compositions, yet some generalizations are possible. Thus the kernel usually makes up most of the seed weight, while the seed coat in which the kernel exists accounts for Ca. 25% of the seed weight and is usually made up of carbohydrates, proteins, and fibre with very low oil content. The size of the seed, the ratio of husk to kernel, and the oil content of the seed are the factors which usually dictate how a seed is processed. The treatment applied to different seeds is, however, essentially the same; the

object being to obtain the best quality of oil at the lowest possible cost. In addition, a safe, useful residue must be obtained.

A broad classification of oilseeds utilizes their oil content as a criterion of assessing the ease with which they lend themselves to industrial operations. Thus we have the low oil-content category which includes those seeds with an oil content of 20% or less. If the oil content is higher, the seed or nut is said to have a high oil-content. Table (2.1) shows the oil content of different oilseeds. It must be stressed here that this classification is somewhat arbitrary. Thus while high oil content seeds have the reputation of being difficult to treat with direct solvent extraction this would depend on how high is the oil content. Cottonseed, for example, would be a high oil-content seed according to the above classification, but in spite of this it is being increasingly processed by direct solvent extraction.

The methods of vegetable oil production are the ones based on mechanical methods, and those employing organic solvents for extracting the fatty material. A number of combinations of these two methods exists. The mechanical methods employ either hydraulic or screw presses. Direct solvent extraction works best with the low oil-content seeds, while high oil-content seeds are either full pressed or prepressed and then solvent extracted. The extraction of solutes from solids by the use of solvents is termed leaching but this terminology is seldom used in the vegetable oil industry, and some authors even call

TABLE 2.1

The Oil Content of Common Oilseeds (61)

this process "washing extraction" (63).

2.2 MECHANICAL PRESSING

2.2.1 Introduction

Mechanical pressing was, for some time, the only method used for producing vegetable oils on a commercial scale. It started with hydraulic presses used for batch production but later was developed to use the high-pressure machines known as screw presses in the continuous processing of oilseeds.

Each method of production has its sequence of operations, and in a typical screw press installation the operations would include cleaning, storage, decortication, size reduction, moist heat treatment (cooking), and pressing. Each of these would be taken in some detail in the following subsections.

2.2.2 Seed Preparation

The main product from the seed is its oil. Although this is recovered in the single step of pressing, yet the key to good pressing is good preparation. Hence all the operations before pressing are lumped in "seed preparation" although cleaning and storage are not included in this group and will be dealt with first.

When the seeds are received at the processing plant, it is advisable to clean them before storage because some of the foreign matter in the seeds tends to generate heat in confined spaces. This leads to increased free fatty

acid levels in the seeds and hence colour fixation of the oil results or high refining losses are incurred, or both may take place. In some cases, e.g. cottonseeds, the seeds may even catch fire. Actual mill practice differs from the desired procedure because, due to high cost, seeds are cleaned when they are moved to the production line.

It is also essential to store the seeds in a manner which prevents breakage or biological deterioration. The temperature and moisture level may be the two crucial parameters to be checked during storage. Details for the storage of different seeds will be found in the literature (64).

The clean stored seeds then take one of two courses down the production line. In the first the seeds are decorticated i.e. the seed coat is removed and the husks are separated from the kernels. In the alternative course, the seeds are processed with their seed coats on. Most manufacturers recommend the first course (65), but the choice depends on many factors. The next step is to reduce the size of the seeds. The need for this may be more appreciated by referring to Fig. (5.1) which shows the ultrastructure of castor seed, a typical oilseed in this respect. An infinite number of oil bodies is found in each seed, and protein bodies are dispersed among these oil bodies. For efficient recovery of the oil, the cells must be ruptured and the "bond" between the oil bodies and protein broken. These aims are achieved by

size reduction and steaming (cooking). Size reduction may be achieved by flaking or rolling. Flaking is used with direct solvent extraction or prepressing followed by solvent extraction, while rolling is usually connected to mechanical pressing. When flaking is applied, cooking is either eliminated or applied in some form before the flaking step e.g. in prepressing. Rolling, however, is usually followed by some form of cooking. There is still a controversy over whether the effectiveness of size reduction is due to grinding or rolling. In modern 3-high and 5-high rolls, the seeds experience the shearing action of the rolls as well as the weight of the upper rolls.

The flaked seeds then proceed to the cookers where they are heated in the presence of moisture. The equipment used is of the stack type and hence the flakes are treated in stages. In the first stage, cooking is done at high levels of temperature and moisture content. These levels and their combinations vary for different seeds, and there are variations in the residence times employed for the various types of seeds encountered. In the rest of the stages the flakes are subjected to venting and higher temperatures so as to reduce their moisture content.

Thus cottonseeds are cooked at a moisture content of 12-15% in the top kettle. Keeping this moisture level, the temperature is rapidly raised to 190^{o}F. Heating is continued at high moisture levels until the temperature

reaches about 220°F or maybe 270°F (66). A final moisture content of 3% is normal for cottonseeds intended for processing in a screw press. Soybeans are reduced to 2½-3% moisture before being sent to expellers, whereas moistures of about 2% are sufficient for copra and sesame (66).

It will be seen that the initial and final moisture contents of the flakes are vital for high-quality meats. It is essential that the final moisture content be the optimum for the seed in question because if the moisture content is higher than the optimum value, the material will be too fluid to press, and excessive undesirable "fottings" pass into the oil. If the moisture content is lower than the optimum, the material is not sufficiently plastic and cannot "flow" in the expeller (67). The total cycle time varies for different seeds but is usually taken to be between 60 and 90 minutes.

For direct solvent extraction the flakes may undergo the same treatment as that mentioned above. Normally, however, meats for direct solvent extraction are mildly heated to 120°-130°F, their moisture content adjusted to 10-11%, and they are then sent to the flaking rolls (68). The purpose of this conditioning is to form thin, tough flakes, and in some cases the flakes are crisped to achieve the desired characteristics.

Although thin flakes are required for efficient oil recovery, the reduction in thickness is limited by the fact that extremely thin flakes cannot withstand the flow

of solvent. Good characteristics of compactness and high drainage rates can be obtained in a bed if the flakes are properly treated.

In the mechanical processing of castor seeds it is recommended to cook whole seeds to 190-200°F at 4.5-5.5% moisture for 45 minutes (69). Sharder (70) reported the operation of a plant for the continuous mechanical pressing of castor beans in which the beans were heated in such a manner as to enter the expellers at 120°F. The resulting cake had a residual oil content of 13% and a moisture content of 6%, and was successfully solvent extracted afterwards. The single- and double-pressing of castor seeds was discussed by Dunning (71). The treatment of seeds varied according to the husk thickness and the nature of the pigment glands in the inner lining of the husk. Thus, medicinal quality oil, No. 1 grade, could be obtained by warming the beans to 150°F and immediately pressing them in a screw press. The cake with a 20% oil content is heated at 200°F to lower the moisture content to 4-5% and when pressed yields a No. 3 oil, leaving 5-7% residual oil in the cake. Problems encountered as a result of pigments in the husk prompted the processing of decorticated seeds. Thus, the kernels were cooked at or slightly below 175°F and the moisture maintained at 15%. The cooked meats were then dried, preferably below 215°F, to a moisture content of approximately 4% and then fed to the presses. The same method of preparation is also applicable to prepressing decorticated castor beans followed by solvent extraction.

Although most of the patented processes for removing castor oil from the beans utilize solvent extraction, some of them refer to the more conventional methods. Thus, Anderson (72) describes a process whereby the seeds are ground, heated to about 105-125^{o}C and then cooled to 60-80^{o}C. The moisture content is adjusted not to exceed 4% and the mass is fed to a continuously operating press thus producing a relatively dry cake.

The cookers, commonly situated above the presses discharge continuously into these machines where the oil is forced out of the flakes by mechanical pressure. The oil is taken for further treatment, and the cake is sent to the bagging sections. Figs. 2.1, 2.2, and 2.3 outline flowsheets for three possible methods of extracting oil.

2.2.3 The Mechanism of Mechanical Extraction

The conventional theory of oil expression from oil-seeds indicates a need for rupturing the cell wall if a substantial amount of oil is to be expressed (66). This view has very recently been re-investigated by Mrema (323) who presented results on expression tests carried out on rapeseed and cashew nut (324). The basis of his argument is that the recent discovery of the porous nature of certain biological cell walls suggests that these pores may act as outlets for the oil when it flows out of the cell under pressure. Transmission electron microscopy confirmed the porous nature of the cell wall in the oilseeds investigated, and analysis of the expression tests, conducted in an

Instron Universal Testing Machine, suggests the rate determining step to be the flow of oil across cell walls in the seed kernel. According to him, the conventional physical and thermal pretreatment of oilseeds, e.g. rolling and cooking, do not appear to rupture the cell wall, and hence a better strategy should be adopted. He suggests applying undrained compression as a pretreatment and claims that up to 35% oil was expressed at ambient temperature without rupturing the cell wall.

There is no doubt that considerable work must be done before these concepts could be seriously applied in industry or even on pilot plant scale. The relevance of this approach to our present study is that the process we employed for removing castor seed husk resembles the undrained compression advocated by Mrema. It would be interesting to determine whether or not the castor seed cells have porous cell walls, and if they have, at what frequency and of what size. More about our results with undrained compression will be presented in chapter four.

2.2.4 Machinery

2.2.4.1 The Preparation Section

The machinery for this section includes equipment for seed cleaning, decortication, rolling and size reduction, and cooking. The equipment is standard and thus the same type would be used for a particular operation regardless of the seed processed, except where the seed or nut has unusual size, shape, or other characteristics. For similar seeds, the specifications would differ with differences in the size of feed and product.

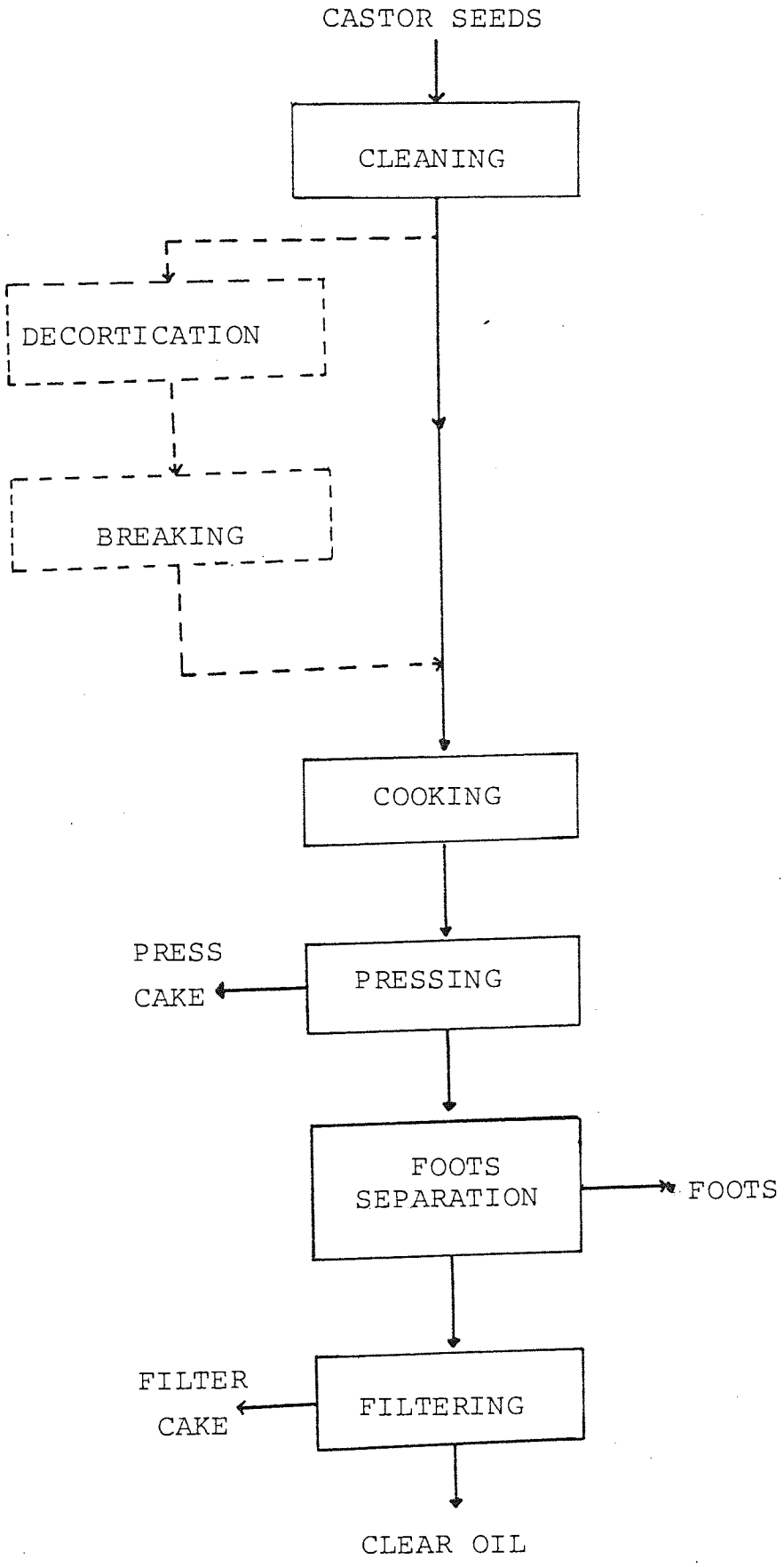


FIG. 2.1 THE MECHANICAL PRESSING OF CASTOR SEEDS

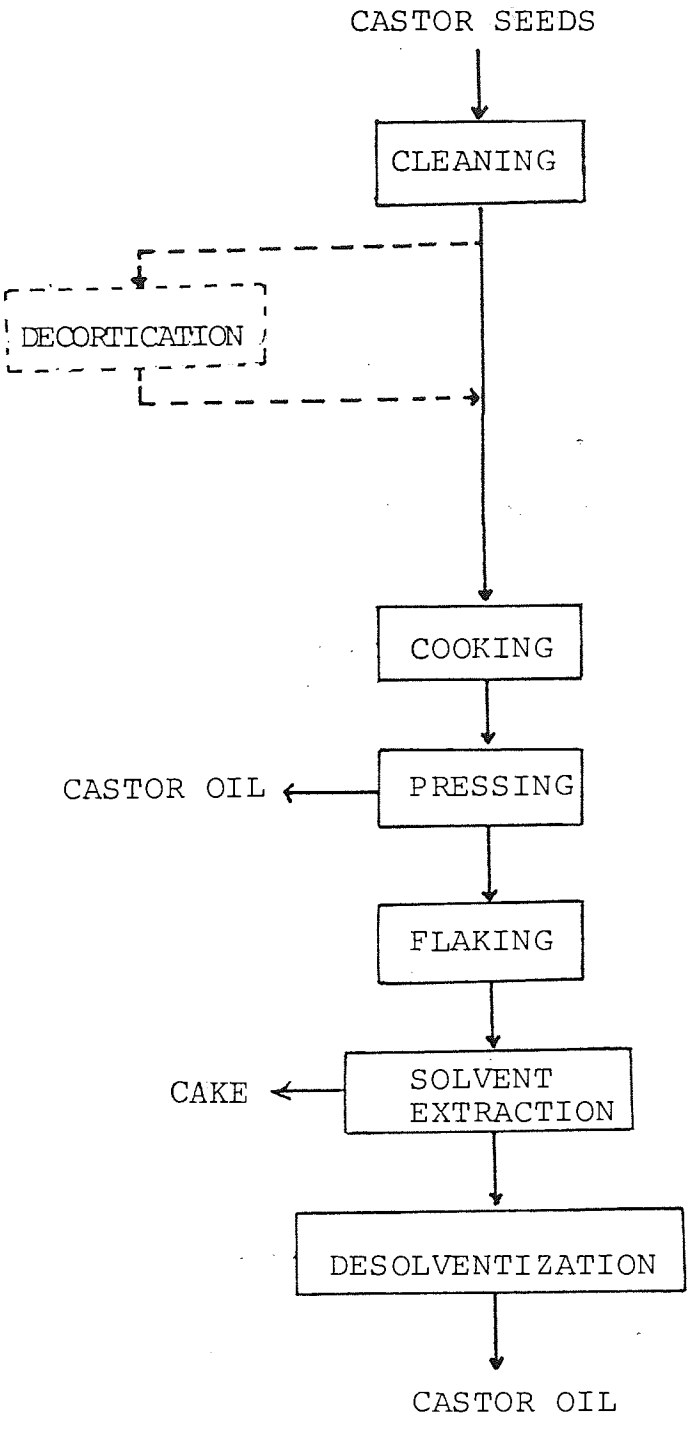


FIG. 2.2 THE PREPRESSING OF CASTOR SEEDS FOLLOWED BY SOLVENT EXTRACTION

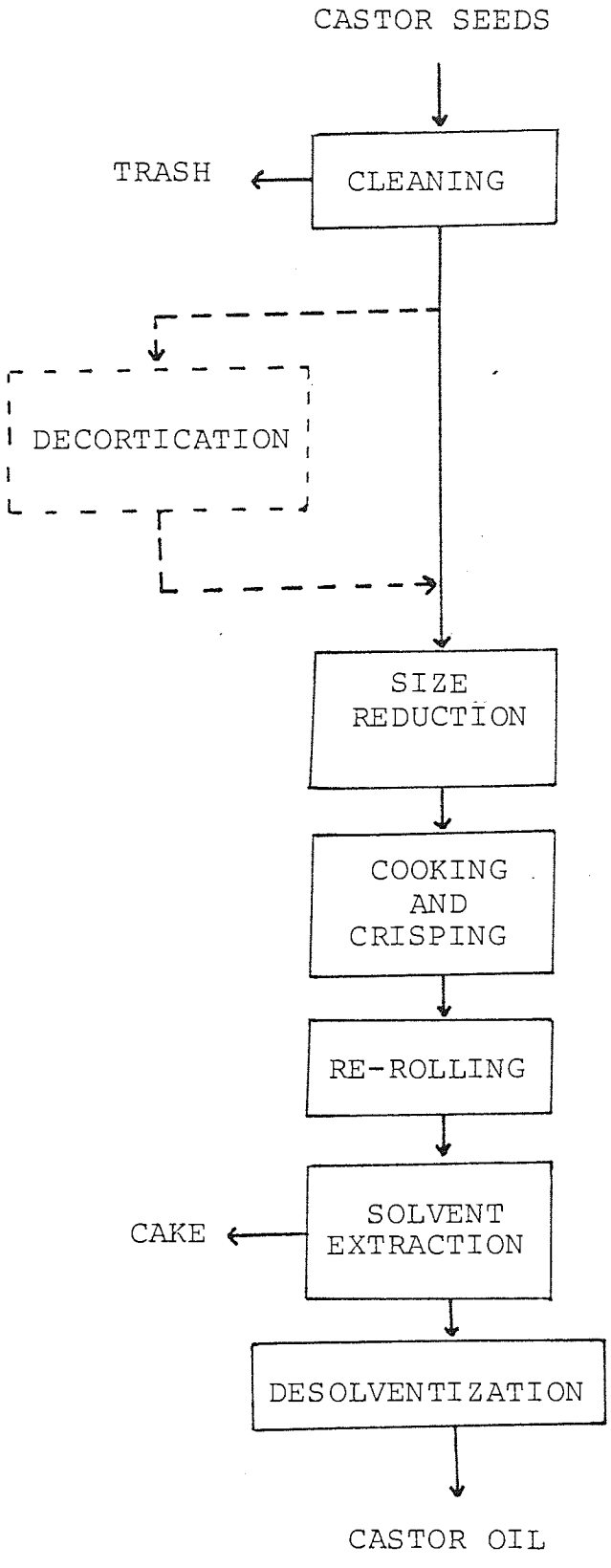


FIG. 2.3 THE DIRECT SOLVENT EXTRACTION OF CASTOR SEEDS

a) Cleaning

The cleaning operation removes different types of foreign matter from the seeds to be processed. Reels, drums, or revolving screens are usually used for removing sticks, stems, and leaves. Metal pieces, nails and other objects are removed by permanent or electro-magnets placed in strategic positions. It is essential to keep these metallic objects away from screw presses since they inflict a lot of damage to the press assembly.

Pneumatic systems for removing light material are also used. They include vacuum boxes, aspirators, and air separators. Such systems are usually supplemented by shakers and gravity tables. Sometimes special problems arise when the foreign matter is of the same size and density as the main product, e.g. small stones in shelled peanuts. Such cases are handled using special equipment, e.g. destoners in the case cited.

b) Dehulling

Removing the seed hull is usually accomplished by one of two types of hullers; the bar huller and the disc huller, and in each there is a stationary member and a rotating one. The bar huller consists of a rotating cylinder equipped with knives on its full width. These knives are longitudinally placed on the cylinder. The stationary member is concave and has knives which are individually adjustable towards the cylinder. The device works by splitting the seeds as they are caught between the stationary and rotating knives. The disc huller

has two discs placed opposite one another, with one rotating and the other stationary, and each has grooves cut radially along its surface. The seeds, fed to the centre of the discs, travel by centrifugal force towards the periphery where the clearance between the two discs is a minimum and hence the seeds are cleanly cut. The operation of these hullers requires that they be run at speeds suitable for the seed being handled. Moreover, the moisture of the seeds should be critically controlled.

Weiss (4) describes a number of hullers designed to remove the shells from castor beans after harvest, and it is desirable to narrow the time gap between these two operations so as to avoid loss of seed due to shattering and reduce the cost of transporting and storing the bulky capsules. Although Weiss does not mention using these machines for decorticating castor seeds, yet they operate on the same principles outlined for the disc huller. The capacity varies according to size, and some machines can hull up to 10,000 kg per hour of castor capsules. The operational problems encountered were mainly caused by the variations in the thickness and strength between the varieties processed. Uneven shelling occurred if the feed was not classified according to wall thickness and capsule strength. Fig 2.4 shows a sectional view of a huller which was successfully used in the Sudan in 1966 for hulling capsules of 'Pacific 6' and 'Cimarron' varieties grown in large-scale plantings (4).

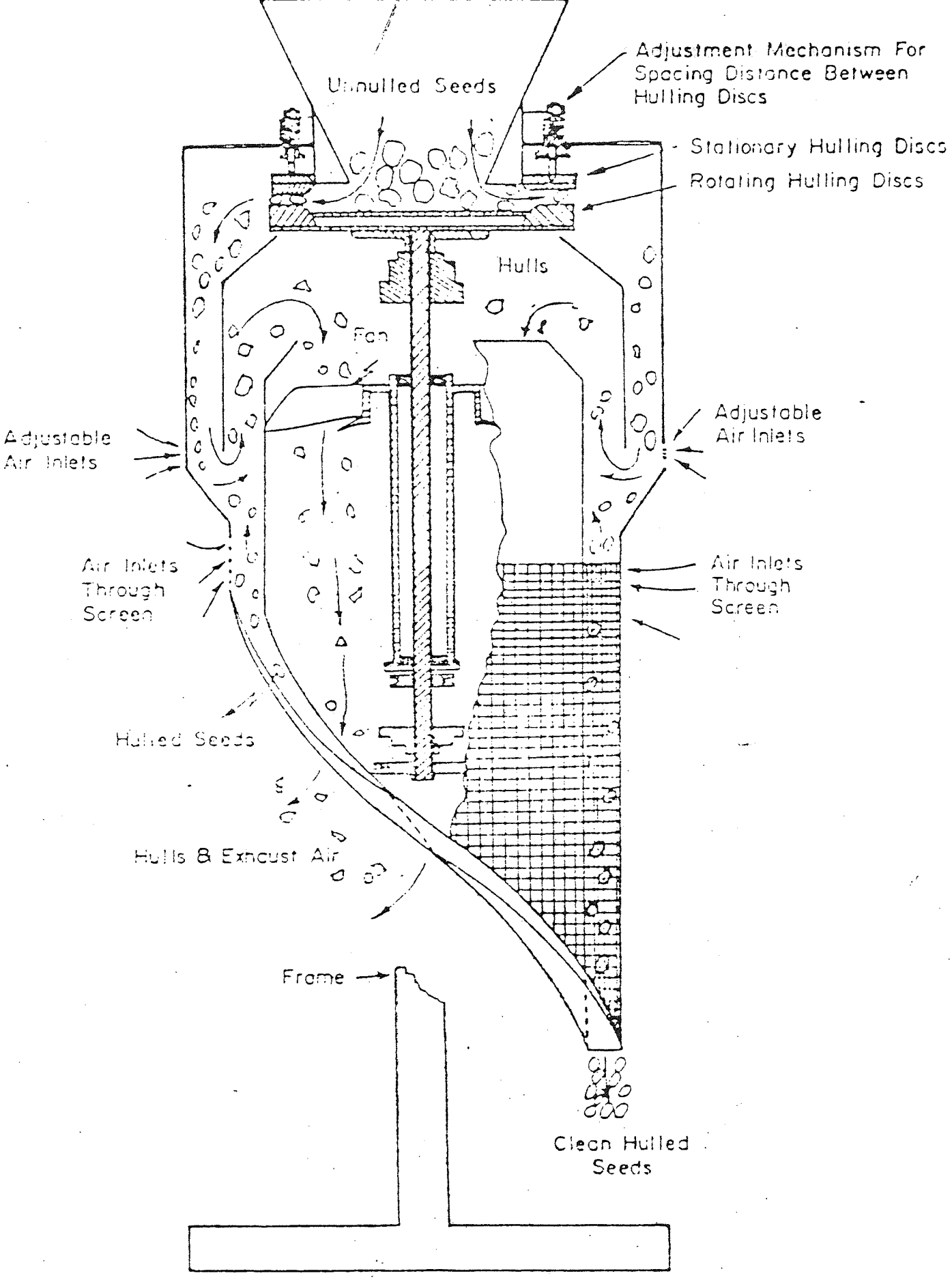


FIG. 2.4 SECTIONAL FIEW OF A DEHULLER FOR CASTOR SEED CAPSULES

Some oilseeds, such as soybeans, require feeding to cracking rolls before further dehulling can be done. After husk removal, the separation of hulls from kernels is achieved by a pneumatic system similar to that described for the cleaning operation. In some cases, grinding plus air aspiration is employed to achieve decortication, but this only applies to seeds which do not lose oil excessively on grinding. For the palm fruit and the coconut, hammer mills crack the outer shells which are then separated from the kernels by floatation or by sending them down an inclined plane. The first method utilizes the fact that the kernels are lighter than the shells and hence would float in a brine solution whereas the rest would sink. This method requires the drying of the shells and kernels before further treatment. In the second method, the mixture of shells and kernels is caused to move down an inclined plane where the kernels would roll freely as they are spherical.

c) Size Reduction

The meats from the previous step proceed to the step of flaking or rolling. Parekh (55) applies "rolling" to the treatment of meats in 3 to 5 high rolls, whereas "flaking" describes the case when the meats are fed to one pair-high smooth rolls laid side by side. For many seeds, this operation starts by cutting the seeds into quarters and eighths, but this is generally considered a disadvantage in the case of castor seeds (69). Some seeds need their moisture content to be adjusted; thus flax seeds and sesame seeds are usually dried before rolling (55),

while it is customary to humidify cottonseeds prior to size reduction (47).

Cottonseeds, flaxseeds, and peanuts are usually processed in 5-high rolls which are placed one above the other with their longitudinal axes parallel. The seeds enter through a feeding mechanism between the two top rolls and are made to pass back and forth between adjoining pairs of rolls. Each roll carries the weight of the rolls above it, and hence the seeds undergo shearing action plus the pressure of the rolls. The top roll is usually corrugated to ensure proper feeding while the rest are smooth. Scraping knives remove any flakes adhering to the face of the rolls. Seeds which are directly solvent extracted, such as soybeans, are flaked between two rolls mounted side by side and provided with springs to adjust the clearance between them and thus ensure the uniformity of the flake thickness.

Rollers mounted vertically one above the other are free to move up and down in machine guides in the sides of the frames (47). Roller bearings are fitted in these crushing rolls and are reported to contribute substantial savings in power consumption. Although size reduction equipment may have high fixed costs, and sometimes high operating costs too, yet if they are efficiently run they will cut the spending in the subsequent processing steps especially the extraction stage.

d) Cooking

Cooking of the rolled seeds is achieved in what is known as stack cookers which are multistage-type equipment made of chambers through which the seeds fall to the screw presses. A cooker is a cylindrical vessel with diameters ranging from 72 to 100 inches and may be 1.5 to 2.5 feet high and would have four to eight chambers. Extending through the entire height of the cooker is a common shaft with stirrers mounted on it for each stage. These stirrers are located close to the bottom of each stage and they sweep the meats so that no portion would be "over-cooked" since this would have adverse effects on the oil yield and quality. The temperature of cooking is controlled through steam jackets located on the sides of the cooker. At the bottom of each stage there is a gate which opens when the height of the meats reaches a certain level, and thus the charge in that stage drops to the next stage. Since the cooker is usually located on top of the screw press, the bottom stage delivers directly to the press.

The regulation of the seed's moisture content is essential in cooking. Hence the top kettle is usually equipped with spray nozzles for moisture addition while the rest of the stages have forced ventilation to remove the moisture during the later stages of cooking. The rate of heat addition in the upper stages is normally higher than in the lower stages. To meet this requirement the steam pressure is maintained at high levels in upper stages but is subsequently lowered to provide

enough heat to maintain the meats at the desired temperature.

It was the normal practice to feed every screw press from one cooker, but it is becoming increasingly common for more than one press to be fed by the same cooker. In using the term "cooker" we refer to the stack type, but other types are known especially the horizontal conditioner. It is rare, however, to find conditioners used in place of cookers, i.e. they are usually used to supplement one form of cooking.

2.2.4.2 The Pressing Section

Today the screw press is the most common equipment available for the mechanical pressing of oilseeds. The batch methods previously used were found uneconomical and less efficient compared to the continuous mode of operation offered by the screw press. Solvent extraction methods were later developed to meet the limitations of screw presses.

The screw press consists of a main wormshaft rotating inside a barrel (or cage). The shaft carries a worm assembly which is made up of a series of worm sections separated from one another by tapered collars. This assembly is housed in a cage made of two halves and lined with lining bars around its periphery. To allow the drainage of expelled air and oil, the bars are spaced apart by spacers which leave clearances of up to 0.020 inches between each two bars. Knife bars are locked in the frames which clamp the cage halves and are meant to minimise meal

rotation when the shaft is revolving. The meats are fed by a high speed feed screw which can be three to four times faster than the main screw. At the discharge end of the machine there is a "choke gear" which adjusts the cake thickness. (73).

The worm sections are made in such a way that the volume of meats handled decreases along the length of the barrel and hence the cake is compressed to release the oil. The pressure exerted on the meats is of the order of 15000 psi and it generates heat which is dissipated by cooling the shaft and cage. This water cooling helps to produce good quality oil and a bright cake whose oil content is as low as 3%. An important factor governing the percent of oil in the cake is the friction coefficient between the meats and the cage lining bars and this may be upset if the material to be pressed is not properly prepared because then excessive "foots" get into the oil and the cake becomes "muddy". The hardening of the lining bars contributes to an improved friction coefficient.

The higher the capacity of the screw press the greater the horsepower required. Thus a 75 HP motor will give a capacity of 25 tons per day oilseeds, whereas a capacity of up to 100 tons per day would require the installation of a 300 HP motor (65). The gear boxes employed and the bearings fitted are governed by the requirement that they should withstand a certain number of working hours before requiring service or replacement.

The main reason is that with high capacities, stoppages are very costly and should thus be avoided at any cost.

2.3 SOLVENT EXTRACTION

2.3.1 Introduction

Being the most efficient method currently available for producing oils from seeds or nuts, solvent extraction has recently received increasing attention. In spite of its efficiency, it is known to work best only for low-content oilseeds. When high oil-content seeds are brought in contact with organic solvents, they disintegrate and produce "fines" which interfere with the extraction operations, and thus these seeds are usually pre-pressed and then solvent extracted. Castor seeds are classified as high oil content seeds and are therefore not directly solvent extracted on a commercial scale. The main drawback against pre-pressing castor seeds is that this would not produce a high quality oil if carried out in screw presses because of the heat generated, no matter what degree of cooling is applied.(71). Hence it is very desirable to develop a process for the direct solvent extraction of castor seeds in order to obtain high yields of top quality oil.

2.3.2 Theory

In order to be able to design equipment that will efficiently extract oil from oil-bearing material it is necessary to know the factors involved in the process. The theory of the solvent extraction of oilseeds was investigated in depth in the 1940's as is evident from articles written on the subject with reference to soybeans,

but the subject is complicated by the difference in opinion regarding the nature of the mechanism of the leaching process. Although oilseeds are similar to one another in ultrastructure, yet it would be appreciated that they would not necessarily have the same properties when we talk about extraction rates and their correlation to the various parameters involved. What follows is a brief survey of the theories advanced to explain the mechanism of the solvent extraction of oilseeds and the various factors which affect the extraction rates.

Basically there are two theories through which researchers in the oilseed industry have attempted to explain experimental data relating to the solvent extraction of oily substances. The first theory advocates that molecular diffusion is the mechanism by which oil is extracted from oilseeds, and the second is the "difficultly soluble oil" theory. The work of Boucher et al (74) simulated the extraction of soybean oil under closely controlled conditions which are listed by Osburn and Katz (75) as:

1) Diffusion takes place through both faces and along one axis only. There is no diffusion sideways.
2) Thickness of solid is uniform.
3) There is a uniform concentration of solute in the solid at the beginning of the experiment.
4) The solvent is maintained at a constant concentration throughout the experiment.
5) The diffusion coefficient is constant.

6) The temperature is constant.

7) The transfer of material at the surface of the solid is sufficiently rapid not to interfere with the internal diffusion process.

8) The porous solid is rigid and inert.

9) The plate is uniform i.e. it has the same structure with reference to any axis.

The porous solid mentioned in assumption (7) refers to the clay plates which were used by the authors because they argued that since oilseed flakes are porous, then if porous clay plates were impregnated with oil the extraction of those plates would indicate the mechanism when flakes are used. Furthermore, the physical properties would be easily controlled and the moisture content can be eliminated as a variable. The authors acknowledge that a modification is necessary to allow for the departure exhibited by soybean flakes from the uniformity assumed in (9). By using velocities which corresponded to Reynolds numbers ranging from 14.7 to 20,400, Boucher et al concluded that the liquid film resistance is negligible compared to the internal resistance within the plates. They presented their results as plots of E, the ratio of the extractable oil content at any time to the initial extractable oil content, versus time of extraction on special coordinates and obtained straight lines. They concluded that the extraction process is one of molecular diffusion because in terms of those coordinates straight lines were obtained (as suggested by Sherwood) when plotting data on the drying of solids

where internal diffusion controls the process. The following theoretical relation was proposed:

$$E = \frac{8}{\pi^2} \sum_{n=o}^{n=x} \frac{1}{(2n+1)^2} \exp\{-(2n+1)^2 (\pi/2)^2 \tau\}$$

where $\tau = D\theta/R^2$

D = Diffusion Coefficient, sq. ft./hr.

R = one half of plate thickness, ft.

θ = time, hrs.

The work of Boucher et al was extended by Osburn and Katz (75) who studied the effect of structure on extraction rates and found that the diffusion theory applies and that different structures produce wide variations in the type of theoretical curves obtained. However, the work of King, Katz and Brier (76) could not confirm the simple diffusion theory for uniform porous solids since it did not correlate the extraction data for soybean flakes. They suggested that the divergence was due to the structure of the flakes and the distribution of the extractable material in the bean. That the irregularities in the structure were a cause of deviation was shown by Fan et al (77) who found that the theoretical relations developed by Boucher et al (74) applied to peanut sections carefully prepared in a microtome. Othmer and Agarwal (78) confirmed the findings of King et al (76) and also found that when soybeans were extracted with hexane the concentration of oil in the extracting solvent had no effect on the total oil extracted or the extraction rate. Later, Othmer and Jaatinen (79) extended this work to solvents

other than hexane, and they concluded that the extraction rate was affected by the concentration of oil in the extracting solvent. The mechanism of extraction they suggested is as follows. Oil from the surface of the flakes and the outer layers of broken cells dissolves when the solvent comes in first contact with the flakes. Concentration differences are set up in the capillaries when the solvent penetrates to dissolve more oil, and capillary flow brings the oil nearer to the bulk miscella where more oil dissolves. The decrease of oil content in the flakes causes a decrease in the concentration gradient and, at the same time, the oil concentration increases in the miscella thus reducing its dissolving power. If the miscella is replaced by fresh solvent, this process is set up again. This mechanism is based on solvent flow being caused by capillary action since the authors have already concluded that molecular diffusion is not involved in the process of vegetable oil extraction.

The "difficultly soluble oil" theory of extraction was proposed by Karnofsky and Coworkers (80) as an alternative to the diffusion concept. They assumed that the oil to be extracted is held in the oil-bearing material by a bond, representing a resistance to extraction, which is slowly soluble. Thus the rate depends, at least in part, on the extent of this resistance. Thus, if the structure is the controlling factor in the extraction, then the extraction of original flakes and reconstituted flakes should proceed at identical rates.

To test this, flakes were extracted, re-immersed in oil, and extracted once more, with the result that the reconstituted flakes were much easier to extract than the original flakes (81). The conclusion, therefore, was that the structure of the flakes did not control the rate of extraction. Furthermore this theory is supported by the observation that repeated extraction of oilseeds yields fractions of oils with varying fractions of nonglycerides whose percentage increased towards the end of the extraction cycle, with the inference that there are some components in the oilseeds which do not extract as readily as, say, the glycerides in those seeds. Othmer and Agarwal (78) have shown that in extracting soybeans with normal hexane there is no point in employing counter-current operation. This supports the soaking theory, and a confirmation of this was made by soaking flakes in the solvent and then extracting them by percolation. Even when soaked in strong miscella, the flakes extracted more readily than without soaking (81) as long as the concentration of the oil in the miscella is below 20%. The industrial application for this is the filtration - extraction process applied to cottonseeds (82) and some other oilseeds, where the flakes are soaked in a single stage extractor for a prescribed period and then taken to a filtration unit to separate the miscella from cake - hence the name filtration extraction.

Not all investigators see the mechanism of oilseed extraction as being necessarily explained by one theory. If diffusion is not the only mechanism, there is no reason

to exclude it altogether from being a factor in the process. Since oilseeds are complex mixtures of many substances in varied proportions, it is logical to expect differences in their rates of extraction. The work of Othmer (78) suggests that changing the solvent affects extraction even when the seed is the same. Furthermore, the properties and chemical composition of vegetable oils show wide variation and these reflect on extraction rates. The situation is best summed up by quoting Becker (83) who stated "The mechanism for extraction of oilseeds appears to be a combination of diffusion, dialysis, and the solution of slowly soluble extractable material. The latter is sufficient to determine the size of commercial percolation extractors".

2.3.3 Machinery (34,66,84)

2.3.3.1 Introduction

Solvent extraction processes and mechanical methods of oilseed processing have some equipment in common before the actual extraction stage. The basic differences between the two methods mean that fundamental differences should be expected in the machinery used by each. The equipment described in this section would therefore exclude the common items which were discussed under mechanical methods, and the emphasis will be on the extraction equipment and the solvent recovery section.

The environment in an oil mill can be kept safe by preventing dust clouds which may lead to explosions. But in a solvent extraction facility the risk is far

greater because of the fire and explosion hazards associated with hydrocarbon solvents. Hence designs for extractors would necessarily be leak-proof and explosion proof (85). Vapour-tight seals and improved solvent recovery systems greatly contribute to the safety of operation. These considerations reflect on the auxilliary equipment needed for coping with the safety requirements.

2.3.3.2 Extractors

a) Batch Processing

Batch methods are advantageous compared with continuous methods because of their low initial investment cost, their ability to process small tonnages, and the possibility of operating them intermittently. They cannot, however, compete with the lower cost offered by continuous extractors of processing per ton of oilseeds because of the large tonnages handled by the latter. The main field of usefulness of batch extractors is in the pharmaceutical industries, in extracting oil from spent bleaching earth, and in similar operations, such as the processing of meat scraps and garbage.

The design of batch extractors varies according to the material they are required to handle. When dealing with substances like castor seed cake, it is usual to employ an extractor consisting of a large drum which is separated into two halves by a metal strainer covered with filter cloth. The charge of material to be extracted is placed on this mat and the solvent is allowed to percolate

through the mass and the miscella drains to the bottom compartment. Some versions of this extractor employ mixing arms to agitate the contents of the drum. Another type of extractor which handles relatively wet material is made of a steel cylindrical kettle fitted with a steam jacket and a vapour-tight cover. The extractor also has a low-speed agitator, and may hold a batch of up to 5 tons. The operation of this type of extractor may also handle materials which can be extracted and dried in the same vessel (66).

b) Continuous Extractors

The continuous mode of operation is the one most favoured in industry because of its economical and technical merits. The classification of continuous extractors can be made either on the basis of how solvent contact with the seeds is achieved or according to the mechanical features of the system being discussed. According to the first method, an extractor is either of the percolation type or the immersion type. In percolation extractors the solvent is sprayed onto a bed of flakes and percolates through as the miscella is drained by gravity. In the immersion type the seed particles are agitated in the solvent and the miscella is later separated from the solids. Each system has its advantages. Thus the percolation system is credited for producing a clear miscella, almost free of fines. It needs, however, careful preparation of the flakes because its efficient

operation depends on the existence of a bed with the right porosity. Although the filtering action is one of the advantages of percolation extractors, channelling and poor solvent distribution can occur; hence attention has to be paid to these factors in the design and operation. When the immersion extractor is specified, there will not be the strict limitation of careful seed preparation as in percolation systems, but there will be the disadvantage that gravity drainage of the miscella with not be applicable. Furthermore, auxilliary equipment is needed for separation of miscella from solids. Also, the immersion extractor would not be recommended in systems where particle size-reduction is not tolerated. The second method of classifying continuous extractors is to group them according to their mechanical design features and as such the following categories are observed:

a) Bucket type

b) Screw conveyor type

c) Tower extractors

d) Horizontal endless-belt conveyor

e) Miscellaneous

a) Bucket type

The bucket type is the oldest successful continuous oil-seed extractor (66). It is constructed like a bucket elevator with baskets having perforated bottoms. The number and dimensions of the baskets are related to the capacity of the extractor. The buckets are raised and lowered very slowly, and those buckets on the upward journey are sprayed with pure solvent while the descending

buckets receive half miscella. When a rising bucket reaches the top of the extractor it is inverted and the exchausted flakes are dumped into a hopper and screw conveyors transport them to meal desolventization units. The empty bucket is then automatically loaded as it starts its downward journey and it also receives a spray of half miscella. The solution draining from the bottom of the last descending bucket is termed "full miscella", this being the strong solution of oil in solvent, and it is pumped to miscella desolventizing equipment. This type of extractor is a percolation type and clear miscellas are usually obtained. There are other versions in which the buckets travel horizontally.

In the Rotocel extractor the baskets are carried in a rotary motion in a horizontal plane, and this extractor is claimed to have all the merits of the traditional bucket-type without suffering from the disadvantages they have, namely bulkiness and lack of flexibility of operation (66). There are between 15 and 18 cells, 1.8-3 m deep with perforated bottoms, and when the solids discharge zone is reached, the bottom of the basket is unlatched, dumping the solids which are then conveyed to the desolventizers. A similar deep bed extractor manufactured by Extraktiostechnik is the Carrousel which is said to have been tested on more than 90 materials. The French stationary-basket extractor differs from the Rotocel in that the cells do not rotate; instead, the moving parts are the solids feed spout, the solids discharge hopper, and the cell bottom unlatching mechanism. This means that

there must be a periodical switching of the location of the fresh solvent feed. Like the Rotocel, the cells are 1.8-3.0 m deep but there are between 12 to 20 cells in the stationary-basket version, and the excellent filtering action of the deep bed is a common feature to both types.

The horizontal bucket extractors have largely superseded the Bollman extractor because they permit one-floor operation and hence reduce housing costs (84). It seems, however, that deep-bed extractors will continue to dominate the markets of industralized nations.

b) Screw Conveyor type

The Hilderbrandt extractor is the best known in this type. It consists of two vertical tubes interconnected at the bottom by a horizontal tube through which a horizontal screw conveyor runs. The seeds are introduced in one limb of the U-tube and the vertical screw conveyor in that section advances them to the horizontal tube where they are moved and picked by the conveyor in the other limb of the U-tube. The solvent is introduced in the side of the extractor receiving the exhausted flakes and thus the miscella leaves on the side where the flakes are fed. The chief objection to this total immersion extractor is the disintegration suffered by the flakes as they are being transported by the conveyors. A modification of this system is the Detrex extractor which employs a horizontal tube connected to a sloping tube with screw conveyors in each of them.

c) Tower Extractors

The extractors in this category are distinguished by the vertical column in which extraction takes place. In the Bonnotto system the column houses a number of horizontal plates which divide the column into sections through which flakes fall and the solvent rises. A central shaft rotates the plates, and scraper arms located directly above each plate sweep the flakes and cuase them to fall through staggered slots in each plate. The solvent is introduced at the bottom of the extractor and flows upwards countercurrent to the flakes. The Allis-Chalmers and Anderson extractors were introduced to modify some features of the Bonnotto extractor, the main difference being the use of stationary plates and rotating scraper arms. Other differences also exist (66,86).

d) Travelling-belt type

This type was originated by De Smet in Belgium and seems to be not so popular outside Europe. Many of the extractors previously described are of European origin, German in particular, with subsequent modifications to suit particular needs and situations. The travelling belt type would mostly be of the percolation category, but it would need vapour-tight housing and pumps to transfer the miscella to the relevant sections.

e) Miscellaneous

Other designs for oilseed extractors are reported in the literature, and the Kennedy extractor is perhaps one of the most famous. It is made of a long enclosed trough

divided into a number of horizontal cells each with a round bottom. Perforated curved rotating blades mounted on wheels are fitted into each cell and they convey the flakes through the extractor countercurrent to the solvent. The conveying system is so designed as to minimize the solution retained by the flakes in passing from one stage to the next. A special wheel in the last stage helps to clarify the miscella by trapping any fines which could have reached so far. The extracted flakes leave by means of an inclined conveyor. A drawing of this extractor is shown in Fig. 2.9.

The Filtrex Extractor is the industrial application of the principle of the filtration extraction process. The main piece of equipment of the unit is a rotary vacuum filter with a shallow bed on which the flakes have a very short residence time, much less than a minute (87). Such short times of contact are made possible by presoaking the flakes in miscella for 45-60 minutes before feeding them as a slurry to the five-stage filter where they are washed with lean miscella and then in fresh solvent. The process is said to be adaptable to a number of oilseeds including those which are usually difficult to handle in conventional equipment (88).

In addition to the types described, other designs were reported but they seem to enjoy little, if any, attention today. Among these are centrifuge extractors, Miag, and Fauth (89).

Not all the types of extractors discussed are in use today. In fact, a large number of them are either obsolete or rarely employed (87). Thus, screw conveyor extractors, such as the Hilderbrand, are not encouraged because their screw cause excessive disintegration of the flakes. This produces fines which may choke the extractor. Tower extractors such as the Anderson and the Allis Chalmers model (Fig. 2.7) are also blamed for suffering from the fines problem. In addition, they are reported to cause backflow of miscella due to its increase in density as it proceeded through the extractor. For these and other reasons, they are no longer being manufactured in the USA, their country of origin.

The extractors currently used commercially for oilseed processing are listed in Table (2.2), and the characteristics of a number of them are illustrated in Figures 2.5 through 2.9.

2.3.3.3 Solvent Recovery

2.3.3.3.1 General

The reocvery of the extracting solvent from miscella and meal is one of the important steps in the process, in fact so important that the ease of recoverability is one of the basic criteria of solvent selection. The choice of the type of recovery system for a particular application is governed by the physical properties of the solvent and the miscella. The significance of removing the solvent from the final products has economic justifications and quality control considerations. Economically, the

TABLE 2.2
The Characteristics of Common Oilseed Extractors

Type	Capacity 10^3kg/day	Size (m) Diameter	Length	Height	Width	Bed Depth	Products and Materials Processed	Extraction Efficiency at L/F	Solid Retention Time (s)
Horizontal Rotary Deep Bed (Rotocel) (Carrousel)	0.2-3000 for soy	Rotocel 3.4-11.3 Carrousel 1-8	-	6.4-7.3	-	Rotocel 1.8-3.0 Carrousel 0.5-2.5	FOS, POS, SC	Soy 97-98% at 0.8-0.85	Soy 1080 FCS 5100
Stationary Basket, Rotating Feed and Discharge	Soy 61-2700 FCS to 1350 PCS to 3400	2.4-10.4	-	9-15	-	1.8-3.6	FOS, POS	Soy 97-98% at 1.1 FCS 96.5% at 1.3	Soy 2700 FCS 3600
Drag Chain, Horizontal Loop	OS1-1500	-	3-24	-	0.13-3.7	0.1-0.76	FOS, POS	Soy 96.3%-98.5% at 0.8-1.0	Soy 2400
Basket Conveyor Vertical Bollman	180-900	-	9.6	14	1.5-2.0	0.5-0.7	Flaked Oil seeds	Soy 97.3% at 0.96	-
Basket Conveyor, Horizontal & Rectangular	FOM to 3000, Lurgi 3-4000	-	-	-	-	0.5-0.7	FOS, HO, COS, CP CR	Soy 97-98% at 0.95-1.1	-
Continuous Belt	10-3000 for FOS	-	7-37	-	0.5-9.5	0.8-2.6	FOS, POS	-	-
Horizontal Rotary Filter Filtrex	45-450	1.8-5.2	-	18.30	-	0.05	Flaked Oil seeds	Soy 59% at 1.1	2700-3600 Presoak, 10 on filter

Key to Table 2.2

COS : Cocoa Shell

CP : Cocoa Pulp

CR : Cocoa Residue

FCS : Flaked Cottonseeds

FOS : Flaked Oilseeds

HO : High Oil-content Oilseeds

OS : Oilseeds

PCS : Prepressed Cottonseeds

POS : Prepressed Oilseeds

Soy : Flaked soybeans

FOM : French Oil Mill Machinery Co.

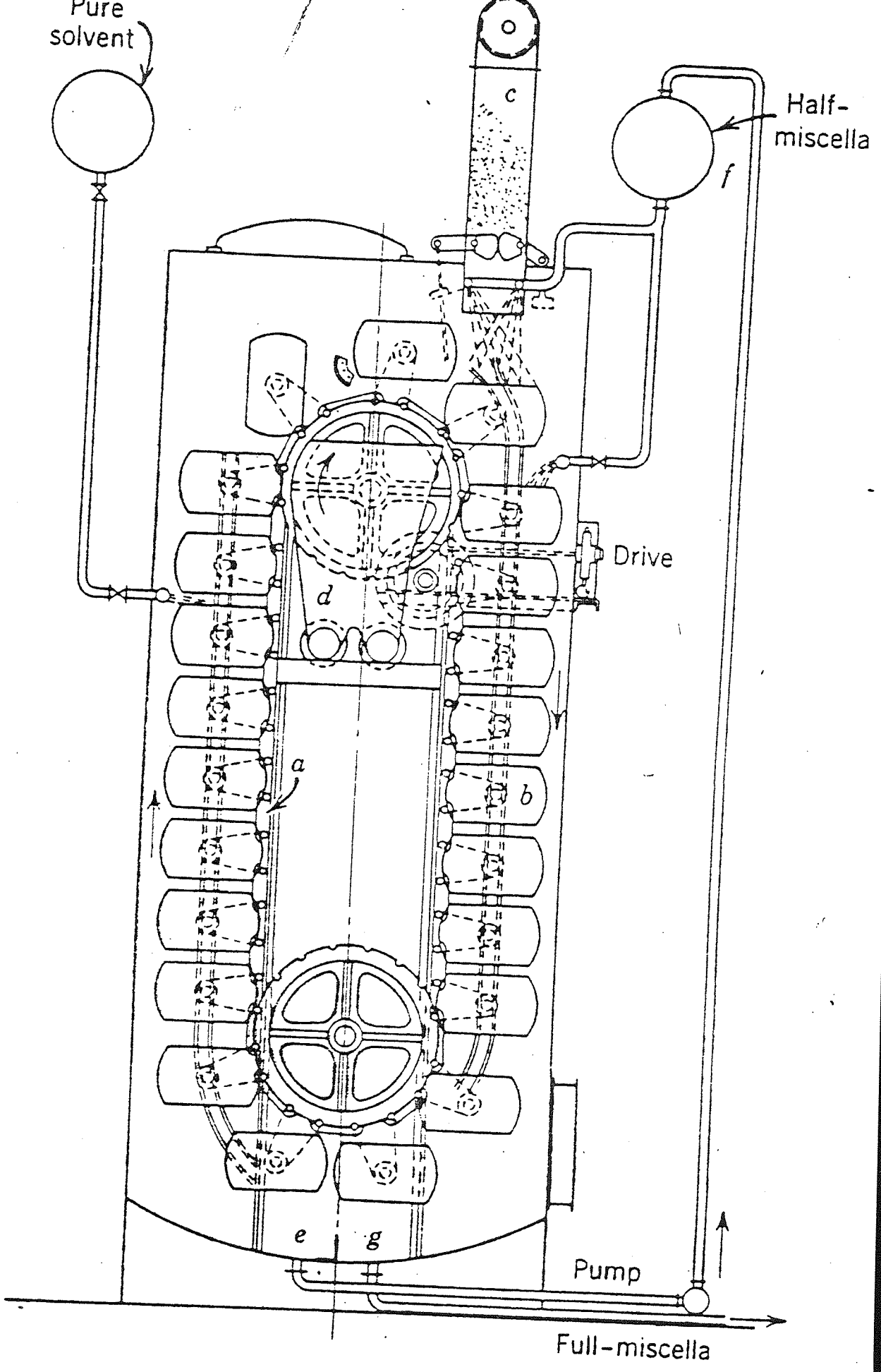


FIG. 2.5 THE BUCKET EXTRATOR

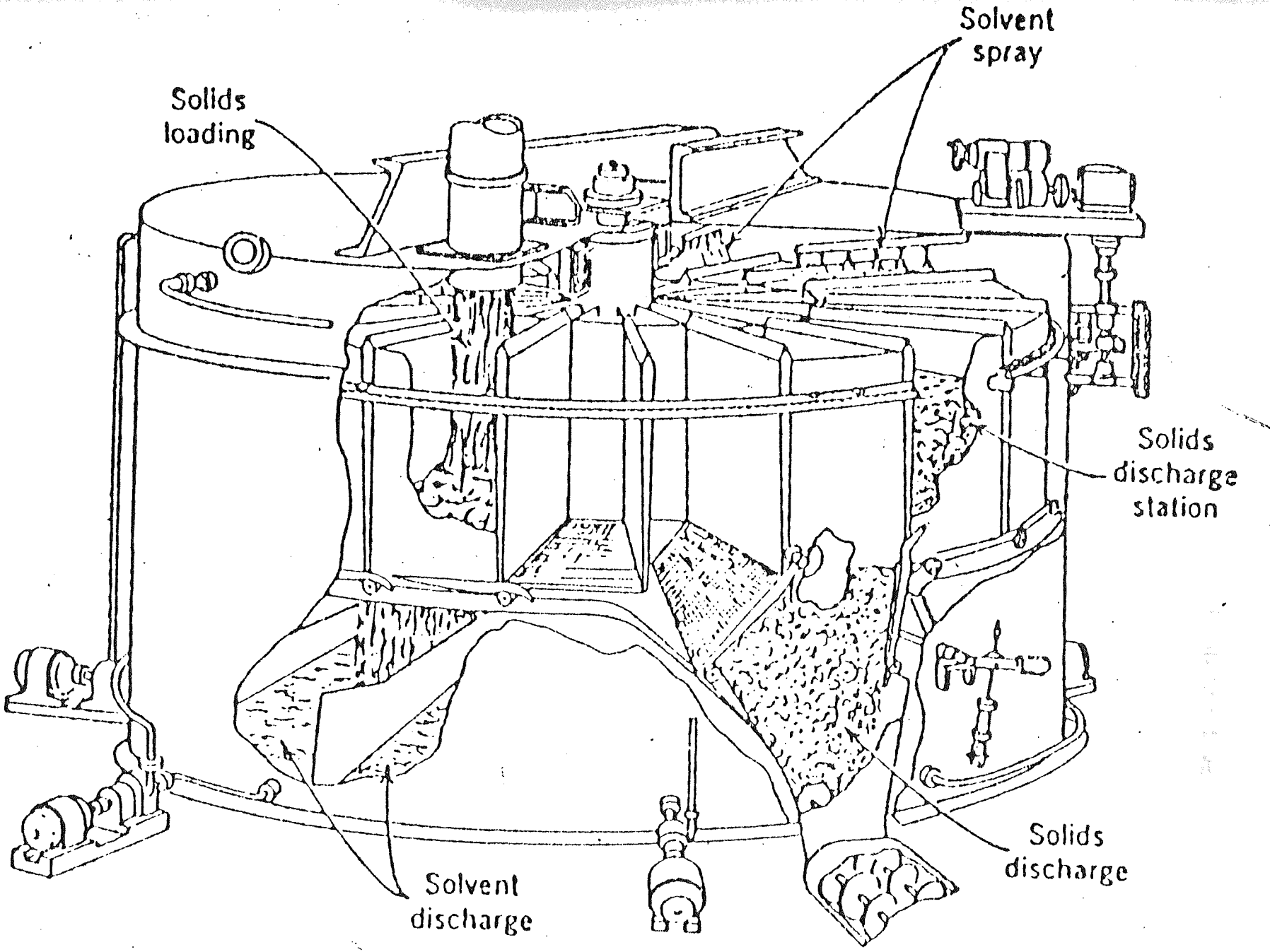


FIG. 2.6 THE ROTOCEL EXTRACTOR

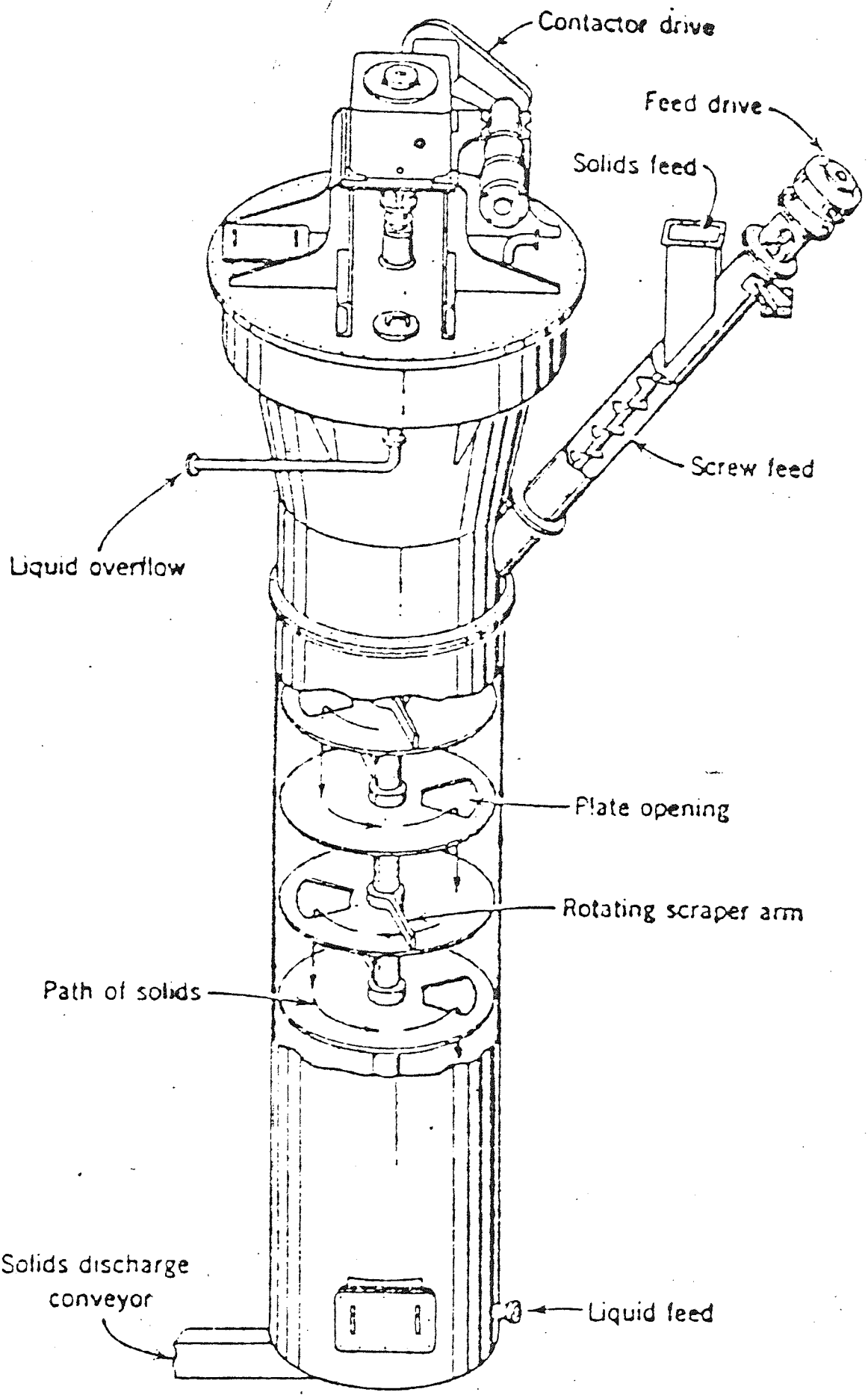


FIG. 2.7 THE ALLIS-CHALMERS EXTRACTOR

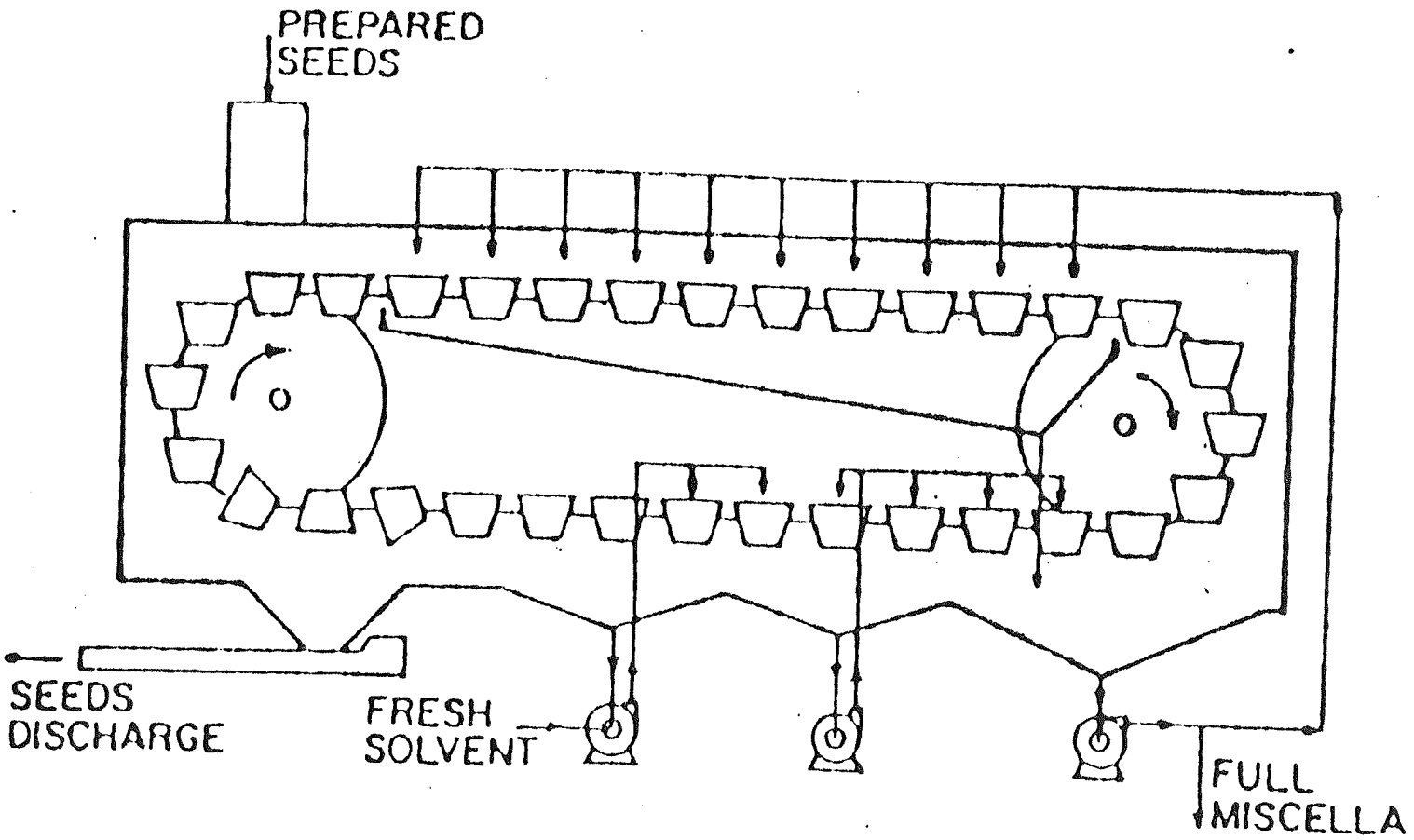


FIG. 2.8 THE TRAVELLING-BELT EXTRACTOR

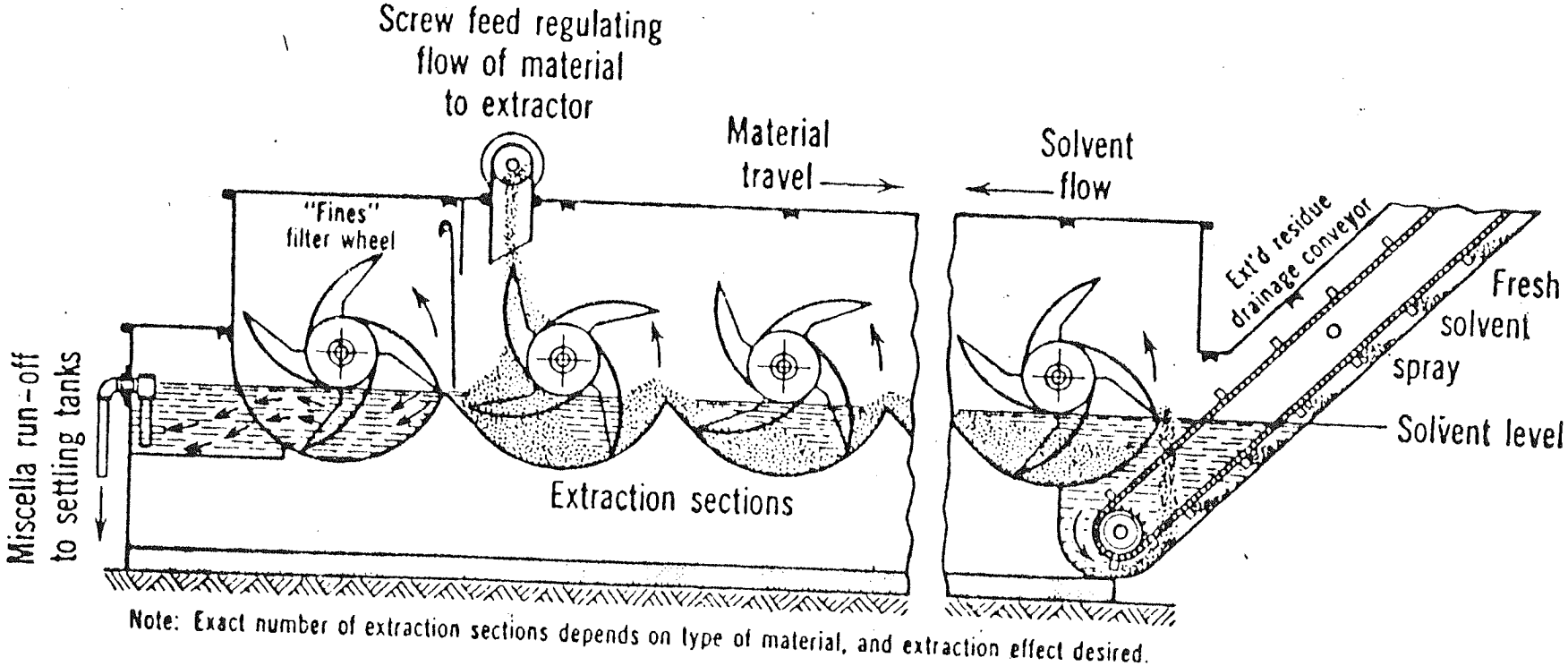


FIG. 2.9 THE KENNEDY EXTRACTOR

solvent is to be reused with the minimum of losses because of the large volumes employed in continuous processes. As for the quality control side, the edible oils should be exhaustively stripped of the solvent for health reasons. With some solvents, their presence even as trace impurities can interfere with subsequent operations, e.g. hydrogenation, as in the case of trichloroethylene (34). Fire and explosion hazards also dictate that the solvent should be removed as completely as is practicable, especially from the meal. In order to reduce the heat damage to the final products, solvent removal should be done at the lowest possible temperature and contact time should also be the shortest possible. These factors reflect on the design of the equipment in this section.

2.3.3.3.2 Recovery from Miscella

The methods previously used for oil recovery from miscella usually consisted of atmospheric evaporators followed by a vacuum packed still provided with direct steam. The current practice is to strip the solvent in a series of evaporators where all the effects maintain some level of vacuum. The miscella is usually filtered before being fed to the solvent recovery system because the presence of fines complicates the operation of the equipment especially the packed still. The fines also entrain some oil and therefore they should be washed to recover that oil. In addition, it is not recommended to circulate a large amount of very fine material through the system since that material does not contribute to the

efficiency of the required separation. At one time there was a tendency to employ centrifuges to effect the separation of fine solids from the miscella, but it seems that there is still a difficulty in handling fine solids if they exceed certain limits. An approach for removing fines which make up to 0.30% of the miscella in commercial systems is to hydrate them by injecting 1-4% water plus a wetting agent (90). The hydrated material plus a small quantity of emulsion are then removed in centrifuges.

A modern oilseed processing facility would have the typical arrangement described as follows. The clear miscella is fed to a pre-evaporator where most of the solvent is evaporated. The equipment utilizes the vapours coming from the solids desolventiser in removing the solvent and is operated under 400 mm.Hg vacuum. A second stage evaporator receives the concentrated miscella (oil content 65-70%) and raises its concentration to about 90% by heating it with steam flowing through the shell side of a tube chest. The miscella is pumped through another steam-heated exchanger to a packed column operated at 450-550 mm Hg vacuum. It is customary to employ disc and doughnut packing in this equipment because of their high performance in handling fines. The boiling point of the miscella decreases as the oil content increases, and vacuum operation is thus essential for working at reasonable temperatures. This is best illustrated by referring to a cottonseed-hexane miscella (66). At 760 mm Hg. pressure, a miscella containing 10% hexane by weight has a boiling point of 231^{o}F, whereas the same

miscella boils at 163°F when the pressure falls to 310 mm Hg.

2.3.3.3.3 Recovery from Cake

The flakes issuing from an extractor are allowed to drain long enough to reduce the solvent retention to 35-40% and are then carried through vapour tight conveyors to the desolventiser. Hutchins (91) described a desolventiser-toaster which would reduce the hexane concentration in the meal to 500 ppm or less and at the same time toast the meal by heating at 100°C and 18-22% moisture.

Chapter Three

Process Analysis and Development

3.1 INTRODUCTION

In this chapter the functions of the main stages of the process will be explained in detail, and the reasons for choosing certain options among possible alternatives will be made clear. The two main characteristics of castor seeds which affect process design are their soft nature and high oil-content. The softness affects the design of storage facilities and the choice of the handling techniques, the aim being to reduce seed breakage to the minimum (69) because damaged and broken seeds yield low-grade oil, cause oil losses, and clog chutes by accumulating dust on the oil so produced. The high oil-content dictates how the seed coat should be removed,how cooking should be conducted, and how the oil is to be extracted. The presence of toxins and allergens in the seed makes safety considerations highly important. The process sequence is affected by the method of treating the hazardous materials and whether it is applied to the intact seeds, the flaked seeds, or the pomace. Finally, the method employed for desolventising the miscella is governed by the physical properties of the oil-solvent solutions. The three sections under which the process stages will be grouped are: seed preparation, solvent extraction and solvent recovery. The treatment of toxins and allergens and its effect on the process will only be outlined in this chapter but will be taken up in

detail in chapter four.

3.2 SEED PREPARATION

3.2.1 Decortication

The practice in the vegetable oil industry is to decorticate oilseeds whenever possible. The reasons favouring decortication can be summarized as follows:

a) Decortication increases the capaicty of the processing equipment.

b) The seed coat usually contains very little oil compared to the kernel, and at the same time exhibits a tougher surface than the kernel; thus it absorbs more oil than it contributes.

c) The presence of hulls reduces the protein content of the cake and increases its fibre content. The hulling of soybeans is practised whenever a high protein content is required. A high fibre content is undesirable because it reduces the value of the cake for feeding purposes.

d) The hulls can serve as a raw material for producing certain chemicals, e.g. furfural from cottonseeds and the bromine from cocoa beans.

These arguments hold for most oilseeds. For the direct solvent extraction of castor seeds, the following additional reasons are given in favour of decortication.

e) It can be argued that the presence of hulls may hinder the rate of the leaching process.

f) The castor seed husk is reported to contain pigments which pass into the oil under the action of heat and moisture (71), so the removal of husks may eliminate the need for extensive bleaching.

g) The abrasive nature of the castor seed hulls and the excessive wear they cause to processing machinery are sources of concern to manufacturers. If the cake containing hulls is used as animal feed, there is always the danger of harming the animal intestines.

h) There are reports that the castor allergen is partly present in the seed hulls (22). Thus, removing the hulls may lower the percentage of the allergen to be eradicated.

Based on the above, the first step in processing the seeds in this research is decortication. For choosing a suitable method of hull removal, the types of decorticators commercially available were studied and this information was presented in Section 2.2.4.1. An examination of the mode of action of each type reveals the need for developing a more satisfactory method in the special case of castor seeds. The main objection to decorticating castor seeds on conventional machinery is that the seeds easily lose oil on impact, and hence stoppages for cleaning become more frequent. More attention is also needed to the question of dust control because of the allergy shown by some people towards castor seeds.

D'Aquin and Co-workers (88) reported a bench-scale process for the direct solvent extraction of castor seeds in which they used for decortication a 12 in. diameter single-pass corrugated rolls and an 18 in. wide aspirated shaker screen, and claimed satisfactory results were obtained. Earlier, Dunning (71) had reported the difficulties caused by the abrasive castor hulls in processing the seeds and mentioned the impact decorticator as being the most satisfactory equipment for hulling castor seeds at that time. However, he also noted the need for cleaning the equipment after 16-18 hours of continuous operation.

In the process reported by D'Aquin et al (88) some of the runs were made using whole (i.e. undecorticated) seeds and it was found that the bleached crude oil never tested darker than the standard grade 1 oil (92) even when whole seeds were processed. The authors considered this result unusual and suggested that the method of cooking they employed may have made the pigments less soluble in solvent. Based on this, they suggested the possibility of by-passing the decortication step on account of its equipment cost, operational costs, and the loss of oil and meats as a result of entrainment in the air stream which is used to separate the hulls from the kernels. As we mentioned earlier, the aim of husk removal is not only the improvement of oil colour, and hence a better approach than that suggested in D'Aquin's paper is to develop a more reliable method of decortication which will be technically and

economically feasible for commercial utilization. An added advantage of such a process would be its potential for processing oilseeds other than castor. For practical application, it is necessary that the proposed method be able to fit within existing process systems with minimum modification to the process sequence.

The approach adopted for castor seed hulling differs basically from that of the conventional methods. It is designed to overcome the difficulties associated with the mechanical methods and to improve the shortcomings inherent in them. Efforts were made to ensure that the suggested process satisfies most of the criteria mentioned above for the success of a new idea in oilseed decortication. The performance of this new technique indicates that it has promising value for the castor oil industry.

3.2.2 Size Reduction

Othmer (78) and others before him proved that solvent extraction of oil from whole or half beans is not possible irrespective of how long contact is maintained between solvent and beans because diffusion is not the predominant mechanism of the process. Hence the seeds must be flaked to enable the solvent to extract the oil. Because castor seeds are not directly solvent extracted in industry, no equipment has been developed for their size reduction, and it is not advisable to use the equipment designed for processing other seeds since castor seeds are much softer and contain more oil than those seeds. At present, solvent extraction of castor seeds is preceded by mechanical

pressing and the resulting cake is flaked prior to solvent extraction. In the direct solvent extraction of soybeans and cottonseeds, the seeds are conditioned before flaking, (91) but this cannot be done in the case of castor seeds because the high temperature employed in conditioning will make the oil very fluid and hence a high percentage of it will be lost during the subsequent flaking. Even in the mechanical pressing of castor seeds, the moist heat treatment is proposed under conditions which would not make the oil too mobile as this may cause excessive flowrates in the expeller (4). An alternative method for flaking oilseeds was employed by Fan and Coworkers (77) in their work on peanuts which involved embedding the nuts in wax and sectioning them on a microtome. This method suffers from the need to separate the wax from the flakes as well as a lack in simulating industrial practice because the resulting flakes do not exhibit the internal disruption caused by industrial flakers which enables more cells to be exposed to the action of steam and the solvent. This method of flaking was attempted for castor seeds but was abandoned because the seed was much softer than the wax, and the latter had to be hard enough to produce coherent flakes. The separation of the flakes from the wax also proved difficult, and neither solvent action nor heat treatment could produce acceptable results.

Another method tested for flaking castor seeds was to employ smooth horizontal rolls, and trials were made with two plastic rolls, one stationary and the other rotated by hand. The performance was affected by the

seed size, its moisture content, and the degree of smoothness of the rolls, but there was always the problem of oil being expressed under the exerted pressure.

It was therefore evident that the seeds must be so treated before size reduction as to minimize the loss of oil afterwards. Such a method was found and was successfully applied for reducing the size of whole seeds as well as of kernels, and will be presented in Chapter Four.

3.2.3 Moist Heat Treatment (Cooking)

The moist heat-treatment (cooking) of oilseeds has become standard practice in the vegetable oil industry because of its beneficial effects on oil extractability and quality (66). Alderks (67) summed the reasons for cooking cottonseeds following

a) To rupture (or finish the rupturing of) oil cells.

b) To increase the fluidity of the oil by increase in temperature.

c) To coagulate (or granulate) the protein aleurone grains. This facilitates the separation of the oil from the proteinaceous and other material.

d) To precipitate phosphatidic materials in order to produce oils with low refining losses.

e) To dry the cooked meats mass to a proper moisture content, optimum for oil expression.

f) To detoxify free gossypol, molds, and bacteria.

These reasons also apply to castor seeds, but the operating conditions have to be modified to suit the properties of castor oil. For the mechanical extraction

of castor oil, the seeds are cooked whole (69,71) as is the case with flaxseed (94), but the bench scale direct solvent extraction of castor seed was carried on cooked flakes as well as cooked whole seeds (88) and the cycle followed the sequence: preheating, moisture addition, cooking and drying.

The heat treatment of oilseed flakes was studied in relation to the oil extraction. Thus the laboratory extraction of cottonseeds with various solvents by Ayers and Dooley (94) revealed that cooking the cottonseed meats increased the rate of oil extraction and improved its colour. According to Good (95), the heat treatment of soybean pieces confers on them a thermoplastic nature that makes them better distorted by flakers and hence easier to extract.

The inactivation of poisonous and anti-nutritional substances is an important objective of cooking. In soybeans, the trypsin inhibitors are rendered harmless by moist heat treatment at 100^{o}C, and at the same time the "beany" and "bitter" flavours are diminished (54). The inactivation of myrosinase in rapeseed meal during cooking is highly essential for raising the quality of the cake (8). The meal from other oilseeds such as sunflower and safflower may need the same treatment as that applied to rapeseed. In the case of castor seeds, the steam treatment achieved ricin inactivation (96) but the meal's content of some essential amino acids was lowered (97). The addition of chemicals like sodium hydroxide to the

flakes during cooking caused a considerable loss of allergen content, and hence the steam treatment was considered a promising method for detoxifying castor cake. Work was carried out on pilot-plant scale (97), but no reports of industrial practices are available on this aspect of castor seed processing. It was found that the analysis of castor pomace samples from processing plants revealed lower ricin and allergen contents than their counterparts which were produced in the laboratory, and this decrease was attributed to the industrial treatment of the seed (98).

D'Aquin et al (88) cooked the castor seeds before solvent extraction. The cookers employed were a 15-lb- capacity steam-jacketed Loomis mixer, and a 1-lb-capacity stainess steel vessel (99). Elamin (100) conducted his cooking runs in a 4-litre-capacity steam-jacketed Morton mixer, Model 'O'.

For the present investigation, a domestic pressure cooker was considered for cooking the comminuted castor seeds. Since the design of this equipment does not allow moisture addition during cooking, the schedule adopted would have differed from that applied by other workers. However, since a new method was adopted for the size reduction of the seeds which was believed to result in cell rupture, the cooking stage was not included in the experimental investigation. It was hoped to implement a technique for studying the moisture uptake by the seeds, but the method could not be fully developed because of the omission of the cooking experiments.

3.3 SOLVENT EXTRACTION

3.3.1 The Solvent

The choice of solvent is most important in an extraction process. A number of factors have to be considered when choosing a solvent for a particular application, and these include the properties of the solvent and the nature of the separation required. The literature on the extraction of the vegetable oils covers a wide range of solvents for the various oilseeds reported, and it will be useful to know their advantages and shortcomings when selecting a solvent for castor oil. It must, however, be emphasized that the unusual chemical properties of castor oil may dictate the choice of certain solvents which are not recommended for other vegetable oils.

The hydrocarbons, aliphatic and aromatic, and the alcohols constitute the bulk of the solvents employed in oilseed extraction. Chlorinated hydrocarbons were also suggested as possible alternatives, but they have not performed as expected. Other solvents, such as ketones, were investigated, but are not popular for oil extraction.

The choice of a solvent involves examining the physical and chemical properties of the potential liquids and weighing their merits and disadvantages. Economic factors have also to be considered. Desirable qualities in a solvent are low specific gravity, low viscosity, and constant - but not too high - boiling point. The low viscosity is specified so that the solvent will be able

to penetrate to dissolve the solute and the solution to diffuse back to the bulk stream. It is also important especially when the solute is highly viscous because then the solution will have reasonable viscosities. A low boiling point and a low heat of vaporization indicate that the solvent is easy to recover at relatively low cost; a low specific heat is also useful in this regard. If the solvent is to be competitive with other candidates for selection, it should be easy to store and transport, and to fulfill this it has to be non-flammable, non-explosive, non-corrosive to common materials of construction, and with a low freezing point. The first two properties are vitally important from an operational point of view. So as not to upset daily plant runs, the solvent should be stable and should also be available at given specifications.

Chemically, the solvent is required to be highly selective for the solute and should dissolve the minimum possible of foreign substances like pigments and impurities. Immiscibility with water is highly desirable as a property of the solvent since it greatly facilitates solvent recovery, especially if the solute is also water immisicible. The solvent should also be non toxic in order to avoid danger to the operators and customers alike.

The economic factors include the cost of solvent, its storage, transport, and recovery. Solvent losses can form a substantial percentage of the product cost, and are usually a function of the boiling point of the

solvent and the leakages resulting from faulty design and operation.

It is obvious that no one solvent has got all these merits, but some have more advantages than others. The most widely used solvent in the vegetable oil industry is the hexane fraction of petroleum naphtha (boiling range 63-66^{o}C). Although the explosion and fire hazards are always real possibilities with this type of solvent, it has passed the toxicity test and is permitted in the extraction of edible oils. The pentanes and heptanes are not as popular as the hexanes partly because of their physical properties and partly because the rate at which they extract oils is slower than that of hexane. Thus, Arnold and Choudhury (101) extracted soybean oil with isopentane, pentane, isohexane, hexane, and found the extraction rate to increase in the order of solvents given. Cyclic hydrocarbons are more favoured in European practice than the straight chain hydrocarbons because of the abundance of the former compared to the latter (66).

The fatty acid composition of a vegetable oil plays an important part in deciding the solvent to be used for extraction. Thus rapeseed oil high in erucic acid can be satisfactorily extracted only by n-hexane. Other solvents give poor results, and the only exception is cyclohexane which gives the same results as n-hexane (102). Castor oil, whose fatty acids are dominated by ricinoleic acid, is generally soluble in polar organic solvents because of the hydroxyl groups of its major fatty acid.

At room temperature, it does not dissolve in hexane, heptane, or the higher straight chain hydrocarbons, but dissolves in them at higher temperatures. Cyclohexane, however, dissolves castor oil at room temperature, and is thus a good candidate for its extraction.

The fire and explosion hazards associated with hydrocarbons prompted the investigation of chlorinated hydrocarbons as solvents for oilseed extraction. Trichloroethylene was selected but in spite of its good solvency a number of disadvantages became apparent in addition to its inherent toxicity. Thus it was found highly corrosive in the presence of moisture, and it produced a cake that poisoned cattle. Small traces of this solvent in the oil were found to interfere with the hydrogenation process (34). Loury and Feng(102) reported dichloroethane and trichloroethylene satisfactory when compared to n-hexane for extracting rapeseed except for the colour which was rather red. Renewed interest has recently been shown in a halogenated hydrocarbon, 1,1,2-Trichloro-1,2,2-Trifluoroethane (FC-113) which is quite similar to hydrocarbon solvents in many of its physical properties (103). When FC-113 was compared to n-hexane as an extractant for soy flakes, the extraction by the former was markedly improved with increasing temperature, while hexane extraction was relatively independent of temperature (104). Similar results were obtained when the flakes were extracted with miscellas from both solvents and when soy meal was extracted with fresh solvents.

The alcohols, especially ethyl alcohol, were suggested as possible oilseed solvents (34). Oils which do not contain oxy fatty acids dissolve in ethyl alcohol at 250°F under pressure (34). Rao and Arnold (105, 106, 107, 108) studied the alcoholic extraction of vegetable oils and reported the solubilities of many in aqueous alcohol. The work of Kaparthi and Chari on the alcoholic extraction of four oilseeds (109) led them to conclude that the solubility of vegetable oils in alcohol is a function of temperature and the water content of the alcohol. The same conclusions were earlier reached by Rao and Arnold (108) who also noted the change of pressure with temperature, the maximum being 20 psig at 95°C. Kaparthi and Chari (109) found that the addition of a good solvent like n-hexane to the alcohol lowers the solubility temperature and increases the solubility of oils. When they investigated ethanol as a process solvent (110) they concluded that the oleaginous material must be of a moisture content less than 1% to prevent the dilution of the alcohol, and this limitation was listed by Schweitzer as one of the disadvantages attendant upon the use of alcohol for extraction (34). The alcohol is also reported to extract nonglyceride material from the oilseed or cake, and the oil must therefore be further purified from phosphatides, carbohydrates, and similar substances. Since the alcohol is miscible with water, the solvent must be recovered from both the extract and the underflow streams, which is not a requirement with the water-immiscible hydrocarbons.

The alcohol does have its advantages; Arnold and Choudhury (111) extracted peanut oil with hexane, absolute alcohol, and 95% alcohol, and found that absolute alcohol was a better solvent than 95% alcohol, and hexane was intermediate. When he and his coworker extracted soybean with 90,95, 98, and 100% alcohol (101), the extraction rate increased with increase in alcohol concentration. Alcoholic extraction produces an oil with good colour and low free fatty acids (110).

Isopropyl alcohol was investigated by Rao and Arnold (108) as a solvent for 14 vegetable oils, and the pattern that emerged was similar to that of ethyl alcohol. An interesting property of 2-propanol is its ability to extract gossypoll effectively from cottonseed, thus presenting an alternative method for detoxifying cottonseed cake (66). In this respect, isopropyl alcohol is superior to the petroleum naphthas which do not dissolve the toxin.

Solvents such as carbon disulphide and carbon tetrachloride which were once used for oil extraction from seed or cake have now been displaced by the petroleum naphthas. Toxicity, flammability, and unpleasant odour are some of the reasons for the phasing out of these solvents and similar ones, like toluene and benzene. Acetone and ethyl ether once found limited application in extracting oil from wet material, but are not recognized as industrial solvents at present.

Mixtures of solvents were also tested for their performance compared to that of the pure solvents. Ayers and Scott (112) determined the effect of adding alcohols to n-hexane on the extraction rate and residual oil in cottonseeds and soybeans. The alcohols employed were methyl alcohol, ethyl alcohol, isopropyl alcohol, and allyl alcohol, all at 5% by volume. All the alcohol-hexane mixtures tended to remove more oil for a given time than pure hexane, but the methanol-hexane mixture was outstanding in that it removed more oil at a faster rate. Although the two oilseeds tested showed similar trends, yet data reported for each exhibited discernible differences, and this emphasizes the influence of the extracted material on how the extraction proceeds. The acetone-hexane-water azeotrope (AHW) was used for extracting raw comminuted cottonseed kernels (113) and glanded and glandless cottonseeds (114). The inference of the second group of workers is that, compared to hexane, the AHW gave much slower percolation rates and yielded much darker crude oils having higher free fatty acid values and higher refining losses. The extraction of cottonseeds with aqueous acetone followed by hexane yielded high quality oil and meal (115). In spite of the usefulness of extracting with mixed solvents, the trend in the industry is still the dependence on pure solvents.

Because of the high oil content of castor seeds, the solvent extraction of the oil was not applied to the seeds but to the cake left after pressing. The solvents were mainly aromatic and chlorinated hydrocarbons, and work in

India was mainly directed to the alcoholic extraction of castor cake. Sharder was the first to report the solvent extraction of castor press cake (70) and he concluded that the oil can probably be economically extracted by the solvent process alone. Prepressing followed by solvent extraction was also reported by Andre (116), Skipin (117), Goldovskii, et al (120) and Perkins (121) who reduced the oil content of the seed to 18% and extracted the residual oil with hydrocarbons, chlorinated hydrocarbons, alcohols, and esters. Eddy (122) treated castor press cake with benzene and acetone and recovered the oil by centrifugation in the presence of water. Benzene, petroleum ether, and dichloroethane were the solvents employed by Chernukhin (118) to extract castor oil, and he found dichloroethane to be the best. When the factory extraction of press cake was later attempted with dichloroethane, equally good results were obtained (119). Hassel (123) tested a number of solvents for potential commercial utilization. He discarded carbon disulphide on account of fire hazard and danger to health. Carbon tetrachloride was undesirable because it decomposed into carbon dioxide and hydrogen chloride whenever water was present, and this resulted in corrosion to the apparatus and the production of coloured products. He reported that chlorinated hydrocarbons were good solvents but they extracted undesirable resins, albuminoids, and colouring matter. Trichloroethylene was the best of the chlorinated solvents but was abandoned after lengthy trials because of inferior products and danger to health. The

results he obtained with dichloroethane were better than those of trichloroethylene, but the former also turned out to be a health hazard, although it showed no tendency to generate hydrogen chloride. He reported that benzene was well suited to the extraction of castor oil from press cake but it produced poisonous vapours. Hassel concluded that the best solvent was well-purified benzine. Swisher and Fiero (124) extracted castor press cake with benzine, benzene, acetone, toluene, and ethyl alcohol. Work on the solvent extraction of castor oil was also reported by Andrè (125) (126), Klein (127), Geyson (128), and Otin and Alexa (129).

Lindenberg (130) (131) (132) investigated the partition coefficient of many solvents between water and different vegetable oils and animal fats. In acetone, castor oil behaved like the other oils, but in ethanol it had a much higher partition coefficient than other oils in the extract phase. He drew attention to the marked effect of the hydroxyl group of ricinoleic acid, the major fatty acid of caster oil.

Various solvents were employed by Otin and Alexa (129) to extract castor oil in a soxhlet extractor. In the order of increasing oil yield, the solvents were ordinary benzene (89.4% oil recovered), cracked benzene, airplane benzene, petroleum ether (boiling up to 60^{o}C) acetone, benzene, ether, and trichloroethylene (100%).

The patent literature gives a description of a number of processes proposed for the solvent extraction of castor

seeds. Thus, Sly (133) leached the seeds with a solvent, e.g. heptane, at a temperature higher than the critical miscibility temperature of solvent and castor oil. The temperature is then lowered until two layers are obtained; the solvent-rich layer is reused, while the oil-rich layer is withdrawn for desolventization.

A somewhat different approach was adopted by Chayen (134) who attempted to avoid the problems resulting from pressing castor seeds, such as the sticking of oil to the rollers. He proposed grinding the seeds with 5-10 times their weight of carbon tetrachloride, trichloroethylene, hexane, benzene, or a saturated aqueous sodium sulphate solution in a hammer mill. The oil is then separated by centrifugation or distillation. The dry residue, he claimed, contained 0.4-1.0% oil. A similar method was earlier proposed by Colbeth (135) who treated the seeds with paraffinic petroelum naphtha in a ball mill, and the slurry thus produced was passed to a holding tank and allowed twenty minutes to settle the fines. The slurry was heated to 160-170°F and the effluent sent to a rotary suction filter. The miscella was then separated from extracted meal and allowed to cool below 41°F but above the temperature at which the oil crystallizes. The two layers thus formed were a top layer of pure solvent, and a bottom layer of 50% castor oil. The solvent was recirculated and the miscella sent to evaporators to remove the solvent. Gathman (136) contacted the castor seeds with solvent at 150-160°F. The resulting slurry was filtered and cooled to 60-80°F, and two layers formed:- a top layer

rich in solvent containing 2.2% oil, and a bottom layer rich in oil with 24.1% solvent. The solvent used was a high-purity grade with a boiling range 98-103°C and containing over 99% isoparaffins including not less than 85% 2,2,4-trimethylpentane. When ordinary commercial grades of the solvent were employed, e.g. commercial heptane, the temperature of operation reduced to 104°F, and when the miscella was cooled to 60-80°F, the two layers obtained were: a top layer, 5% oil in solvent, and a bottom layer, 43.6% solvent in oil.

The application of the filtration-extraction process to castor seeds was reported by D'Aquin et al (88) who also reported that heptane gave results comparable to hexane for extracting castor oil.

The alcoholic extraction of castor oil from the cake was reported by Indian investigators. Thus, Chatterjee (137) preferred alcohol extraction to pressing in hydraulic presses for the following reasons.

i) With completely decorticated seeds, the oil obtained is No. 1 oil

ii) The yield is 4% greater

iii) The nitrogen content of the cake is 25% higher

iv) The manufacturing cost is lower; recurring costs such as those due to bag replacement are eliminated.

Chatterjee and Saxena (138) employed denatured alcohol (10% methyl alcohol) and were able to extract 99% of the oil in castor cake in four laboratory extractors.

For the extraction of edible oils, n-hexane is almost the universal solvent in commercial practice. In spite of the fire and explosion hazards associated with it, it surpasses ethyl alcohol in its good solvency for these oils at ambient temperature. It is in the area of extracting hydroxy-acid oils, such as castor oil, that ethyl alcohol excells the straight chain hydrocarbons, and this is where its real potential lies as a commercial solvent for oilseeds.

The extraction of natural products, oilseeds included, with supercritical gases has been studied (139,140,141). In this extraction technique, the solvent power of compressed gases is utilized, together with the other properties which the gas acquires when it is in the supercritical state, namely high density and low viscosity. Therefore, the dense gas phase will display solubilities comparable to those of the liquid state, yet its penetration into the matrix of the natural substances to be extracted will be faster and deeper than what can be achieved by the corresponding liquid solvents (140).

Extraction with liquid or supercritical gases is attractive because of a number of advantages, although the high pressures involved may in some cases be a setback. Thus, the relative ease with which the solvent can be removed in this type of operation is a definite merit in food applications, which require the end product to be free of any contaminant. Also, the lower temperatures employed leave the heat-sensitive substances, e.g. proteins, undamaged. When carbon dioxide is the gas

chosen for the extraction, toxicity problems associated with some organic solvents do not arise. Its availability and non-polluting nature are important advantages for utilizing resources without polluting the environment.

The use of carbon dioxide, in the liquid or supercritical state, for the extraction of natural products has been reported. A historical account was given by Grimmett (142), and the subject was also discussed by Jolly (143) with emphasis on flavour extraction. Stahl et al (144) described the extraction of oilseeds with carbon dioxide under various conditions. The seeds studied were soybeans, sunflower seeds and rapeseeds. The temperature and pressure largely influenced the amount of carbon dioxide needed for extraction. At a pressure of 250 bar, liquid carbon dioxide at 20°C was as suitable a solvent as the supercritical gas at a temperature of 40°C and a pressure of 250 bar, but when the pressure exceeded 300 bar supercritical carbon dioxide had a higher dissolving power than the liquid. For the extraction of sunflower seeds, the amount of carbon dioxide needed at 260 bar was four times the amount required when the pressure was raised to 700 bar. Varying the temperature and pressure also resulted in the recovery of fractions differing in colour, taste, and odour, thus indicating a potential for employing the process for refining crude oils. The separation of monoglycerides from a mixture of glycerides was described by

Peter and Brunner (145). Acetone was added as an entrainer to enable the alteration of the relative volatilities of the components in the mixture, and propane was the compressed gas chosen. The results indicate the feasibility of operating such a method on a large scale, and the economics of the process are given. Stahl et al (146) discussed the microextraction of sunflower seeds and sesame seeds with carbon dioxide at 90 bar.

Although no reports are available on the extraction of castor oil seeds with liquid gases, it is possible to adapt the techniques already mentioned to castor oil extraction if a suitable solvent is found. This point will be elaborated in relation to the process developed in this research when the results are discussed in Chapter Six.

3.3.2 The Leaching Process (150,151)

The two main modes of operating leaching processes are the percolation and immersion systems. A mixed system, analogous to the prepressing-solvent extraction combination, was developed for extracting oil-rich seeds which are usually difficult to handle in either type alone (147). The laboratory methods for determining the rate of extraction of oilseeds are based on the two above methods (148). The percolation method is carried out on a modified soxhlet extractor, and it determines

the amount of oil removed from a single sample of oil-bearing material by fresh solvent percolating through the bed by gravity. The test material is prepared in such a way as to enable it to pass solvent without channelling. The moisture content of the sample is determined and the extraction rate, expressed as the percentage of residual oil in the sample as a function of time, is plotted on moisture-free basis on log-log coordinates. The criterion for comparison between different extraction conditions is the time required to reduce the oil content to 1%.

The batch cocurrent method is the second laboratory technique applied to those materials which cannot be extracted by percolation because of their small particle size. It permits extracting the test sample with miscellas of various oil concentrations, rather than by fresh solvent, and can therefore be used to simulate stage-wise operation. The lipid and moisture contents of the material to be run are determined, and known quantities of the sample and the extraction solvent are charged into a flask and agitated. Samples of miscella are withdrawn at the times desired and analysed for their oil content to monitor the progress of the leaching process. The percent unextracted lipids is calculated by a material balance after making allowances for miscella retained in the cake.

The principal types of operating methods for leaching systems are the single-stage system, the multistage cocurrent (parallel) system, the continuous multistage countercurrent system, and the batch countercurrent multiple-contact system. The continuous countercurrent system is perhaps the most important mode of operating leaching batteries on a large scale, and the calculation procedure to be outlined here will be based on this system.

Three types of methods are frequently referred to: algebraic, graphical, and analytical. They are all based on the concept of the ideal stage defined as the stage from which the resultant solution leaving is of the same composition as the solution adhering to the solids leaving that stage. The components of a leaching system are assumed to consist of solute, solvent, and inert, insoluble solids. The freely-flowing stream leaving each stage is known as the overflow, while the solids are referred to as the underflow.

For the purpose of designing a solid-liquid extractor, data is needed on extraction rates, amount of solution retained by the solids at varying concentrations, percolation rates, and wetting rates. The holdup of bound liquor by the solids includes static and dynamic retention; the former is the amount of solution remaining

on the solids after draining indefinitely; the dynamic hold up refers to the quantity of solution actively percolating through the solids. Solution holdup and extraction rates enter into the determination of the number of stages and affect the size of the extractor. The solvent-to-solids ratio is determined after knowing the solution holdup (149). The minimum time that must be allowed before the solution thoroughly wets the solids is determined from the wetting rates. The percolation rate fixes the amount of percolating solution per stage and hence the size of the stage pumps. The results of such studies will determine whether to extract by immersion or percolation.

Constant underflow means that the amount of solution retained by the solids is constant as they leave each stage. However, in many cases the amount of solution retained is variable, giving rise to variable underflow. Modifications must then be made for the accommodation of this variation in the calculations.

The algebraic method of calculating leaching operations can be summarized as follows:

1. Calculate the quantities and compositions of all the terminal streams using a convenient basis.

2. Compute material balance and stream compositions for a terminal ideal stage, using equilibrium and solution-retention data.

3. Repeat calculations for each successive ideal stage until one is found which corresponds to the desired conditions.

The graphical solution of leaching problems has the advantages that it simplifies the calculations and permits the visualization of the process variables and their effect on operation. A number of methods were described (152), but most users seem to prefer the right angled triangular diagram or a modification of the Ponchon-Savarit H-x diagram used in distillation calculations. The constant underflow case is represented in these diagrams by a straight line parallel to the overflow axis, while a curve describes the variable underflow situation.

The analytical methods were developed for counter-current leaching calculations with certain restricting assumptions. Later, modifications were made to suit the different operating conditions met in practice.

A discussion of the application of these methods can be found in numerous texts (152, 153, 154, 155).

3.4 THE TOXINS AND ALLERGENS

3.4.1 Significance of their Deactivation

The hazardous substances in the castor bean were briefly described in the preceding chapter, and will be discussed in more detail in Chapter four. In this section the emphasis will be on explaining why it is necessary to make these components innocuous.

For the solvent extraction of the castor seeds to be economically feasible, the by-product, i.e. the pomace, must sell at the best possible price and be able to compete with other raw materials in various markets. This of course assumes that the main product, castor oil, retains a well-established market. The toxicity and allergicity of the pomace are the factors that limit its large-scale utilization, and hence they must be removed at acceptable costs that would not ruin the economics of the process.

The health hazards associated with these substances are extremely serious. The cake produced by the mechanical pressing of the seeds contains a rather large percentage of residual oil, yet it is capable of causing community illnesses and occupational epidemics. Therefore the cake from a direct solvent extraction process, with a residual oil content of 1% or less, will be much more

unsafe, and the urgent need for control measures becomes more obvious.

An extensive literature exists on the inactivation of the toxic factors in the castor seed. Although ricin is said to be detoxified in commercial practice, Panzani and Layton (156) seem to dispute this statement, and it is definite that the allergen is not totally eradicated. New methods are therefore needed for inactivating the cake; also, some of the essential nutrients were damaged in the reported treatments, this being another reason for the desire to improve the existing practices.

3.4.2 Inactivate or Extract?

The harmful components of oilseeds are usually dealt with in one of four ways:

a) Inactivate them so that they become an inert part of the cake.

b) Transform them into a harmless form that can be extracted or distilled.

c) Extract with a suitable solvent or a mixture of solvents

d) Reduce or eliminate through selective plant breeding.

The last option was referred to earlier in the thesis, and is a long-term venture. A frequently-cited example to illustrate the first alternative is the moist heat treatment of cottonseed flakes to detoxify the gossypol present in them. The "debittering" of apricot and almond

kernels is an example for the second option; the coarsely-ground kernels are soaked in acidified water, pH 6.5, for two hours at 55°C. The glycoside responsible for the "bitterness" is completely hydrolysed under these conditions and the products of hydrolysis are removed by steam distillation (157). Aflatoxin contamination of peanuts was remedied by solvent extraction. The main parameters were the polarity of solvent, the temperature, and the moisture content of the treated cake. Systems that gave promising results included acetone-water (90:10 V/V), 2-propanol-water azeotrope (87.7-12.3), and 2-propanol-water (80:20) (158).

Commercially, the castor cake has always been detoxified by inactivating its harmful constituents. Ricin and allergen preparations were prepared in the laboratory by a number of workers for studying detoxification processes, but no such attempts are reported commercially or even on a pilot plant scale as a means of detoxifying the cake. Waller and Negi (98) have isolated ricin, ricinine, and the castor allergen from the same lot of pomace in the laboratory in the hope that the resulting product would be suitable for feeding purposes. They stated that the end product was non toxic and could serve as a source of protein, but the technical and economic feasibility of the process on a large scale was not discussed.

The advantages of extracting the allergens and ricin are said to be that the cake will be completely free from them, but the attendant problems and disadvantages are not to be underestimated. Thus, to obtain the complete removal of these components from the cake, elaborate extraction procedures must be set up, which may prove expensive and time consuming and may involve the loss of a portion of the protein. The different conditions needed for recovering each of the active components mean that at least a two-step process is required. The safe and economic disposal of the toxin-laden solvents will prove a major problem since some form of treatment must be applied before the effluents are allowed to enter the drainage system. It is also possible that these considerations will affect the economics of the finished product.

The potency of the allergens and ricin calls for the minimum of handling for them in any concentrated form. It is therefore best to deactivate without extracting them from the cake because of the technical hazards and the economic difficulties involved.

3.4.3 Pre- Versus Post-Extraction Treatment

Since the inactivation of the toxicants was preferred to extracting them, it remains to decide whether this will be before or after the solvent extraction step. The only available report on treating the flaked seeds before

extraction was made by Gardner et al (96); almost all the other available trials were reported on the defatted cake.

Castor oil is the main product derived from the seeds, and hence it is usually recovered first and the by-product is then freed from toxicants. Therefore, any detoxification before extraction must be closely monitored for its effect on the oil. In some of the experiments of Gardner and his coworkers, the oil was actually liberated during the treatment prior to extraction, but the quality of the extracted oil was not described, and thus the impact of pre-extraction detoxification on the oil cannot be gauged.

The merit of detoxifying the unextracted flakes is that the pomace will be safe to handle from the moment it is produced, but this depends on the efficiency of the deactivation process. A limitation is imposed by the nature of the detoxifying technique and the operating conditions that prevail in it, i.e. a method for treating the pomace may not be suitable for application to the whole beans or the unextracted meats.

It is therefore suggested that the deactivation treatment is to be carried out after the oil has been extracted, the necessary precautions being taken to contain the dust from the untreated cake. At the same time, it is worthwhile investigating the techniques of pre-extraction deactivation because of the advantages to be gained from the success of this approach.

3.4.4 The Choice of Assay Method

In order to be able to measure the extent of deactivation of castor cake, a reliable assay must be available. Bioassays and immunological tests are utilized for determining proteins, and use could be made of them for quantifying ricin and the castor allergens. Because of the variety of methods reported, this section will look briefly at choosing the most suitable among them for this purpose.

The toxicity of ricin is the property most employed for finding the level of the toxin in castor cake. Animal experiments are conducted on mice, guinea pigs, or rabbits; standard conditions are set up to ensure the uniformity of the tests. The interpretation of the results is made in one of two ways: the LD_{50} or LD_{48}; the former is the dose required to kill 50% of the animals in a batch, and the latter is the dose that results in death in 48 hours. Corwin (159) discussed the application of this bioassay technique and extended it to include the dose-survival time curve method. The basis of this method is that for each species, given a particular route of injecting toxin, there is a relation between the quantity administered and the survival time of the animal. Thus, a certain survival period will indicate a certain toxin level as read from such a curve. Clarke (160) introduced a method based on the fact that the serum from an immune animal will neutralize

the toxicity of ricin. The material to be tested is extracted with hydrochloric acid and the extract is divided into two parts; from each part a series of dilutions are made so that the resulting identical solutions (A and B) represent 1, ½, ¼, ⅛, . . . etc of the concentration of the original extract. 0.1 ml. of a normal (i.e. not immune) serum is added to each tube of series A, and 0.1 ml. of serum from an immunized animal is added to each tube of series B. Both series are then injected into two groups of mice (0.5 ml. intraperitoneally). If the mice in series A show an appreciably greater mortality than those in series B, the original material must have contained ricin, as the immune serum will protect against no other substance. This method is claimed to detect as low as 1 ppm of ricin in cattle feed.

The bioassays described are in vivo techniques. Alternative in vitro methods can be chosen from those which capitalize on the antigenic nature of ricin, i.e. its ability to raise anti-ricin serum if injected in an animal. This antiserum can be reacted in test tubes with ricin in what is known as the precipitin test. Calibration curves are drawn from which the toxin concentration in a test sample can be determined. Animals are not required for the actual test as is the case with Clarke's method. The precipitin test can be carried out in gels, and two possible techniques which can be employed for assaying ricin are the Mancini technique or the Laurell's rocket technique (see

section 4.5.2.3).

The hemagglutinating activity associated with the castor bean lectins was for sometime used to test for ricin although it was shown that the toxicity and hemagglutinating ativity are two unrelated properties, i.e. the latter is not a measure for the former. In addition to its being non specific, because a number of lectins are agglutinins, this method is also semi quantitative and is affected by many variables.

An enzyme-linked immunoassay of ricin was reported recently and is claimed to be a sensitive method (161). Ghosh et al (162) reported what they described as a rapid and sensitive assay for castor bean lectins. The method is based on the sugar specificity of the lectins which enables them to inhibit the activity of an enzyme conjugated to a galactose residue.

It is desirable that the method of assay be as simple as possible, especially that in this research it is meant for use in an engineering project. Hence the animal experiments were considered unsuitable, and emphasis was made on in vitro techniques. Hemagglutination assays for ricin were ruled out for the reasons mentioned earlier. An immunoassay was considered the most suitable because of its inherent specificity and sensitivity, but the radio-labelled or enzyme tagged versions were thought to be too involved for the present investigation. The Mancini technique was adopted as the one that satisfies the criteria

for a routine assay at a reasonable cost. Its use for assaying the allergen was also one of the factors that supported its selection.

The assay techniques for allergens are described in Chapter four of this thesis; here, two techniques will be elaborated upon for their importance in allergen quantification, namely the paper radioimmunosorbent test (PRIST) and the radioallergosorbent test (RAST). The method chosen from among the various techniques will then be indicated.

PRIST is an assay for the amount of lgE circulating in the blood. The basic operational steps are as follows.

1. Couple antihuman antibody (raised in sheep) to a paper disc; add the patient's serum.

2. lgE in the serum reacts specifically with the coated disc; wash; add radioactively labelled (I^{125}) labelled antihuman lgE antibody, which couples to the lgE.

3. Rewash; measure the radioactivity on the disc. The bound radioactivity is directly proportional to the amount of lgE in the serum sample.

lgE specific for a given allergen can be measured with RAST. A typical procedure is as follows. Take the specific allergen to be CB-1A.

1. Couple the CB-1A to a solid phase, e.g. a paper disc. The patient's serum is then reacted with the coupled allergen.

2. If IgE specific for CB-1A is present, it will fix to the allergen in an antigen-antibody reaction. Remove unreacted IgE by washing.

3. React radioactively labelled antihuman IgE antibody with the particles. After further washing, the radioactivity in the centrifuged particles is measured to give the amount of IgE specific for CB-1A.

Although RAST and PRIST are widely used, they could not be adopted for this work for reasons similar to the ones given in the case of ricin. Animal experiments, such as PCA tests, and scratch tests could not be considered as they require the practitioner to be licensed. Considerations of similar nature apply to assays such as the Schulz-Dale technique. The method chosen, the Mancini technique, was also suitable for assaying ricin, and this simplifies the overall analytical effort by eliminating the need for setting up two different assay procedures.

3.5 SAFETY

The safety precautions normally observed in the solvent extraction of oilseeds arise from the flammable nature of the hydrocarbon solvents used. The extraction of castor oil from seed or cake involves an extra hazard due to their content of toxins and allergens. The first type of hazard is properly

covered in the literature, and will therefore be only briefly discussed. The second type, however, is the one that is more involved and deserves a more lengthy discussion.

Fires, solvent explosions and dust explosions can be avoided by keeping the three ingredients of fire separated from each other at all times. These ingredients are fuel, oxygen, and a source of ignition. Below a certain concentration of solvent or dust in an atmosphere, no explosion occurs because the heat of combustion is insufficient to propagate it; above a maximum limiting concentration, an explosion cannot be produced because of lack of sufficient oxygen. Ignition can be caused by sparks from flames, hot surfaces, and spontaneous combustion. Therefore the basic safety procedures aim at reducing the concentration of solvent vapours or of dust below the lower limit and at the same time to prevent sparks and other causes of ignition from taking place. The use of spark-proof tools and proper earthing systems are mandatory. The design of equipment should ensure tightness to avoid solvent leakage. The desolventization of cake and miscella should be regarded as hazardous as the solvent extraction operation. Fire-fighting equipment must be installed and inspected regularly. The relevant codes spell out these and other precautions in more detail (335).

The biological hazards in the castor seed and cake are ricin and the castor allergens. The precautions are necessary at all the stages of the process, and they will be discussed for seed and cake handling, the analysis of cake for toxins

and allergens, maintenance of equipment, and disposal of waste substances.

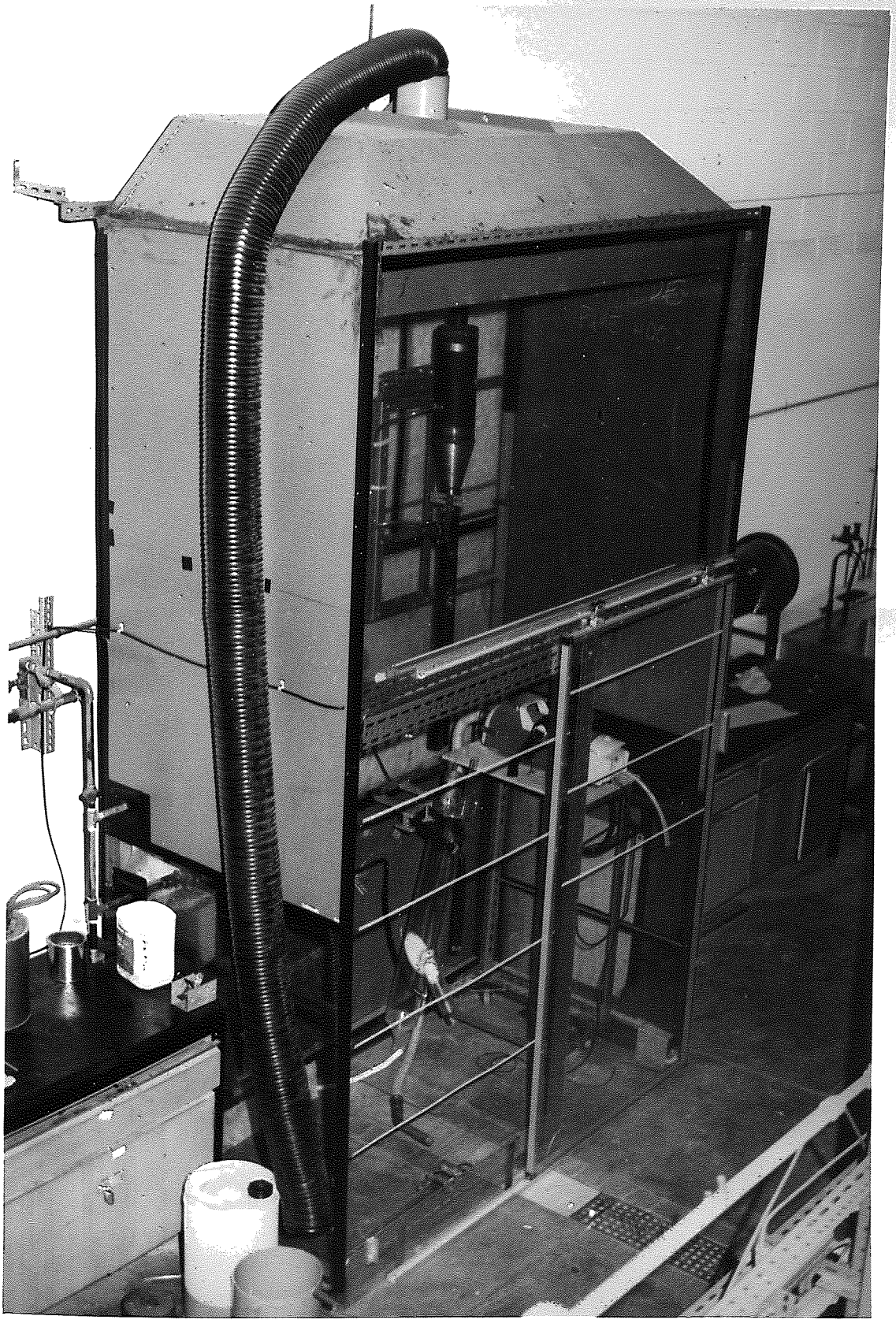
The beans should be stored where they are accessed only by authorized personnel. The storage and handling area should be as dust-free as possible, and the collected dust should be collected in equipment such as wet pressure filters equipped with filter bags and should then be disposed of as chemical waste, e.g. by incineration.

The handling of the cake should be controlled even more closely than the beans because it is more hazardous. The efficiency of dust collection in this section of the process must be as high as is feasible, and no discharge shall be allowed to the atmosphere before proper treatment, as it can otherwise constitute a major environmental hazard. The cake is to be sold or disposed of only after it has been certified safe by the quality control section, and for that purpose records must be kept of the various batches treated. An autoclave is a convenient means for inactivating castor cake for disposal, but its performance must be monitored by regular inspection of the installed measuring devices, e.g. temperature and pressure gauges.

The method of assay for these hazardous substances can be chosen according to the circumstances. For this research, extensive discussions which involved the University Safety Officer and Her Majesty's Inspector of Factories (336) resulted in the adoption of a cabinet of the type shown in fig. 3.2 for carrying out the analysis. The manufacturers -

Hi-Tech Scientific Ltd., Salisbury, Wiltshire, England - stated that the cabinet was tested to comply with British Standard BS 5726:1979, and was check tested by the Public Health Laboratory Service in Porton Down. This equipment was to be housed in a specially designed laboratory which will conform to the safety standards required when handling dangerous pathogens. Originally, the work on the preparation and extraction operations was to be carried out in the rig shown in fig. 3.1 which was constructed by raising a cyanide hood so that there was adequate space and ventilation for working inside. A pipe with perforations was introduced at the base of the wall inside the rig to provide additional air if required. The entire construction could be kept under negative pressure by connecting it to a suitable exhaust system. The recommendation of the Factory Inspector was to carry out both the process and analytical work in the special laboratory referred to.

A system was also proposed for the maintenance of equipment used for processing the castor seeds which requires job sheets to be filled in,stating the steps taken to ensure the safety of the equipment before dismantling. In addition, all items that came in contact with the seed or cake and are to be disposed of should be classified as chemical waste and treated accordingly.



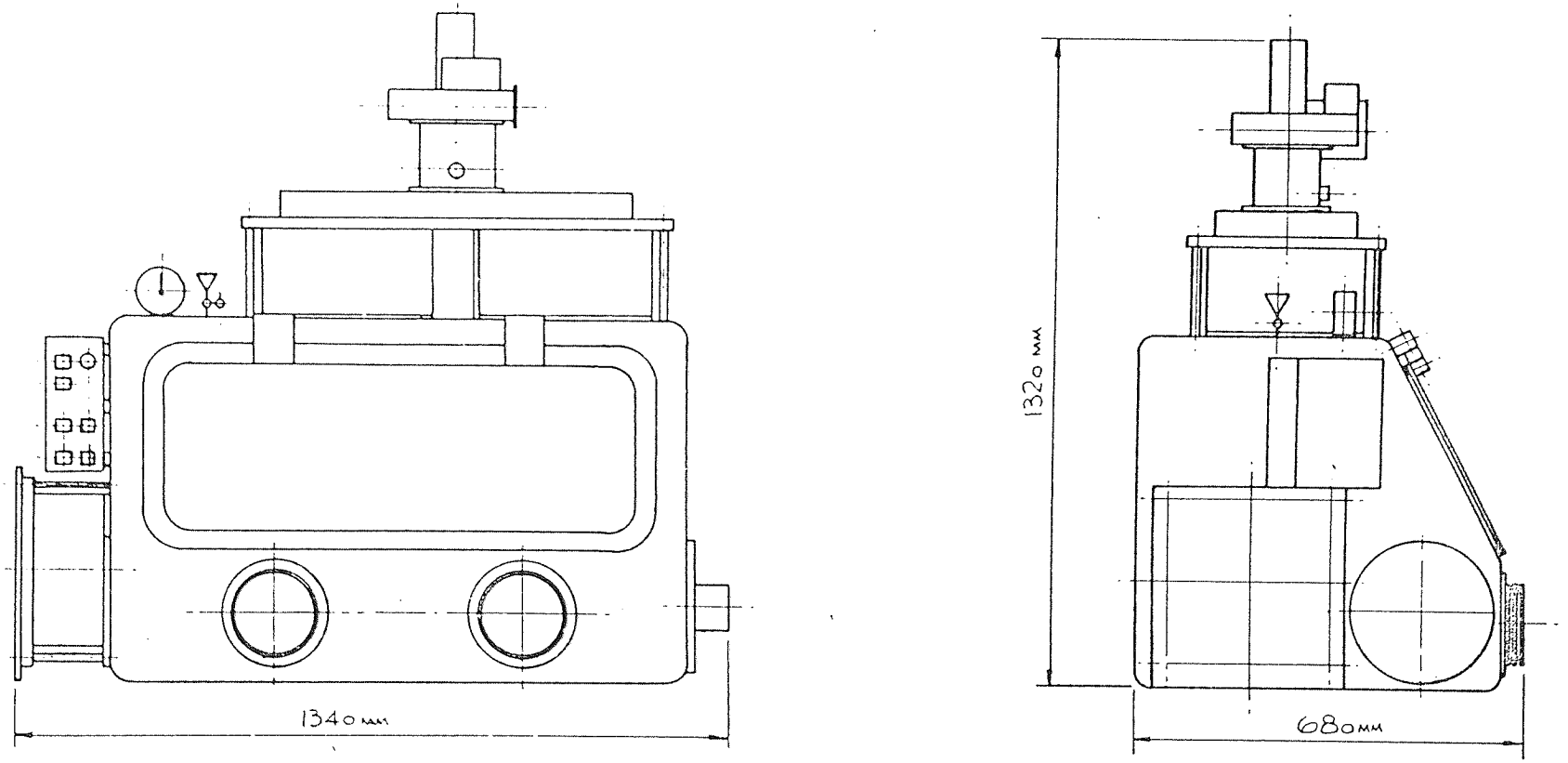


Fig. 3.2 Sectional Views of the Biosafe (Class III) Cabinet Chosen for Use in Assaying Ricin and the Allergens

Chapter Four

THE TOXINS AND ALLERGENS IN THE CASTOR SEED

4.1 INTRODUCTION

The two major harmful components of the castor seed are ricin and the group of allergens. Although these substances may have a physiological role during seed germination, their presence in the cake resulting from solvent extraction is highly undesirable. Since these toxins and allergens are proteins, a brief introduction to the nature and properties of this important class of living matter is given in Section 4.2 . Section 4.5 on immunochemical principles is included to help in understanding the methods of protein quantification. Section 4.4 on protein synthesis is thought to be mandatory since it is now established that ricin exerts its toxic action by inhibiting this process in host cells.

4.2 PROTEINS

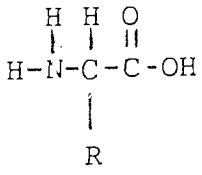
4.2.1 Amino Acids

Proteins, carbohydrates, lipids, and nucleic acids are the major molecules from which biological systems are constructed, but proteins are by far the most important and widely varied among them. All these organic molecules have carbon, hydrogen and oxygen as common elements. Proteins are distinct in having nitrogen in their structure. The variations in the types of proteins are required for the various roles they play in living organisms, examples being the acceleration of chemical reactions by enzymes, regulation of metabolic activities

by hormones, oxygen transport to the blood by haemoglobin, and iron storage by ferritin. These variations are possible because proteins are polymers made up of amino acid residues, and the uniqueness of a protein is due to the sequence in which the individual amino acids are assembled in its chain.

Amino acids are organic acids which have an amino (NH_2) group in addition to the functional carboxylic group. The amino and carboxylic groups are both joined to the first, or α, carbon atom (see drawing) which is different for different amino acids. There are twenty naturally-occurring amino acids specified in the genetic code that is universal to all organisms, and these are listed in Table 4.1 together with their common abbreviations.

To form a protein, the first step is to join the amino acids of that protein in a peptide unit which results from attaching the amino group of an amino acid to the carboxylic group of the next acid, and the bond so formed is called a peptide bond. A number of peptides joined together will result in a polypeptide, and a protein is made up of one or more polypeptide chains.



"R" represents a side chain, and different side chains give rise to different amino acids. The side chains may be polar or non polar, aromatic or carboxylic, and they may be charged or without a charge.

TABLE 4.1

The Important Amino Acids

Name	Abbreviation
Alanine	Ala
Valine	Val
Leucine	Leu
Isoleucine	Ile
Proline	Pro
Phenylalanine	Phe
Tryptophan	Trp
Methionine	Met
Glycine	Gly
Serine	Ser
Threonine	Thr
Cysteine	Cys
Tyrosine	Tyr
Asparagine	Asn
Glutamine	Gln
Lysine	Lys
Arginine	Arg
Histidine	His
Aspartic Acid	Asp
Glutamic Acid	Glu

4.2.2 Protein Structure

There are four levels of protein structure, the first three of which are met with in any single chain polypeptide, but the fourth, the quaternary structure, occurs only in proteins with two or more polypeptide chains. The first three levels of the complexity of a protein's structure are the primary, secondary, and tertiary structures, and each has its significance in the functions of the substances involved.

The primary structure defines a unique protein because it describes the number and sequence of amino acids from which the protein is built. Any change in the amino acids sequence may drastically alter the properties of the protein. The secondary structure results when the polypeptide chains are folded into specific coil structures held by disulphide (S-S) and hydrogen bonds. The arrangement of these coils in specific three dimensional orientations gives rise to the tertiary structure. The primary and secondary structures affect the details of the tertiary structure and the way it is brought about, and this level of structure in turn governs the cellular role of the molecule. Some proteins display a quaternary structure which may be required for the protein's activity and which occurs when the molecule in question has more than one polypeptide chain.

4.3 OILSEED PROTEINS

Vegetable proteins are generally known to be of lower biological value than animal proteins, yet attention

has recently been increasingly focussed on oilseed proteins because of views on the expected shortage in proteins for feeding the increasing population of the World. There are certain important advantages for some vegetable proteins over animal proteins, and an example is afforded by soyabean proteins whose yield per acre is significantly greater than that of beef, Table (1).

Oilseeds, like other seeds, have two types of proteins, namely metabolic and storage (163). The former include enzymatic and structural proteins, whose role is to regulate the normal cellular activities. Storage proteins, on the other hand, are mainly concerned with providing the necessary elements on which the developing seeds grow. The synthesis of storage proteins, like the other proteins of the seed, is part of the functions of metabolic enzymes. The reserve proteins of seeds are localized in protein bodies, or aleurone grains, which are spherical organelles each bounded by a single membrane and contains many hydrolytic enzymes which function following germination (13,20). In castor seeds, the protein bodies have an amorphous protein matrix together with inclusions of phytin globoids and protein crystalloids. The matrix proteins are soluble in water and have very diverse low molecular weights. The crystalloids are mainly composed of proteins with molecular weights between 50,000 and 60,000 daltons and are water insoluble but soluble in salt solutions. Thus, the matrix proteins are albumins whereas the crystalloids are globulins. Furthermore, germination experiments showed the crystalloids to be the

TABLE 4.2

The number of days of protein supplied by one acre of land to meet the requirements of a moderately active man.

Source	Days
Soya	2224
Wheat	877
Maize	354
Beef	77

storage proteins since they were rapidly degraded into smaller units and later into amino acids to be used in the growth of the new plant. Evidence was furnished (13) to the effect that the main storage protein in castor is the castor bean allergen, CB-1A, and is based on immunological studies in addition to comparing CB-1A to the known storage proteins in seeds, whose main characteristics are:

a) They are highly amidated, i.e. they have high contents of aspartate, glutamate, and arginine.

b) They have low molecular weights

c) They can be distinguished by the pattern of their solubility in water.

Thus, albumins are soluble in water and in dilute and moderately concentrated salt solutions, but are insoluble in saturated ammonium sulphate solution. Globulins are insoluble in water or in concentrated salt solutions, but are soluble in weak salt solutions. In seeds other than those of Gramineae, the classical storage protein is a globulin of high molecular weight (165).

The immunological evidence is that rabbit antisera raised against CB-1A precipitated the storage protein of castor seed, thus indicating that the two are identical.

4.4 PROTEIN SYNTHESIS (166,167,168,169,170)

4.4.1 Significance

The cells of living organisms are constantly changing due to the dynamic roles they play in cellular activities. Thus, there is a constant turnover of molecules and

structures in living beings, yet there is exact duplication of proteins every time they are synthesized. This remarkable authenticity of protein structure is made possible by the genetic code carried in the DNA of the nucleus. The complex process of protein synthesis is briefly outlined here to show how ricin intoxication takes place.

Polypeptide synthesis requires the presence of certain components which have to follow a prescribed process, the components which must be present being:

1. Amino acids
2. Site of synthesis, the ribosome with its proteins and mRNA
3. A message to be translated; carried by mRNA
4. Means for transferring the amino acids for synthesis; furnished by tRNA
5. Enzymes:
   a) For amino acid activation
   b) Peptide polymerase system
6. Energy source for carrying out the various operations; provided by ATP
7. GTP for the synthesis of the peptide bond
8. Initiation and transfer factors
9. Inorganic cations, e.g. Mg^{2+}, K^{+}.

The process involves transferring the amino acids to the sites of synthesis, their activation, and insertion in the polypeptide chain which is later elongated, and the process is eventually terminated.

4.4.2 The Components

4.4.2.1 Nucleic Acids

This major class of biological molecules is so named because it accounts for most of the constituents of the nucleus, the active centre of the cell. The major nucleic acid in the nucleus is Deoxyribonucleic acid (DNA) which is the genetic material of all living organisms. A molecular double helical model was proposed by Watson and Crick (171) and the molecule described by this model has two strands of sugar-phosphate "backbone". The strands, on the outside of the molecule, are oriented in opposite directions, together forming a double helix. Nitrogen-containing bases cross connect the sugar-phosphate strands, and these are the two purines: adenine and guanine, and the two pyrimidines: thymine and cytosine. The base pairing is such that adenine is always connected, by two hydrogen bonds, only to thymine, while three hydrogen bonds always connect guanine only to cytosine. The sugar of DNA is the 5-carbon 2-deoxyribose.

The second nucleic acid is Ribonucleic Acid (RNA) and is the tool through which DNA mediates protein synthesis in the cytoplasm. Chemically, RNA differs from DNA in that its sugar is the pentose:ribose. Molecules of RNA are linear, single-stranded polymers of mononucleotides. RNA is synthesized in the nucleus, using one of the strands of DNA as a template, and thus the base-pairing rules for DNA apply to RNA except that in the latter uracil occurs instead of the thymine in DNA. There are three kinds of RNA, ribosomal, messenger, and transfer; their structures and functions vary, but the basic function for RNA is to

carry the genetic message of DNA to the cytoplasm.

4.4.2.2 Ribosomes and rRNA

Ribosomes are dense, membraneless, spherical granules about 100-150 Å in diameter which contain as much as 80% of the RNA of the cell, and are asymmetric aggregates of two subunits which combine to form the intact molecule. The size of the ribosome is defined by its sedimentation coefficient in Svedburg unit (S). In plants, the ribosome subunits are 40S and 60S, and the intact active ribosome unit formed from these subunits has a sedimentation coefficient of 80S. The number of proteins in each subunit varies with the source of the ribosome, e.g. mammalian, bacterial, etc. Ribosomes are the templates upon which the decoding takes place of the information leading to protein synthesis; clusters of ribosomes known as polysomes are used for this purpose. rRNA does not carry genetic information, and its precise function and how it fits in ribosomes is not yet clearly determined.

4.4.2.3 Messenger RNA

The linear sequence of the four bases of DNA provides the genetic information of the molecule, and this information is transferred to RNA molecules by synthesizing the latter from the former. RNA molecules also have their genetic information stored in the sequences of their four bases and this is what confers a specific amino acid sequence on a given protein. A messenger RNA molecule

is produced from a DNA double helix as illustrated in Fig. 4.1. Note that only one chain of the DNA is copied, and that the direction of "transcription" is as shown. This process is catalysed by an enzyme, RNA polymerase. The base-pairing rules for DNA apply here with the exception that the thymine of the DNA bonds with adenine of RNA, and the thymine in DNA is replaced by uracil in RNA. Thus a segment of DNA like the one shown in Fig. 4.1 when transcribed will produce the corresponding mRNA chain shown in the figure with the specific coded message whose translation means the synthesis of a polypeptide chain with the given amino acid sequence.

4.4.2.4 Transfer RNA

Transfer RNA is responsible for carrying the amino acids to the ribosomes where they are incorporated in the synthesized polypeptide chains. This molecule is the smallest of the three RNAs, and has a "cloverleaf" three dimensional structure that is common to its various forms. The tRNA molecule has unique features not possessed by rRNA or mRNA, and these seem necessary to enable it to perform its complex function which will be explained when discussing the process of protein synthesis.

The rRNA molecule is L-shaped; the short segment is capable of specifically binding one and only one of the 20 amino acids. The long segment recognizes a specific codon of mRNA, and this recognition follows the rules of base pairing between the three bases of the mRNA codon and a complementary set of bases on tRNA called

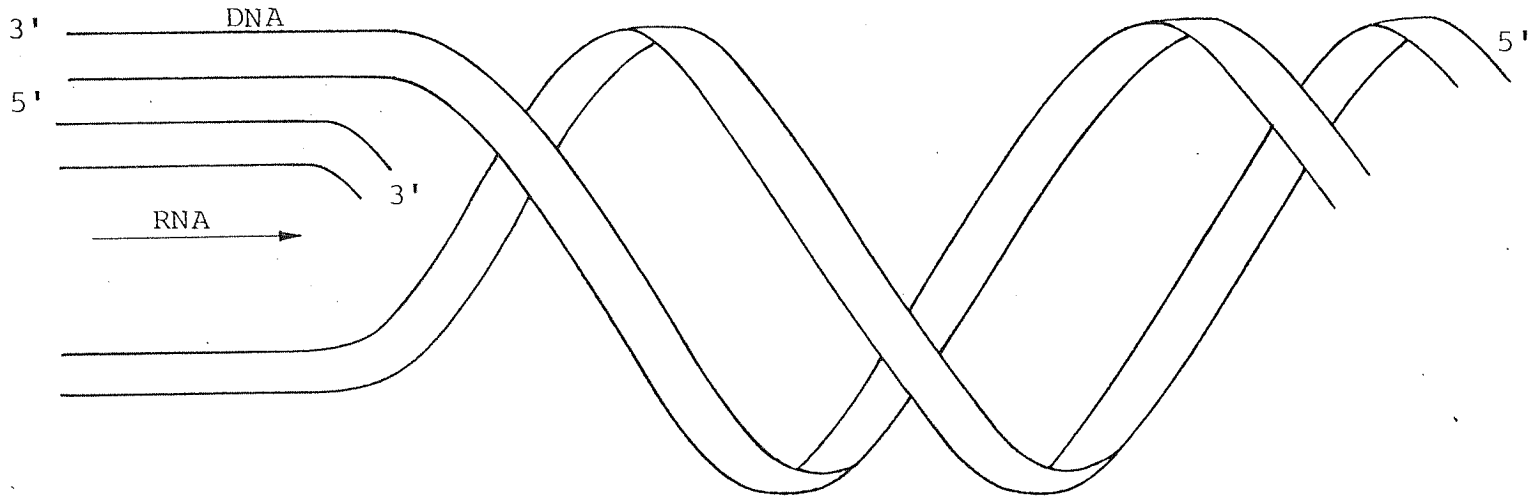


FIG. 4.1 THE TRANSCRIPTION OF DNA ON RNA

the anticodon.

4.4.2.5 The Genetic Code

After proving that DNA is the genetic material and that the genetic information resides in the vertical sequence of its bases, the next step was to "decipher" the message, i.e. to know which amino acid is coded for by a given sequence of DNA bases. The first problem to be solved was to know how many mRNA nucleotide sequence (or codons) code for a particular amino acid. If one base formed the codon, then only four bases would code for the twenty amino acids, while if the codon was made of two bases, sixteen codons would exist for the twenty amino acids. It is clear that both propositions would lead to ambiguity in the code. If, however, a triplet codon is envisaged, the number of codons will be sixty four, more than enough for the twenty amino acids. The experimental evidence suggests the triplet codon to be the most likely.

Nirenberg (172) was the first to suggest in 1961 that the triplet codon UUU stands for the amino acid phenylalanine, and subsequent findings resulted in producing the "coding dictionary" as we know it today, Table 4.2.

The essential features of the genetic code as proposed by Crick (173) are that it is triplet, commaless, and nonoverlapping. The terms "commaless" and nonoverlapping" are meant to indicate

TABLE 4.3

The Genetic Dictionary in Terms of mRNA

First Base	Second Base G	Second Base A	Second Base C	Second Base U	Third Base
G	GGG Gly	GAG Glu	GCG Ala	GUG Val	G
	GGA Gly	GAA Glu	GCA Ala	GUA Val	A
	GGC Gly	GAC Asp	GCC Ala	GUC Val	C
	GGU Gly	GAU Asp	GCU Ala	GUU Val	U
A	AGG Arg	AAG Lys	ACG Thr	AUG Met	G
	AGA Arg	AAA Lys	ACA Thr	AUA lle	A
	AGC Ser	AAC Asn	ACC Thr	AUC lle	C
	AGU Ser	AAU Asn	ACU Thr	AUU lle	U
C	CGG Arg	CAG Gln	CCG Pro	CUG Leu	G
	CGA Arg	CAA Gln	CCA Pro	CUA Leu	A
	CGC Arg	CAC His	CCC Pro	CUC Leu	C
	CGU Arg	CAU His	CCU Pro	CUU Leu	U
U	UGG Trp	UAG End Chain	UCG Ser	UUG Leu	G
	UGA End Chain	UAA End Chain	UCA Ser	UUA Leu	A
	UGC Cys	UAC Tyr	UCC Ser	UUC Phe	C
	UGU Cys	UAU Tyr	UCU Ser	UUU Phe	U

how the code is read. In their original proposition, Crick et al suggested that the code is nondegenerate, i.e. for every amino acid there is only one codon that codes for it. It is now known that the code is highly degenerate, so that more than one codon code for the same amino acid. Only two of the twenty amino acids have one codon each, and these are methionine and tryptophan.

4.4.3 The Process

The biosynthesis of a polypeptide chain involves transferring the amino acids to the ribosomes where they are assembled in chains that are in turn assembled into proteins elsewhere in the cytoplasm. The stages of this process will be briefly explained in the subsequent subsections.

The amino acids are transferred from their intracellular pool by tRNA molecules, but before this each of the 20 amino acids must be activated. The activation is a reaction between a specific amino acid and adenosine triphosphate (ATP) catalysed by an enzyme, amino acyl synthetase. At least 20 different types of this enzyme must be present in a cell since each amino acid needs a specific enzyme for its activation. The complex formed in the reaction is referred to as an activated amino acid.

The activated amino acid does not attach directly to mRNA for translation of the genetic message. It has first to be attached to the tRNA molecule, and this is

brought about by the same enzyme which activated the amino acid. The tRNA is considered as an adaptor molecule and it has a strong chemical affinity for the mRNA nucleotide. The short segment of the tRNA L-shape enzymatically binds the activated amino acid, while the long segment is recognized by the appropriate mRNA nucelotide.

The complex formed by an activated amino acid and a tRNA molecule then diffuses to the ribosomes where the actual synthesis starts. As mentioned previously, the ribosome is made of a small subunit and a large one, and it is to the smaller subparticle that the mRNA is attached. Each ribosome has two sites referred to as the aminoacyl (A) and peptidyl (P) sites. A charged tRNA, i.e. one that carries an activated amino acid, is first placed at the A-site on the smaller subunit of a ribosome. The ribosome is moved along mRNA, thus transferring the amino acid from the A-site to the P-site by the action of elongation factor G, also known as translocase. The vacant A-site is next occupied by a second charged t-RNA whose anticodon must be complementary to the codon of the mRNA at that site. An enzyme then synthesizes a peptide bond between the carboxyl group of the amino acid in the P-site and the amino group of the second amino acid in the A-site. The tRNA is now uncharged, and it is released when the ribosome moves a distance of three nucleotides (one codon) along mRNA. This movement shifts the occupied codon to the P-site, thus permitting

the entry of a new amino acid to fit the new codon in the A-site. This series of events is repeated until the ribosome comes across a chain-terminating signal on mRNA. Specific proteins, known as release factors, recognize these stop signals, and as a result the ribosome dissociates into its two component subunits after leaving the mRNA and its completed polypeptide chain is enzymatically released. The ribosome subunits and the uncharged tRNA are free to associate in future with other subunits and amino acids for the synthesis of other polypeptide chains.

Protein synthesis is started by initiation factors (IF1, IF2, IF3), and the energy for the completion of the various steps is provided by Adenosine Triphosphate (ATP). The splitting of Guanidine Triphosphate (GTP) is required for the synthesis of the peptide bond. The available data suggests that the mRNA is read in the 5'→3' direction, and the polypeptide chains are synthesized starting by the amino group terminal and finishing by the carboxy group terminal. The completed polypeptide chains are then available to be incorporated in the appropriate protein molecule.

Polypeptide chain elongation, i.e. the addition of new amino acids to the growing chain, is an important step in protein synthesis and deserves special mention because the action of ricin affects the process at this stage. Protein elongation factors (EF) combine with GTP forming a complex which interacts with the incoming aminoacyl-tRNA, and the whole new unit is introduced in the A-site

to take part in chain elongation. The ribosome should be able to bind the aminoacyl-tRNA complex and to hydrolyse GTP in the presence of the elongation factors. If the hydrolysis of GTP does not take place, protein synthesis stops because the peptide bond formation is discontinued and hence the A-site is blocked because the amino acid in it cannot be transported to the P-site. As long as the A-site is engaged, no further t-RNA-amino acid complexes can enter it, and hence protein synthesis stops.

The vital importance of protein synthesis to living organisms shows how serious its inhibition can be, and its complexity highlights its vulnerability to inhibiting agents. It is not surprising, therefore, that a potent inhibitor like ricin should exhibit the extreme toxicity that is now associated with it. The interference of the toxin with this process is discussed in conjunction with its structure and toxicity in the sections to follow.

4.5 IMMUNOCHEMICAL PRINCIPLES (174,175,177)

4.5.1 The Immunoglobulins

Immunology is the discipline that studies normal and pathologic responses. The substances stimulating such responses are called antigens. The injection of an antigen into a host animal leads to the increased production of serum globulins with antibody activity specifically directed against the foreign agent. If the animal is subsequently exposed to the same antigen, the specific

antibody neutralizes its action. This is the basis of immunity conferred by vaccination or inherited naturally from the mother.

Antibodies, also known as the immunoglobulins (lg), are proteins, and five classes of immunoglobulins have been described: lgG, lgA, lgM, lgD, and lgE. Some of the characteristics of these serum proteins are given in Table 4.4 The immunoglobulins vary in their physico-chemical properties and in their concentration in serum, but they all have the common feature of immunologic specificity of each against the eliciting antigens. The basic structure of the immunoglobulins is represented by the structure of lgG, the most abundant of the serum globulins, illustrated in Fig.4.2. Four polypeptide chains make up this Y-shaped molecule: two identical heavy (H) and two identical light (L) chains joined by disulphide bonds. Treatment of the molecule with the enzyme papain splits it into two main fragments: fragment antigen binding (Fab) and fragment crystallizable (FC). Being polypeptides, the immunoglobulin molecules have an amino terminal end and a carboxyterminal end. The amino group resides in the Fab fragment, and the carboxyterminal end is in the FC fragment.

The Fab contains both heavy and light chains, and the antigen binds to this end of the lg. The FC contains only heavy chains and it also has some carbohydrates in its composition. The lgG is the only immunoglobulin that

Table 4.4 Some Properties of the Immunoglobulins

	IgG	IgA	IgM	IgD	IgE
Molecular Weight	150,000	150,000-600,000	1,000,000	200,000	190,000
Svedberg "S"	7S	7S-17S	19S	7S	8S
Normal Serum Level, mg%	1,200	200	100	3	0.01-0.14
% Total Antibody	76	16	8	trace	trace
Half Life (days)	21	5	5	3	2
Placental Passage	+	-	-	-	-

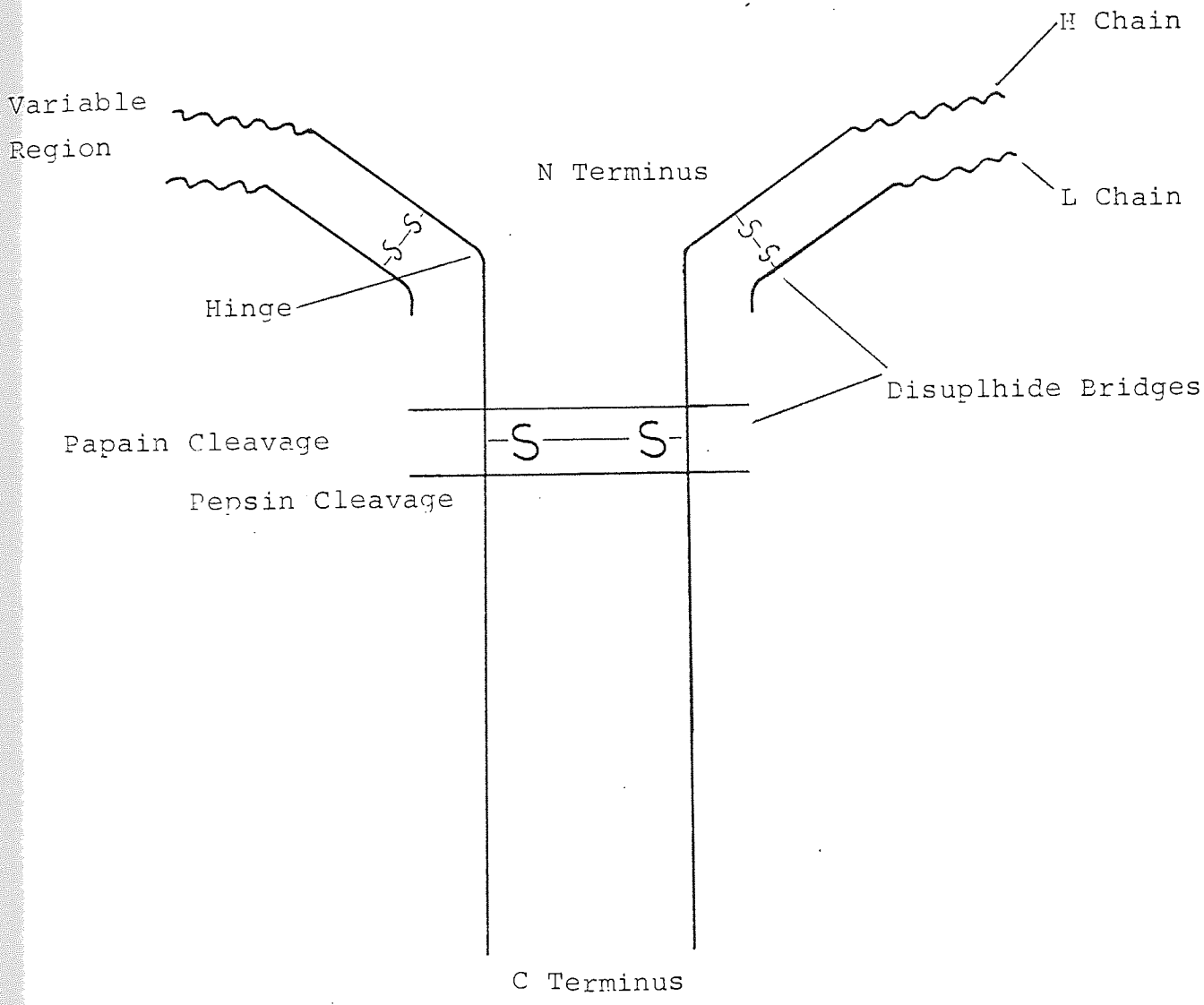


Fig. 4.2 The Structure of the Immunoglobulins

passes the placenta, i.e. is transferred from mother to offspring, and this function is performed under the direction of the FC.

The primary amino acid sequence of the amino-terminal regions of the heavy and light chains varies from molecule to molecule. These varilable, or V, regions distinguish each molecule and are the reason for the specificity shown by the antibody against the antigen that induced its formation. There are five types of heavy chains and two types of light chains in the five classes of lg, and it is possible to utilize this for designating the molecules and their components. The presence of a "hinge" in the Fab enables the free movement of its limbs.

The other immunoglobulins do not differ from this structure except in that lgM is a pentamer and that lgA can exist as a dimer or a polymer in addition to its monomeric form. lgE (reagin) is important in allergic reactions of the anaphylactic type.

4.5.2 Antigen-Antibody Reactions

4.5.2.1 Introduction

Techniques for diagnosing diseases and quantifying antigens and antibodies were developed by utilizing the reactions that can take place between the antigen and its corresponding antibodies. The nature of the various tests will be briefly explained and their sensitivity indicated. The material of this section was applied to the choice of

method of assay of ricin and the castor bean allergen presented in Chapter three of this thesis (see section 3.4.4). The three types discussed first, namely agglutination, precipitation, and toxin-antitoxin reactions, are procedures that involve the direct demonstration of the reaction being carried out. In the remaining types, the interaction can be visualised or quantified using indicators or special equipment, e.g. dyes and radioactivity counters.

4.5.2.2 Agglutination

Agglutinating antibodies, or agglutinins, bind to the cells which they agglutinate, e.g. red blood cells, with the result that clumping occurs and masses are formed that can be detected visually or under a microscope. There are numerous factors involved in the agglutination process, thus affecting its accuracy, and this may explain why the technique is more useful qualitatively than quantitatively.

4.5.2.3 Precipitation

While agglutination involves cellular antigens, precipitation is associated with soluble antigens. It is a specific reaction in which certain antigen-antibody ratios must be used to reach the equivalence zone, i.e. no excess of either antigen or antibody.

Precipitation in gels, first reported by Oudin in 1946, greatly enhanced the potential of this technique. Agar gel is placed in a test tube containing an antiserum. The

gel is allowed to solidify and then the antigen solution is added. Keeping the tube vertical, the antigen diffuses through the gel, establishing a concentration gradient that decreases from top to bottom. Since the speed of migration depends only on antigen concentration, it is possible to quantify the latter under standardized conditions. Modifications of this method are numerous, one of the most popular being double immunodiffusion in tubes. In this version, the antiserum-impregnated gel is covered with gel free of antiserum, and on top of this second gel either free antigen solution or agar-incorporated antigen is added. The antigen and antibody diffuse towards each other on the neutral gel until equilibrium is established.

Test tubes were later replaced by agar plates with wells drilled in them and this enabled performing qualitative as well as quantitative analysis. In the qualitative Ouchterlony technique, the antiserum is placed in a central well and the antigens to be tested are placed in wells around it. If two identical antigens (that produced the antiserum used) are placed in the outer wells, the precipitation lines join and fuse - a reaction of identity. If two different antigens are tested, the lines cross - a reaction of nonidentity. If the two antigen have features in common and one of them is the immunizing antigen, the precipitation lines fuse and a projection is noted that extends the line formed by the immunizing antigen, Figure 4.3

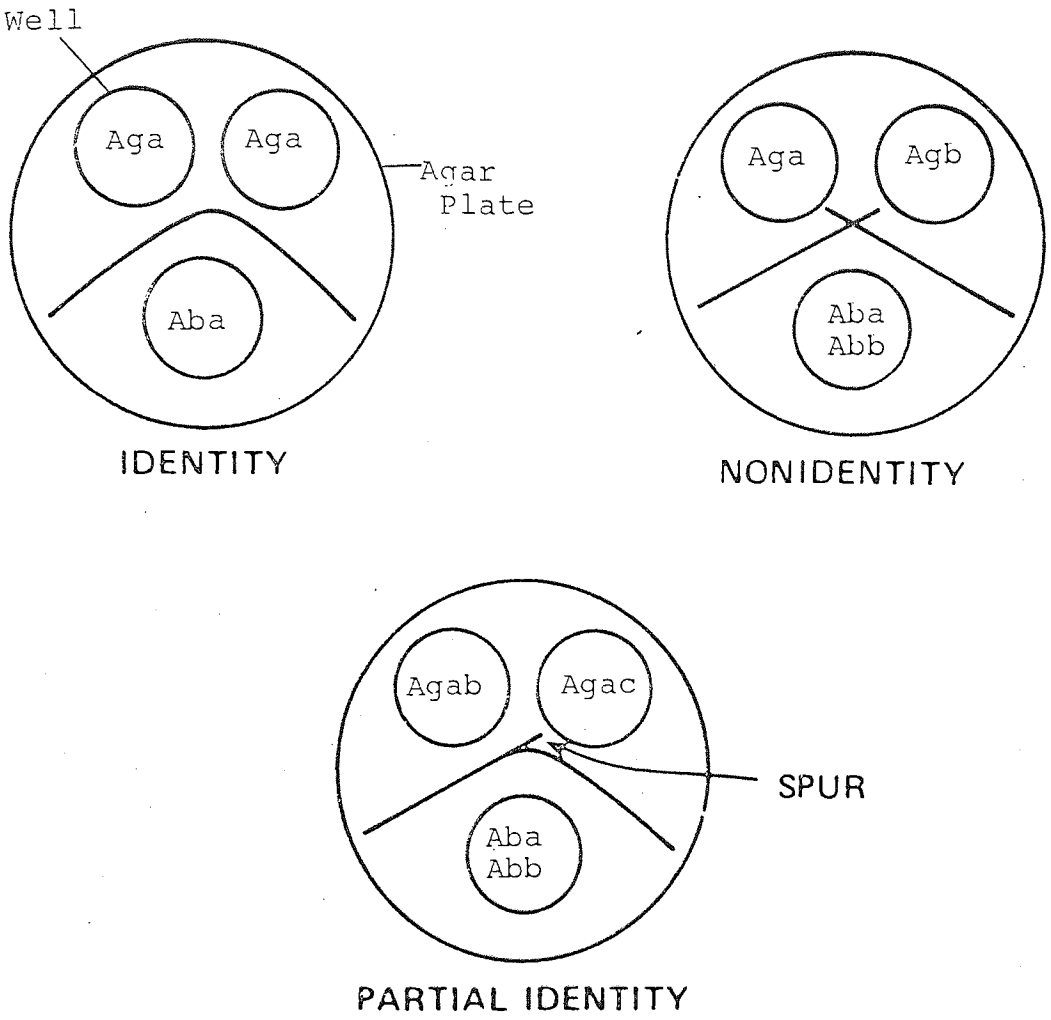


FIG. 4.3 TYPES OF IMMUNODIFFUSION PATTERNS

Ag: antigen

Ab: antibody

Quantitative single radial immunodiffusion is illustrated by the Mancini technique, Fig.4.4. The antiserum is impregnated into the agar and the antigen is placed in wells drilled in the plate. The diffusion of the antigen into the agar results in the formation of rings whose diameter is proportional to the initial antigen concentration. The results can be greatly influenced by factors such as the choice of buffer, incubation times, extent of well filling, and others (174). Hence reference standards are always included on the same plate with the samples whose antigen concentration is to be determined. After equilibrium is reached, the ring diameters of the standards are plotted against their initial antigen concentration to yield a straight line, Fig.4.5, which can be used for reading off the unknown concentrations. On occasions, the amount of antigen in the test sample is so low that the precipitates which develop are not visible directly or by staining. They are made visible by washing the gel and then applying a second antiserum carrying a radioactive material. The radioactivity is then specifically bound to zones of precipitation and is visualised by exposing the plate to photographic film (176).

For resolving highly complex antigen mixtures, immunodiffusion is coupled to electrophoresis in the technique known as immunoelectrophoresis. In this procedure, the electrophoresis is performed first; an electric current is applied to the antigen mixture which is placed into a well cut in an agar plate. The various proteins separate according to their electrophoretic mobility; after sufficient

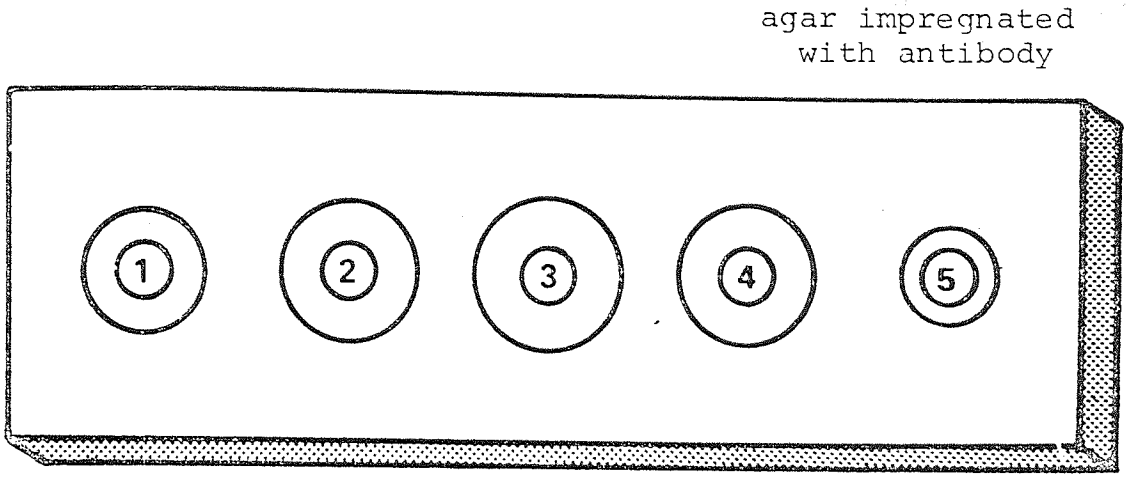


FIG. 4.4 QUANTITATIVE RADIAL IMMUNODIFFUSION

Wells 1,2,3: standards

Wells 4,5: test samples

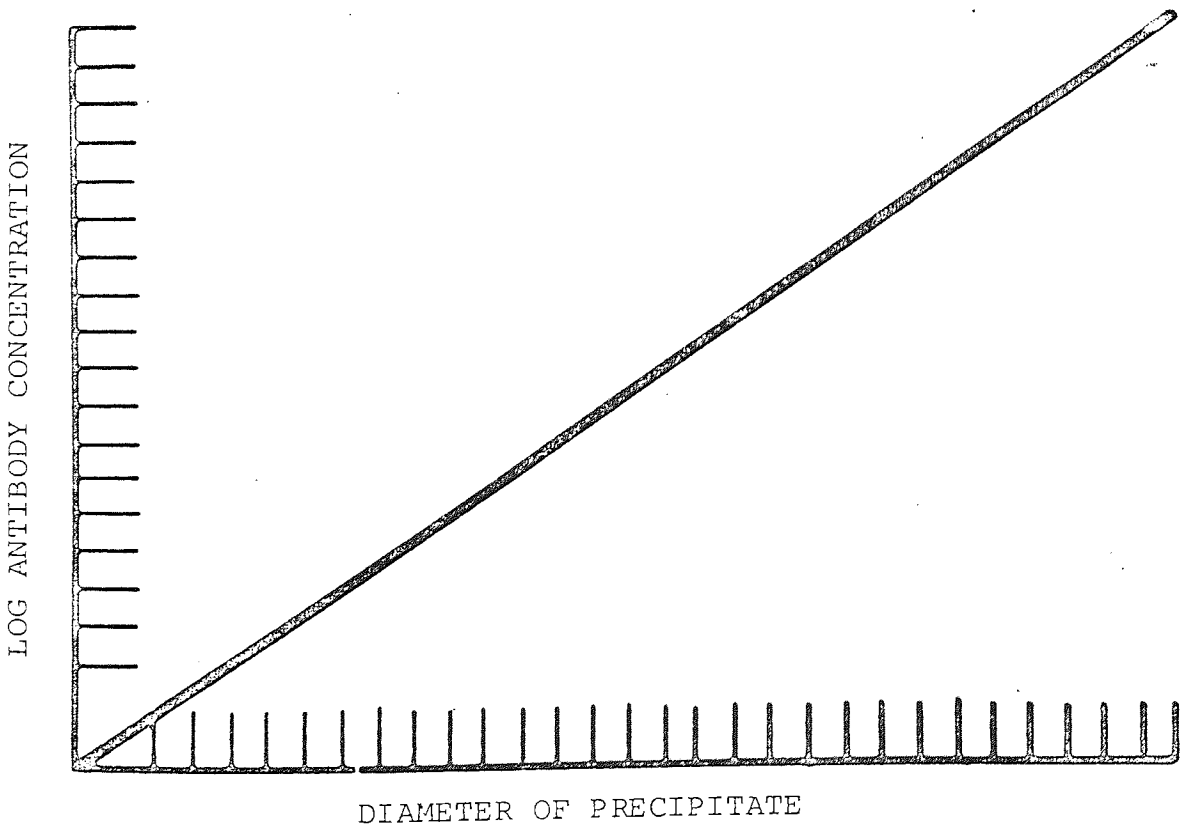


FIG. 4.5 STANDARD CURVE FOR QUANTITATIVE RADIAL IMMUNODIFFUSION

time has been allowed for this process, the electric current is switched off. A ploy specific serum is then added in grooves running parallel to the direction of antigen migration and the antigens and antibodies are allowed to diffuse freely towards each other, forming precipitates similar to those described in the Ouchterlony technique, including standards for identification. A radioimmunoelectrophoretic technique, similar to radioimmunodiffusion, can be set up to study those antigens which give rise to very faint or invisible precipitates (177). The incorporation of electrophoresis in immunodiffusion assays greatly shortens the time of the test and helps to achieve a sharper separation of the protein mixture.

Another combination of electrophoresis and immunodiffusion can be seen in the electrodiffusion procedure, also known as the Laurell "rocket" technique - a reference to the shape of the antigen-antibody product. In this method, the electrophoretic migration of antigens takes place in antibody-containing agar on a glass slide. The migration of antigen towards the anode is visualized by its reaction with the antibody in the agar. The resulting pattern resembles a rocket, and the height of each peak is proportional to the concentration of the antigen that produced it. Quantification is effected by including standards and plotting a graph of their rocket heights versus antigen concentration (174).

4.5.2.4 Toxin-Antitoxin Reactions

This group of reactions is based on the fact that an antitoxin (i.e. the antibody raised against a toxic substance) will neutralize the toxin in vivo or in vitro. Thus, if a toxin is injected in an animal immunized against its action, no harmful effects will occur because the antibody will neutralize the toxin in vivo. However, if the animal is not immunized, reactions will take place in the body, and their effect will depend on the dose of the toxin; a small dose may produce immunity, whereas a large dose may be fatal. Carmichael (178) utilized this observation to demonstrate the immunizing action of ricin.

Toxin neutralization may also take place in vitro. Thus, if the antibody is added to the toxin in a test tube, the injection of a small portion of the mixture in an animal will not be harmful because the deleterious effects of the toxin were abolished. This is the basis for the Clarke's test for ricin which was explained in Section 3.4.4.

4.5.2.5 Radioimmunoassay

The RIA is a sensitive and specific technique that was originally developed for measuring the levels of insulin in the blood. The basic principle of the technique can be explained as follows. Given a radiolabelled antigen (Ag*), an unlabelled antigen (Ag), and an antibody, then in the absence of Ag, the unlabelled antigen, a certain amount of

the labelled antigen will bind to the antibody. The amount of antibody-bound labelled antigen (Ab-Ag*) will decrease when the unlabelled antigen is present, and the amount of labelled antigen displaced from the antibody is proportional to the amount of unlabelled antigen present in the system.

A known amount of labelled antigen is added to a known amount of a specific antibody together with an unknown amount of the unlabelled antigen and the ingredients are allowed to react. After separating the resulting antigen-antibody complexes, the radioactivity of the various fractions is measured. By determining the activity in the unbound labelled antigen, one can calculate the per centage of labelled antigen bound to the antibody. If varying amounts of unlabelled antigen are allowed to compete with the labelled antigen, the resulting curve will enable determining the concentration of an unknown (unlabelled) antigen.

In spite of the technical merits of the RIA, the use of radioactive isotopes poses a number of problems, namely cost, the short shelf-life of the reagents, the complexity of the auxilliary equipment, and the special safety measures that must be observed in the handling and disposal of the reagents (179).

4.5.2.6 Immunofluorescence

Instead of isotope labelling, as in RIA, immunofluorescence utilizes the tagging of the antibody with a fluorescent dye. The antigenic determinant present in the test specimen can then be detected by applying the labelled antibody, which will bind to the specific antiserum, and examining under UV light. Although now an established diagnostic tool, this technique has its drawbacks; it is reported to be time consuming, not easy to automate, and the reading of the results is usually subjective (179).

4.5.2.7 Enzyme-Linked Immunosorbent Assay

ELISA was developed to meet the need for an assay that is as sensitive as RIA but free of the risks inherent in it. It comes in various sytems for assaying both antigens and antibodies. One such variant of ELISA, the double antibody sandwich technique for measurement of antigen, is carried out as follows:

1. Antibody specific for the antigen to be measured is attached to a solid phase which is then washed.

2. The solution being tested for antigen is then bound to the surface of the solid phase support and washed to remove any unreacted material.

3. The specific antibody, labelled with enzyme, is then applied, followed by washing.

4. Finally, the enzyme substrate is added. The colour change resulting from the substrate degration is proportional to the amount of antigen in the test solution.

This type of assay requires that the antigen or antibody be attached to a solid-phase support without loss of activity; the enzyme linkage should also be possible without impairing immunological or enzymatic properties. The reagents for this technique are reported to be cheap to prepare, and they have a long shelf-life (179). The resulting assays are sensitive and the results can be determined with simple equipment.

It is possible to implement a method of assay for ricin and the castor allergens from the techniques already described. The criteria for making such a choice were discussed in the preceding chapter, together with an elaboration on some of the techniques used in assaying allergens.

4.6 RICIN AND THE CASTOR-BEAN AGGLUTININ

4.6.1 Introduction

In 1888, Stillmark (180) discovered the presence of a toxic protein in the castor-oil seeds, which he named ricin. He also observed that the isolated substance agglutinated erythrocytes. For some time, it was thought that the toxic and agglutinating properties both existed in the same molecule, but the work of Jacoby (180) and later of others (181) proved the two properties to be unrelated i.e. toxicity and agglutinating power are

attributable to two separate, though somehow related substances. At present, the name ricin is exclusively given to the toxic protein, while the agglutinin is termed the ricinus agglutinin or the castor bean hemagglutinin (CBH). Ricin and the ricinus agglutinin were shown to have closely related structures (180). Interest in ricin continues to be immense because it is a lectin, a class of biological compounds that bind carbohydrates and possess agglutinating properties (182). The lectin nature of ricin resides in one of its chains, the B-chain. Lectins have proved to be valuable tools in the study of cell surfaces. Recently, ricin was introduced into the field of cancer research because of its ability to suppress the growth of malignant cells (183).

4.6.2 Preparation, Purification, and Structure

After Stillmark, the classic work of Osborne et al (184) on ricin remains the most famous, and their method is still widely used today. They precipiated the toxin from defatted castor seeds with sodium chloride solution. The precipitate was redissolved in water, dialysed against water, and the toxin was precipitated using ammonium sulphate solutions. Other significant contributions resulted from the work of Kobert (12), Karrer (12), Kabat (185), and Schone (12). Later, Ishiguro and his coworkers (186) isolated a ricin fraction, ricin D, which was very toxic but showed no hemagglutinating or proteolytic activity.

Various ricin "types" were described by Funatsu (181),

the differences arising mainly from the starting material and the methods employed. Thus, ricin B_1 was isolated by Kabat et al (185) using sodium sulphate precipitations. The homogenity of this preparation was demonstrated electrophoretically, ultracentrifugally and immunochemically. An amorphous ricin preparation was isolated by Le Breton and Moulé, and it was designated as ricin Tb. Although crystallization of ricin was attempted for long (187), the success in achieving this is attributed to Kabat (185) and Kunitz and McDonald (188) who found the material heterogeneous even after repeated crystallizations. Ricin D is also crystalline, but unlike the other crystalline ricins, it is homogeneous ultracentrifugally and electrophoretically. It is interesting to note that the type of ricin as well as the ease of its recovery could be correlated by Funatsu (181) to the size of the beans.

The early ricin preparations were purified by fractionating with various reagents, of which sodium sulphate was found to be the best (12). The superiority of sodium sulphate over sodium chloride as a precipitant is because it gives better fractionation, is less sensitive to pH changes, and it precipitates the toxin more completely. The modern methods of ricin purification utilize the separating power of techniques such as affinity chromatography, ion exchange chromatography, and gel filtration to separate ricin from the agglutinin (189,190,191). Electrophoretic and chromatographic techniques

are discussed by Jaffé (192,193). Nicolson (194), Brown and Hunt (195), and Sela (196). Thus, the separation in sepharose 4B columns takes advantage of the fact that both ricin and the agglutinin bind to sepharose beads and can be later separated from one another by gel filtration because the molecular weight of the agglutinin is twice that of ricin, by sucrose gradient centrifugation because of density differences, or by ion exchange chromatography because of differences of charge. Olsnes and Pihl prefer ion exchange chromatography (197) to the column techniques employed by Nicolson et al (198) because of the better separation achieved and the larger amounts of protein that can be applied.

Flowsheets showing the preparation and purification of ricin are presented in Fig. 4.6 and Fig. 4.7.

The fractionation of the castor seed lectins gives rise to two products, usually referred to as RCA_I and RCA_{II}. Ricin is thought to be identical to RCA_{II} which has a molecular weight of 60,000-65,000 (196,197,198) and is a toxic, nonagglutinating lectin (193), while the agglutinin is probably the same as RCA_I, with a molecular weight of 120,000. The pure agglutinin is virtually non toxic to animals but shows toxicity towards cells in culture (180,197).

Ricin is composed of two polypeptide chains linked by disulphide bridges. The 'A' chain is called the effectomer and has a molecular weight of 32,000, while

Fig 4.6 The Olsne's and Pihl (197) Procedure for isolating ricin

Fig 4.7 Flowsheet for the Procedure of Ricin D Purification (181)

the 'B' chain, or the haptomer, is slightly longer and has a molecular weight of 34,000. The reduction of the toxin with 2-mercaptoethanol breaks the disulphide bond holding the two chains of the molecule (181,183). The A-chain is then separated from the B-chain by passing the two through a DE-52 column, where the A-chain passes through but the B-chain is retained and is later eluted with a salt gradient (181,183). After further treatment it is possible to obtain each of the two chains in a high state of purity. Extensive research on the intact toxin and its isolated chains revealed information about their biological and immunological properties and mechanism of action.

The amino acid composition of ricin (198,199,200,201,202) and of the castor bean agglutinin (198,199,201,202) has been determined as well as the composition of the isolated chains of ricin (199, 200,201,202) and the agglutinin (203). The data reported by Olsnes et al (199) is given in Table ; the authors observed that their results are in general agreement with those of other workers. Table 4.5 gives the amino acids of ricin.

The aminoterminal and carboxyterminal amino acids of the ricin A-chain were found to be isoleucine and serine, while those of the ricin B-chain were reported as alanine and phenylalanine, respectively (201,204,205). Funatsu et al (181) reported the primary structure of ricin D, and a schematic representation of the main chain structure according to their data is shown in Fig. 4.8 . The two polypeptide chains constituting the whole ricin molecule were denoted as the alanine (Ala) and isoleucine (Ile)

TABLE 4.5

The amino acid composition of ricin chains

Amino Acid	A-chain	B-chain
Aspartic Acid	27.3 (± 0.3)	43.6 (± 0.2)
Threonine	18.1 (± 0.2)	23.4 (± 0.2)
Serine	20.0 (± 0.9)	21.6 (± 0.3)
Glutamic Acid	31.2 (± 0.6)	23.9 (± 0.3)
Proline	16.9 (± 0.7)	16.0 (± 0.2)
Glycine	18.5 (± 0.6)	23.0 (± 0.3)
Alanine	25.4 (± 0.2)	17.2 (± 0.1)
Half Cysteine	1.2	6.0
Tryptophan	N.D.	N.D.
Valine	13.7 (± 0.4)	16.9 (± 0.6)
Methionine	2.2 (± 0.5)	2.9 (± 0.3)
Isoleucine	19.4 (± 0.1)	16.3 (± 0.1)
Leucine	23.1 (± 0.2)	26.9 (± 0.3)
Tyrosine	14.9 (± 0.9)	12.5 (± 0.5)
Phenylalanine	14.3 (± 0.1)	4.8 (± 0.3)
Histidine	3.6 (± 0.6)	2.3 (± 0.1)
Lysine	1.9 (± 0.7)	7.9 (± 0.3)
Arginine	20.4 (± 0.9)	14.8 (± 0.5)

Values given are residues per chain.

N.D. = Not Determined

TABLE 4.6
The Amino Acids of Ricin (%)

Amino Acid	Funatsu (181)	Olsnes* (183)	Tavasolian** (337)	Lin (202)		Karrer (312)	Raven (12)
				Ricin D	Ricin E		
Glycine	7.0	7.4	5.4	7.1	7.5	0.0	-
Alanine	7.0	7.6	5.4	7.5	7.9	1.0	1.0
Valine	6.0	5.5	2.9	6.3	6.7	2.0	2.0
Leu + lle	15.0	11.8	8.8***	17.1	16.9	16.0	16.0
Phenylalanine	4.0	3.4	4.6	3.5	3.4	0.4	0.4
Tyrosine	4.0	4.9	2.3	4.2	4.0	2.7	2.7
Cystine	2.0	1.3	2.9	1.9	1.7	1.0	1.0
Serine	7.0	7.4	6.6	7.1	6.3		-
Proline	5.0	5.9	7.1	5.6	5.4	4.6	4.6
Hydroxyproline	-	-	-	-	-	0.0	-
Aspartic Acid	12.0	12.7	8.3	2.1	12.1	2.0	2.0
Glutamic Acid	9.0	9.9	12.6	9.0	8.6	20.0	20.0
Tryptophan	1.0	1.3	-	-	-	0.4	0.4
Arginine	6.0	6.3	6.6	7.1	7.1	11.7	11.7
Lysine	2.0	1.8	3.2	1.9	2.3	6.3	6.3
Histidine	1.0	1.1	2.0	1.2	1.3	0.0	-
Theonine	7.0	7.4	6.0	7.1	7.5	-	-
Methionine	1.0	0.9	12.2	1.2	1.3	-	-

TABLE 4.6 (continued)

Amino Acid	Funatsu (181)	Olsnes* (183)	Tavasolian** (337)	Lin (202)		Karrer (312)	Raven (12)
				Ricin D	Ricin E		
Humin	-	-	-	-	-	1.8	-
Ammonia	-	-	-	-	-	2.8	-
Peptide Anhydrides	-	-	-	-	-	2.O	-
Unidentified	-	-	-	-	-	3.3	-
Amide N	-	-	-	-	-	-	2.3

* Calculated as percentage from the original data

** Given as mole %

*** Given as 7.1 Leu and 1.7 lle

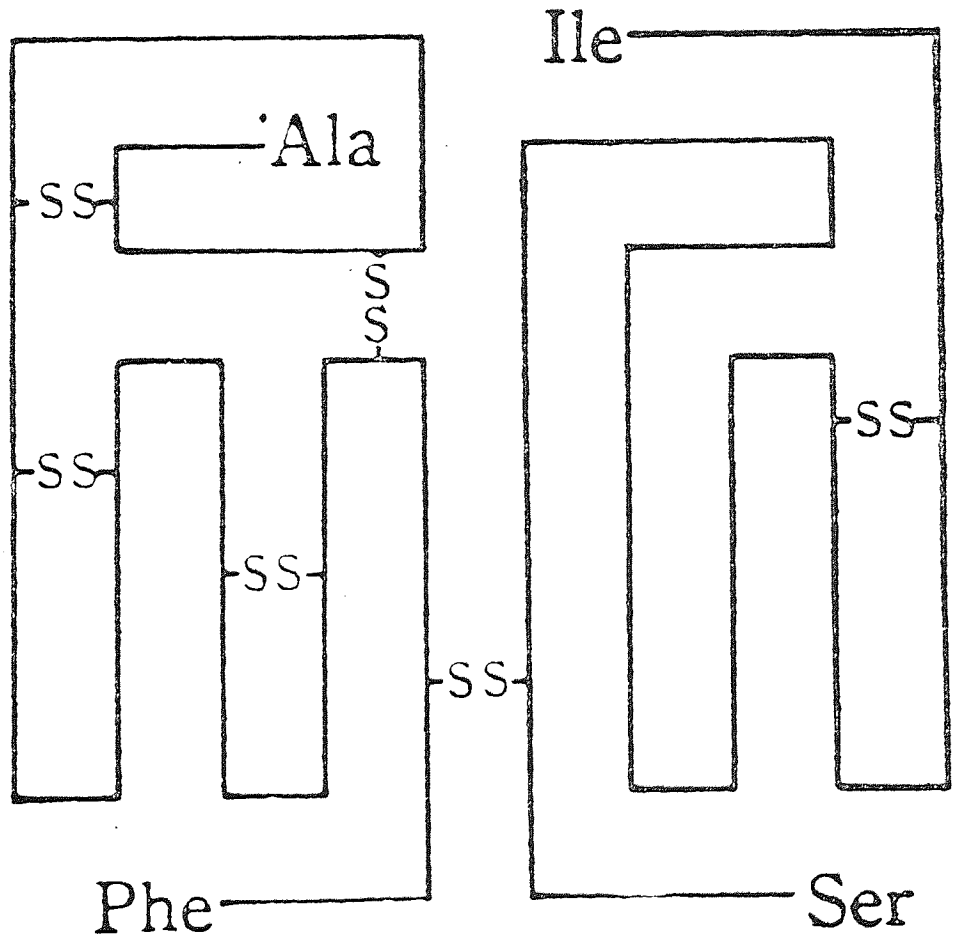


FIG. 4.8 A SCHEMATIC REPRESENTATION
FOR THE MAIN CHAIN STRUCTURE OF RICIN D

chains. Based on electrophoretic studies and amino acid determinations, the opinion of the authors is that the Ile chain is a common subunit in the different ricins studied, whereas the Ala chain varies according to the type of ricin. Ishiguro et al (206) isolated a ricin variant whose A-chain (Ile-chain) was very similar to that of ricin-D, but in which the B-chain (Ala chain) differed considerably from its counterpart of ricin D in chemical composition. The aminoterminal sequences of the ricin A- and B- chains were determined by Li et al (207).

The castor seed agglutinin is a tetramer with four subunits, two of which have molecular weights of 29,500, and the remaining two have a molecular weight of 37,000 each (189,197,198,208). The pair of light chains (A'-chains) appear to be linked to the two heavy chains (B'-chains) by disulphide bonds (180). The treatment of the agglutinin with 2-mercaptoethanol in the presence of galactose releases the A'-chains from the B'-chains, and if the resulting preparation is submitted to ion exchange chromatography, the light chains are separated from the heavy chains (203). The B'-chain passes through the column, whereas the A'-chain is bound and can later be eluted with a salt gradient.

Studies have revealed that the agglutinin and ricin are closely related. Qualitative immunodiffusion tests have indicated a strong cross reaction between ricin and ricinus agglutinin when tested with antiserum raised against the toxin or the agglutinin (108). In addition

to the immunological evidence, chemical studies confirmed this close relationship between ricin and the agglutinin. Thus, the amino acid composition reported (197) shows that the heavier of the two agglutinin chains is probably identical to the B-chain of ricin, while the lighter chain is homologous but not identical to the ricin A-chain.

The carbohydrate content of ricinus agglutinin was reported by Olsnes and Pihl (197) who also mentioned the considerable differences existing in the literature as to the type and amounts of carbohydrates present in ricin. According to these authors, the sugars in the ricinus agglutinin are mainly mannose, together with some glucose and glucosamine. The same pattern was also found for ricin, although the actual amounts bound to the toxin were considerably less than the agglutinin. The distribution of the sugars between the ricin chains showed that most of the carbohydrates are bound to the B-chain. Funatsu (181) found the sugars bound to ricin D to be only mannose and glucosamine, and more carbohydrates were isolated from the B-chain than from the A-chain. Because ricin and the agglutinin are lectins, it is usually speculated that these carbohydrates are involved in binding the lectins to sugars, but Olsnes (183) reported that this is not the case.

4.6.3 Toxicity

The most important characteristic associated with ricin is its extreme toxicity. To illustrate this, Balint (12) mentions the statistic by Rompp that if

ricin is carefully extracted and purified, then one kilogramme is lethal to 3.6 million people, whereas the same quantity of HCN is lethal only to 16000. According to Fuhrman, (209), ricin is the most toxic substance of plant origin.

The toxicity of ricin is what makes the castor oil seeds poisonous to humans and animals, and Balint states that about 700 cases of intoxication in man are reported (12). The number of seeds that may be lethal to an individual depends on the way they were taken, i.e. chewed or swallowed. In the cases known to the author, no death happened but diarrhea and dizziness were experienced. In the case of animals, different species reveal varying degrees of resistance to the effect of the toxin; the hen is the most resistant, while the horse is the most susceptible.

Osborne et al (184) noted a correlation between the body temperature of frogs and the amount of toxin needed to effect death, such that the animals show very high resistance at low temperatures and vice versa. The route through which the toxin is introduced into the animal's body also affects the time before which intoxication occurs, but however high the dose may be a certain lag time has been observed between injection and appearance of symptons (197). Oral administration is the least effective since a certain proportion of the toxin is lost in the alimentary track, and an intravenous injection may be 100 times as effective as a similar dose taken by mouth. Intraperitoneal

injection is less effective than the intravenous route, subcutaneous injection is less effective still.

Depending on the dose administered via a specific route to a certain type of animal, e.g. mice, rabbits etc., there is a relation between the dose and the survival time of the animal (160,197) and this forms the basis for a method of bioassay for ricin; Fig. 4.9 .

The minimum lethal dose (Mld) for ricin was determined by a number of workers. Comparison is not readily possible owing to the different types of animals used, the route of injection of toxin, and the method of calculating the results. The two common methods of expressing the toxicity results are the Mld_{48} and the Mld_{50}; the former refers to the dose that causes death in 48 hours, while the latter specifies the dose that kills 50 percent of the animals tested. Other ways of expressing the toxicity may be encountered as can be seen from Table 4.7 which gives the toxicity results reported by various investigators. The table reveals the wide differences between the toxicity of the ricin fractions tested in animals, and it also shows mice and rabbits to be the two species most commonly used in animal experiments. It could be argued that the methods of preparing and purifying ricin affect the purity of the final product and hence its properties, especially toxicity. The isolation of different types of ricin from small and large castor oil seeds could offer a good explanation for the variations in the toxicity of the many ricin preparations studied.

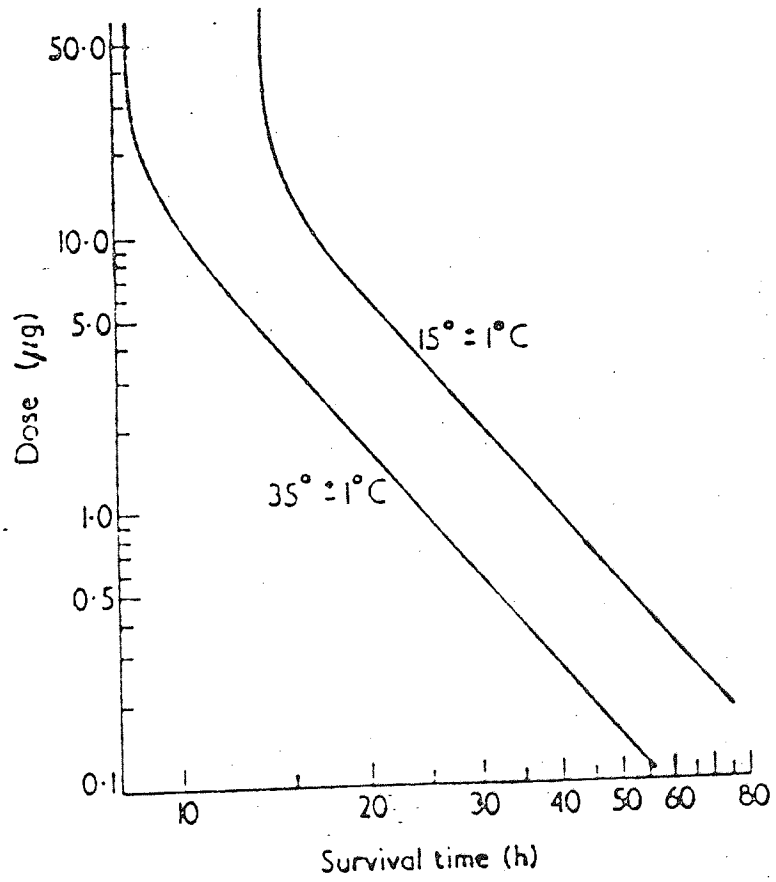


FIG. 4.9 DOSE-SURVIVAL TIME FOR RICIN

TABLE 4.7

Lethal Doses of Different Ricin Preparations

Investigator	Toxicity As Reported	Mg/kg	Animal	Route	Reference
Ehrlich	0.03 mg/kg	30			
Muller & Jacoby	0.5-0.6 mg				
Flexner	0.02-0.03 mg/kg; 6 wks.survival	20-30	guinea pig		Osborne (184) (1905)
Brieger	0.01 mg/kg	10	rabbit		
Osborne	mld 0.00/mg/kg	1	rabbit	subcutaneous	
Waller	5 mg/100g body wt. Crube 15 mg/100g body wt. Peak 1 9 mg/100g body wt. Peak 2 15 mg/100g body wt. Peak 1 3 mg/100g body wt. Peak 2	50 150 90 150 80	rat		Funatsu (181) (1972)
Funatsu et al	0.00 mgN/g body wt. (Ricin D)	6.25	mouse	intrapentoneal	
Cushny	40 mg/kg	40	rabbit		Balint (12) (1974)
Olsnes	0.065 mg LD_{50}		mouse	intravenous	Olsnes (183) (1977)

/Continued

TABLE 4.7 /continued

Investigator	Toxicity		Animal	Route	Reference
	As Reported	Mg/kg			
Tavasolan	0.001 mg/g MLD_{48}	1000	mouse		Tavasolan (337) (1978)
Kabat	Min.quantity to kill 20g mouse in 24h = 0.40 mgN	125	mouse	intrapentoneal	Kabat (185) (1947)
Kunitz and McDonald	0.015 mgN/g body wt. MLD_{48}	94	mouse		Funatsu (181) (1972)
Mosinger	10 mg/kg kills in less than 96h 7.2 mg/kg kills in less than 48h 1.5 mg/kg kills in less than 72h	10 7.2	mouse rat rabbit		Rao (22) (1970)
Funatsu	0.015 mg/g body wt. MLD_{42} crystalline ricin	15	mouse		
Flexner	0.0001 mg/kg	0.1	rabbit		
Fuhrman	0.032 mg/kg	0.032	mouse		Fuhrman (1967)

Equally at variance, and maybe for similar reasons, is the ricin content in the castor oil seeds. Thus Waller and Negi (98) report that their preparation of crude ricin amounted to 1% of the oil-free pomace, compared to a value of 1.5% found by Osborne et al. Funck (98) records a yield of 2.8-3.0% ricin in castor oil seeds, while Moriyama reports a yield of 0.5% (210). Olsnes (183) obtained 120 mg of pure ricin from 100 g of decorticated seeds. Ishiguro reports a crude ricin yield of 4.6% of defatted seeds (186) and a yield of 0.2% of defatted seeds for the purified ricin (206). It is clear that the degree of purity and the fraction isolated affect the values reported. It was also noted (96,98) that castor pomace from an industrial source contained less ricin than a laboratory prepared pomace, thus indicating a degree of detoxification due to processing.

The symptoms of intoxication in humans and animals are well documented (12) and so are the pathological changes revealed by the post mortem. In experimental animals, a lag time was observed between toxin injection and the appearance of symptoms. No antidote is known to ricin poisoning after the lag period has elapsed. Some inhibitors of ricin toxicity were listed by Corwin (160), who emphasized the critical importance of the order in which the toxin and the inhibitor are administered. Thus the inhibitor is effective only if administered before the toxin or before the symptons of intoxication set in. Ricin antiserum offers the best protection against the toxin,

followed by some amino acids and their derivatives. Balint's study of the therapy of ricin intoxication (211) was directed at influencing the factors that play a significant role in the process. He considers his most important conclusion to be the finding that the substances glutathione and glycine have very strong protective effects against ricin poisoning. Other chemicals and drugs were tested and the more promising among them are indicated.

4.6.4 Mechanism of Action

According to Olsnes (183), the inhibition by ricin of protein synthesis was first demonstrated by Lin et al (212) for cells in culture, and this mechanism was later confirmed by other workers (213,214,215,216). More was known about how the toxin exerts its effect after its structure was elucidated, and the functions of the two ricin chains are now known in great detail.

The toxicity of ricin towards intact cells requires the presence of the intact toxin, i.e. both chains are necessary for the biological activity of the protein. This was demonstrated by treating the toxin with 2-mercapto-ethanol which releases the A- and B- chains of ricin by reducing the disulphide bonds between them. It was found that the individual chains were far less toxic than the intact toxin, and when the 2-mercaptoethanol was removed and the chains reunited, the toxicity was fully restored.

Studies on the isolated chains revealed that the toxicity was associated with the A-chain only, and it was

thus evident that the B-chain must have an important function, since it was non toxic but must be present if the A-chain is to perform its toxic role. It was found that the B-chain binds the toxin molecule to cell surfaces, and that the binding is the first necessary step in the intoxication process. The B-chain is thus a lectin; it was demonstrated that ricin binds to free galactose or to lactose, and to galactose residues in sepharose 4B particles. The presence of any of these two sugars inhibits the binding of ricin to cell surfaces, and when this takes place the toxic action is strongly reduced.

Although the toxin is thought to bind to a carbohydrate moiety of the cell membrane, the exact structure of the receptor is not identified. Similarly, it is known that ricin attacks cancer cells but there is no known reason for the increased sensitivity of malignant tissues. One hypothesis is that the altered membrane properties of these cells may be responsible for their increased uptake of the toxin compared to normal cells.

The A-chain is an enzyme that is a potent inhibitor of protein synthesis. Its target of action is the ribosomal 60S subunit, and it functions by a catalytic mechanism such that one toxin molecule inactivates a large number of ribosomes (183,218). The inhibitory action of ricin is at the level of the peptide chain elongation; it happens in such a way that it prevents the efficient binding of elongation factor (EF-2) to the ribosomes. The result is that the activities of the ribosomes on GTP that depend

on EF-1 and EF-2 are impaired. The action of the toxin on ribosomes can be stopped at any time by adding the appropriate antiserum (183), and this is taken as a clear demonstration of the enzymatic nature of the A-chain.

After the ricin molecule is anchored to the cell surface by the B-chain, the A-chain must be released from the molecule before it can act on the cell. Evidence for this was obtained by treating a toxin molecule in such a way that the bonding between the chains became irreversible, whereupon the process of protein synthesis was not affected by the presence of the toxin (183). This fits well with the observation of a lag period between the administration of ricin and the manifestation of intoxication. It is not known whether the A-chain is liberated at the cell surface or inside the cell, but the former possibility is favoured by Olsnes (218) because the toxin is known to undergo considerable conformational changes in the presence of lactose; it is therefore possible that even greater changes take place at the receptor site to enable the free A-chain to enter the cell. This is further supported by the results of the immunological studies which indicate that the A-chain in particular undergoes conformational changes when it is liberated from the B-chain. In conclusion, it should be mentioned that a number of problems in this area are still unsolved, especially the mechanism of toxin uptake and the role of the receptor in this process.

Similar considerations apply to the castor agglutinin in that the heavy chains (B'-chains) are the ones used to attach the molecule to cell surfaces, while the A'-chains (light chains) are responsible for the agglutination associated with it.

4.7 DETOXIFICATION OF CASTOR BEAN AND CAKE

4.7.1 Introduction

Considerable attention has been paid to the question of detoxifying and deallergenizing the castor cake because of its potential as a source of nitrogen for fertilizers and in animal feed. The investigations were mostly carried out on the cake or extracts from it, but sometimes work was done using castor seed flakes. Because ricin was isolated and studied before the castor seed allergen, detoxification trials were mostly directed at removing the toxic effects of the cake. After the publication of Spies' pioneering work on the allergen (219), work on the castor pomace was extended to include the inactivation of the allergen.

The methods employed can be classified as follows:

1) Physical Methods
   - (a) Steaming, with or without adding chemicals
   - (b) Ultrasonics and ultraviolet radiation
2) Chemical Methods
   - (a) Organic Chemicals
   - (b) Inorganic Chemicals
3) Microbial and Enzymatic Methods

The choice of a particular method involves a number of factors, but the basis for all the methods is the relation between the biological function of a protein and its structure. Thus, a toxic protein like ricin has a unique three dimensional structure responsible for its toxicity, and if this structure is altered the protein loses part or all of its activity. The physical methods are expected to achieve the change in structure by raising the energy level of the molecules, thus causing an irreversible change in structure and function. Chemical detoxification seeks to react with the active sites of the toxin to deny them access to receptor sites in host cells. Microbial and enzymatic digestion hydrolyses the protein, which therefore loses its toxic effect. Since these methods were tried in the absence of a detailed knowledge about the structure of ricin and the allergen, the procedures chosen were those which would hydrolyse proteins, or those which were successful in detoxifying similar toxins. Even when the structure of a protein is known, it is still vital to know the reactive groups through which the protein exerts its function. Dasgupta and Sugiyama (220) have discussed the technique of selective modification of amino acid residues in the neurotoxins (e.g. tetanus and Botulinum). In order to determine the active residues, one or more kinds of amino acid residues are chemically treated and the number and type of residues affected are related to the changes in the three dimensional structure of the molecules and their biological activity. Work of a similar nature was

undertaken by Funatsu and his group (221) on ricin, but no such attempts are reported for the castor allergen.

A consideration of high significance in connection with the detoxification of castor cake is to ensure that the treatment to be implemented should not destroy the nutritive value of the pomace. For the results to be meaningful, the assay procedure has to be sensitive, specific, and reproducible. The difficulties associated with setting up such a procedure, the hazards involved in handling ricin and the allergen, together with the inedibility of castor oil are thought to be some of the reasons why published research in this area is not to the standard of that carried out on edible vegetable oils. This claim is substantiated by referring to the efforts made to elucidate the nature of the trypsin inhibitors and the flavour bodies in soybeans (222), the work on gossypol (10) and the aflatoxins (345), and the extensive research undertaken to verify the effect of erucic acid on health and how to lower its content in rapeseeds (54), these being some examples of the problems enjoying close scrutiny and concentrated endeavour for reaching quick solutions. It is hoped that chemical engineering research in this field will provide the necessary stimulation for improving the technology of castor oil extraction and producing better qualities of castor pomace.

4.7.2 Physical Methods

Since its discovery towards the end of the nineteenth century, ricin was known to be detoxified by heat treatment, and the early literature on the subject contains many references to articles and patents covering this method with modifications or additions. Thus, Nagel (223) boiled powdered castor pomace with 6-7 times its weight of 10% salt solution for 6-8 hours with constant stirring. The contents were then filter-pressed and the solids were washed with 10% salt solution until a sample showed no precipitation on heating. The resulting cake is reported to have been used as animal feed. Carmichael (178,224) boiled aqueous ricin solutions in order to confirm the results reported by others on the effect of boiling the toxin. According to him, the boiled solutions were non toxic, so that injecting a dose in rabbits of as many as 500 lethal doses did not harm them. Tangl (225) heated the pomace at 140^{o}C for 60-90 minutes, and the resulting feedstuff was well utilised by sheep. Rudolph (226) boiled aqueous solutions of the powdered cake, with frequent changes of the water. This was followed by hot water washing, filtering and drying, and the material so produced was said to have made a good animal fodder. The defatted castor meal was detoxified by Borchers (227) in flowing steam for 15 minutes, and 95% reduction of ricin was reported as measured by the hemagglutinating activity. No toxic symptoms were noted in chicks fed the detoxified

meal, but no improvement in growth took place over the controls fed untreated meal. When the pomace was treated as before and then treated with 10 volumes of 1% salt solution, stirred for 3 hours, filtered, and dried, no hemagglutinating activity was noted, but growth of chicken fed the treated cake was only half that of the controls fed the basal ration. An extensive study on the detoxification of castor pomace was undertaken by Kodras (229) who investigated various combinations of steam pressure and residence time, as well as chemical methods, and tested the products on rats and chicks. His results (228) are given in Table 4.8 . He found the toxicity of solvent-extracted castor pomace to be higher than that of expeller castor pomace. He also reported that essentially complete detoxification of ricin with minimal damage to the nutritive value of the pomace was achieved by autoclaving for 15 minutes at 125°C (20 psi steam). To resolve the contradiction between Borchers' results and those reported by Kodras, Young (230) extended the work of the latter with a view to determining whether there was a second substance in the castor cake that is stable to boiling ethanol and which depresses growth in chicken. He confirmed the findings of Kodras and found no evidence to support the reports of Borchers. Castor pomace suitable for feeding guinea pigs was produced by Petrosyan and Ponomorov (231) by boiling the toxic cake for 1-2 hours. Jenkins (232) heated castor pomace in 12 psi steam for 15 and 120 minutes. From the various pressure-time combinations employed, he

TABLE 4.8

A Summary of Kodras' Experiments on Castor Pomace Detoxification

Type of Pomace	Treatment	Result of Feeding	% Pomace in Feeding	Animal	
				Number	Species
Expeller Pomace	4 psi steam (30 min)	All survived			
	20 psi steam (15 min)	All survived			
	20 psi steam (60 min)	All survived			
Solvent Extraction	20 psi steam (5 min)	2 died in 10 days; 1 survived	5%		
	20 psi steam (10 min)	1 died in 18 days; 2 survived			
	20 psi steam (20 min)	All survived			
	(30 min)	All survived			
	(60 min)	All survived			
	20 psi steam 12.5g pomace+7ml H_2O; 15 min	All survived			Rat
	12.5g pomace+20ml H_2O;60 min	All survived			
	12.5g pomace+30ml H_2O;60 min	All survived			
	0.2N Hcl overnight, then neutralize	All survived			
	0.2N NaOh overnight, then neutralize	All survived			
	3% H_2O_2 overnight	All survived			
	Dry Heat(140°;30min)	All died in 10 days	10%		
	Excess H_2O(80°C; 30min)	2 died in 8 days; others in 11			
	Excess H_2O (80°C; 60 min)	All survived			

/continued.

TABLE 4.8 /continued

Type of Pomace	Treatment	Result of Feeding	% Pomace in Feeding	Animal	
				Number	Species
Solvent Extraction	20 psi steam; 10 min	All survived			Chick
	30 min	All survived			
	60 min	All survived			

concluded that heating for 1 hour in 15 psi steam virtually destroys ricin. The feeding experiments conducted indicated that the growth rate and food conversion in rats fed a diet containing 23.2% of autoclaved castor meal (10 psi for 1½h) were significantly lower than with control animals.

Cooking the unextracted castor seed flakes and the castor pomace under different conditions of temperature and moisutre constituted one group of the experiments reported by Gardner et al in an effort to find a promising type of treatment for detoxifying castor seeds or cake (96). They found that dry heating of pomace at 205°C for 125 minutes was an acceptable method, but it caused the meal to be charred, indicating extensive heat damage. Earlier, Kodras (229) reported that heating the pomace spread in layers one inch in depth at 125°C in a steam autoclave for 15 or more minutes destroyed ricin, as was proved by animal experiments. The autoclaved pomace was friable and could readily be incorporated in animal rations. Kodras also reported that dry heating of the solvent extracted pomace at 140°C for 30 minutes was inadequate for detoxification because all the rats tested with it died in 10 days (228).

Organic or inorganic chemicals may be added to the pomace before steam treatment. Alternatively, the pomace may be extracted with sodium chloride solutions or other extractants, followed by steam treatment. Thus, Massart (233) extracted the castor oil cake with a solution

containing alkali halides and alkali hydroxides. The cake was subsequently treated with steam in an autoclave. Alkaline treatment of the pomace was also patented by Thörl who treated the cake with alkali solutions of at least 0.8% strength calculated as NaOH. After acid neutralization, the extracted residue may be used as fodder (234). Gardner et al (96) extended the alkali treatment to castor seed flakes during the cooking operation. They added chemicals such as sodium hypochlorite, sodium hydroxide, formaldehyde, sodium chloride, and hydrochloric acid. Their conclusion is that mild moist cooking of flaked meats can completely detoxify ricin, in the presence or absence of chemicals.

The other major physical method attempted for detoxifying ricin is the use of ultraviolet radiation. Carmichael (224) reported the work of Dreyer and Hansen (235) who irradiated ricin with ultraviolet rays and caused it to lose its agglutinating property. Carmichael investigated the effect of ultraviolet radiation on the toxicity or ricin, and for this purpose he employed aqueous ricin solutions and mercury vapour lamp operating at a voltage of 70 volts and using a current of about 4 amperes. The solutions treated were in 10 cc. quantities and were placed in open petri dishes 25 cm. away from the lamp. An exposure of one minute under these conditions was sufficient to inactivate the toxin so that two lethal doses injected into mice did not cause any symptoms of intoxidation. Three lethal doses of solutions treated as above for five minutes did not cause ulcers or

sloughing off of the skin when they were injected into mice.

After proving that the rays from the mercury vapour lamp detoxified ricin solutions, Carmichael set out to find which wave lengths were responsible for this activity. He passed the rays through filters and the wavelengths of the filtered rays ranged from 203 to 275 pico metres. Solutions of ricin concentrations 0.02 mg/cc were placed in glass dishes enclosed in brass cylinders with a brass lid which had an opening for passing the radiation to the ricin solutions. A cylinder closed on one end by a quartz plate was placed on top of the brass cylinder, the quartz end downward, and served as a container for the filters. The solutions were agitated every few minutes during the treatment, and the effect on ricin was assayed by injecting the resulting solutions into mice. Carmichael concluded that ultraviolet light with wave lengths from 225 to 254 pm was quite effective in detoxifying ricin.

Balint (236) attempted to detoxify ricin by means of ultraviolet radiation. Ricin solutions were placed in open shallow glass vessels and the rays were produced by a low-powered table sunlamp fixed at a height of 50 cm. above 1% solutions. The effect of exposure was monitored on the absorption curve from a spectrophotometer. The non-irradiated toxin showed an absorption peak at 280 nm, while the toxin irradiated for 90 minutes showed a peak at the same frequency which was considerably lower. After

240 minutes of irradiation, this secondary peak was no more detectable. Since an absorption peak at 280 nm is characteristic of a protein, its disappearance would mean that a major change has taken place in the molecule being considered. Balint did not dismiss the alternative possibility, namely that the two chains of ricin may have been uncoupled as a result of the treatment. Animal experiments using the irradiated toxins confirmed that they have lost their biological activity and could be tolerated by mice when 30-50 lethal doses were injected into them.

The work of Harvey and Loomis (237) on luminuous bacteria revealed that these microorganisms could be inactivated by exposure to supersonic waves for 30 or more minutes. Nakahara and Kobayashi (238) proved that a mere 2 minutes exposure to supersonic waves of 530,000sec^{-1} was sufficient to inactivate a purified vaccine. Balint (12) reported the attempts made by Tachibana (329) to deactivate ricin by ultrasonic waves.

4.7.3 Chemical Methods

4.7.3.1 Organic Chemicals

A number of organic chemicals were employed to detoxify ricin. Since the early 1920's, the discovery was made that diphtheria toxin could be completely inactivated by low concentrations of formaldehyde (239), and similar action for this chemical was noted on other toxic proteins (298). The striking difference between

ricin and the other toxic proteins mentioned is that the former is not completely detoxified when treated with formaldehyde, but a 1000 fold reduction in toxicity takes place. Balint (236) could not confirm the claim by Meyer and his coworkers that ricin, like all proteins, could be detoxified with formaldehyde.

The list of organic chemicals tested for inactivating ricin includes aldehydes, benzene derivatives, acids, and a variety of organic salts and other organic compounds such as dyes. Thus Balint (12) refers to the experiments of Ohya who tried to influence the toxicity of ricin by treating it with aldehydes and benzene derivatives. According to Kraus (187), ricin shows a remarkable stability towards oxidizing and reducing agents, but this stability is affected by pH changes or the presence of halogens. The observations made by Carmichael (224) that oxidizing agents readily inactivate ricin seem to contradict the statement of Kraus. Olsnes (183) also made a remark similar to Carmichael's. The stability of ricin over a wide range of pH was reported by Olsnes and Pihl (197) and by Taira et al (221). Hence, it may be that the combined action of oxidizing agents and pH change was responsible for Kraus's results for he succeeded in detoxifying ricin completely in 24 hours with benzo-quinone especially at pH 5.4-7.9, but he failed to inactivate the toxin by treating it with maleic acid. Again a seemingly different result was obtained by Funatsu et al (240) who reported that the maleylation of ricin D

with maleic anhydride almost abolished its toxic and cytoagglutinating activities.

Moriyama detoxified ricin using phenylhydrazine, potassium cyanide, and semicarbazide (187). Carmichael (241) reported that sodium ricineolate inactivated the toxin. Kraus, basing his work on reports that deficiency in vitamin C increased the susceptibility of guinea pigs to diphtheria toxin, attempted to modify the activity of ricin with ascorbic acid but the result was negative. He obtained similar results with methylnaphthaquinone which was administered to animals in the hope that it would counteract the hemorraghic effects of the toxin.

Grabar and Koutseff (242) and Kraus (187) noted that ricin was considerably detoxified when treated with ethyl alcohol. Kraus also reported the detoxification of ricin picrate with aqueous solutions of acetone and of dioxane. Ambekar and Dole (243) studied the effect of extracting castor oil by various organic solvents and they concluded that ethyl alcohol and chloroform diminished the toxicity of the resulting cake, while hydrocarbons such as n-hexane and benzene do not seem to affect the level of the toxin in the cake. They also treated the castor cake with chloroform and ethyl alcohol and succeeded in bringing about its detoxification. Extracting the cake with hot heptane did not bring about appreciable detoxification, but toluene treatment holds some promise for detoxifying ricin.

Natural products were also investigated as possible detoxifying agents. Because of the known effects of ricin on the liver, Clementi and De Gaetani studied the action of the bile on ricin. The precipitate resulting from the addition of 6% bile to acidified ricin was centrifugated from the supernatant, and both phases were injected in guinea pigs. The animals receiving the redissolved precipiate died, indicating that the toxin, or part of it, was carried down by the action of bile.(244) Carmichael (224) and Kraus (187) acted with dyes on ricin; the former employed congo red, an acid stain, and neutral red, a basic stain, while the latter applied methylene blue at pH 5.1. Congo red had a weak detoxifying action, but the other two failed to achieve any detoxification.

4.7.3.2 Inorganic Chemicals

The work of Carmichael (178,224) was the first systematic effort for detoxifying ricin by means of various agencies. He found that all the oxidizing agents tested destroyed ricin's activity, but at different rates. Thus, with potassium permanganate the reaction was very fast and proceeded to completion, while hydrogen peroxide solution was slow in deactivating the toxin. Delga (245) also detoxified ricin with dilute potassium permanganate. It is interesting to note that Carmichael detoxified ricin using a 30% hydrogen peroxide solution, whereas Boquet (246) achieved its rapid detoxification with a 1% hydrogen peroxide solution in the presence of copper sulphate (1 mg/l) and later (247) in the presence of nickel, manganese, or iron (as sulphates). Kodras (229) incubated castor cake suspensions in water at room temperature with 3% hydrogen peroxide. All the rats fed this product survived, indicating that detoxification occurred. Corwin et al (248) also reported that ricin's toxicity was readily destroyed with 3% hydrogen peroxide solution.

The toxicity of ricin solutions was abolished by treatment with ozone but the method was found more difficult to control than the permanganate method because of the number of parameters involved, namely the percentage of ozone, its solubility, the temperature of the test, and the duration of treatment (224).

The halogens were investigated as possible detoxifying agents for ricin, and a wide variation in their behaviour was noted. Thus, Carmichael (224) inactivated ricin solutions using chlorine water, but success with chlorine gas depended upon the concentration of the toxin in solution and on the duration of the treatment. He also achieved detoxification with bromine water and bromine gas. Moriyama (249) also detoxified ricin preparations with bromine water. As for iodine, Carmichael reported that its solution destroyed the toxic action of ricin. Avery (250) reversed the detoxifying effect of iodine on ricin by the addition of sodium thiosulphate solution, whereupon part or all of the iodine was removed and a partial restoration of toxicity took place. Kraus (187) suggested the ricin-iodine reaction as a specific test for the toxin to replace animal experiments. The chief difficulty he mentions is the problem of reactivating the precipitate so formed; he did not attempt the thiosulphate method of reactivation because of the poor results reported by previous workers. Clark and Clarke (251) found that iodine destroys the lethal factor in ricin, the toxicity being partially restored by the subsequent action of sodium thiosulphate. Delga (245) succeeded in detoxifying ricin with dilute iodine solutions but there was no mention for toxicity reversal. Funatsu (181) reported that the iodination of ricin at pH 7.0 and $0^{o}C$ resulted in the complete loss of toxicity. Olsnes and Pihl (197) confirmed the results of Lin et al (252) who

detoxified abrin and ricin with periodate, but they stated that this happened only at high concentrations of this reagent.

Kabat and Mayer (298) stated that dilute sodium hypochlorite solutions readily inactivate ricin, but Delga (245) earlier had failed to achieve a detoxification with this chemical. Unlike the already-mentioned oxidizing agents, ferric chloride at pH 1.1 and ferricyanide (pH 7-9; 3.03) had no effect on ricin (187).

Acids, alkalis and salts were also evaluated for their action on ricin. Kodras et al (229) and Ambekar and Dole (243) incubated castor cake in 0.2 N HCl for 24 hours. Ambekar and Dole washed the treated cake with water to remove the acid, but no such treatment was reported by Kodras et al who obtained positive results since all the rats fed the end product survived. As for Ambekar and Dole, some of the pomaces treated were completely detoxified but some were not, thus indicating differences due to variations in processing techniques. Kodras et al obtained similar results with 0.2N NaOH, but some alkali soap was formed and an emulsion produced during the course of the treatment. Salts such as sodium chloride, sodium sulphate, and ammonium sulphate did not detoxify castor cake, but disodium hydrogen phosphate was effective in rendering the cake harmless as was proved by feeding experiments (243).

4.7.4 Enzymatic and Microbial Methods

Proteins are hydrolysed by enzymes and some strains of bacteria. After the discovery of ricin, extensive work was carried out to prove its proteinaceous nature, and part of this was to digest it with proteolytic enzymes. Thus, Müller (12) experimented with pepsin on ricin and noted only a small decrease in the toxicity of the final product. Tryptic digestion of ricin was attempted by Jacoby (224) but there was no loss of toxicity, and he concluded that the toxin was not a protein. The classic work of Osborne et al (184) resulted in very toxic ricin preparations and they were able to hydrolyse the toxin by tryptic as well as peptic digestion. The toxin lost its toxicity with the progress of hydrolysis, and similar results were obtained by Karrer (224). Olsnes et al (199) found that pronase in high concentrations was the only proteolytic enzyme which had some effect on ricin. Partial hydrolysis of ricin by nagarase was noted by Funatsu et al (115), but they could not digest the toxin with pepsin or trypsin. Kobert (12) demonstrated that trypsin does not decrease the toxicity of ricin, and Schone (12) confirmed that trypsin, pepsin, or papain do not affect the toxicity of ricin.

These conflicting reports may be attributed to the variations in the ricin preparations tested and the conditions under which the treatments were made. In fact the

detoxification of castor pomace with proteolytic enzymes was patented (253), and it is claimed that the resulting product is suitable for human or animal consumption. In his procedure, Darzins defatted the castor seeds and processed the cake to obtain dry flour. Water was added to make a solid:liquid ratio of 1:3 and the slurry was agitated for three hours, after which the water was drained. In the second mixing, the wet flour was suspended in water in the same ratio as before and the bacteria was added at the level of 20 mls of culture for every 4-5 kg of meal. The bacteria was of the clostridium strain from sewage. The contents were incubated for 72 hours at 20-45^{o}C and a pH of 7.5-9.0. Calcium hydroxide at 0.5-1.0% (by weight) of the charge enabled pH regulation without soap formation. At the end of the incubation period, the digested mass was autoclaved for 1 hour at 100-120^{o}C and dried. The final product, containing 32% of non-toxic semi-digested boiled proteins, was reported as a high-value feed for both mammals and fowls.

Zimmerman (2) reported a private communication to the effect that the Germans developed detoxification methods for castor cake which enabled them in World War II to use the pomace in a variety of foods and feeds. The source says that this was verified at the close of the war by a committee of the Joint Chiefs of Staff, and that the products included soy sauce, bouillon cubes, and soup stock. As there is no mention for the method of treatment adopted, it may be possible to link this to the type of process described above.

4.8 THE CASTOR BEAN ALLERGENS

4.8.1 General

The castor bean allergens belong to a family of low molecular weight glycoproteins that have the ability to generate reactions in susceptible individuals, leading to skin eruptions or respiratory symptons. Early reports on the castor seed attributed its allergy to ricin, but it was later established that the toxin is not responsible for the allergenic reactions caused by the seeds or the pomace.

The term "allergen" refers to a substance present in the diet or the environment that is ordinarily harmless but is capable of causing such diseases as asthma, hay fever, eczema, and gastrointestinal upsets upon contact with a previously-sensitized individual (254). Three types of allergy can be distinguished: atopic, delayed and the an anaphylactic type.

In the first type, also known as the immediate-type allergy, the patient manifests his responses immediately after exposure to the offending agent, usually from a few minutes up to sixty minutes. Delayed allergy, on the other hand, sets in from a few hours to ninety six hours after exposure. Anaphylaxis, which may be systemic or local, occurs in seconds to minutes following the exposure. The reactions to this type are usually violent, sometimes fatal.

The initiation of atopic allergy is due to a specific reaction between the allergen and reagin, also called immunoglobulin E (lgE), while delayed allergy is initiated by the interaction of the allergen with the small lymphocytes. Anaphylactic allergy is triggered by the specific reaction of the allergen with immunoglobulin G(lgG) or lgE.

Allergy to the castor seed and the other allergenic oilseeds can be classified under the immediate-type hypersensitivity, in which the combination of the antigen with lgE leads to the release of the so called mediators, the most significant among which is histamine. These substances increase capillary permeability, thus causing increased nasal discharge; they also cause the contraction of the smooth muscles which results in constricting the breathing passages, this in turn leading to wheezing and asthma attacks (175,255).

The allergenic nature of the castor bean and castor pomace was known before the First World War (42), but it was not until later that methods for the preparation of the allergen were reported (42). Many oilseeds, e.g. cottonseeds and flaxseeds, have always been associated with allergy, and efforts were therefore directed towards devising methods for extracting, purifying, and characterizing allergens in order to be able to design processes for their inactivation. Work was started in 1939 at the U.S. Department of Agriculture, Washington, D.C. by J.R. Spies and his coworkers on the allergens from cottonseeds,

castor beans, and other oilseeds. Another group led by Layton later tackled this problem in Albany, California, with emphasis on the clinical aspects. The chemistry of atopic allergens and the work of these two American groups was discussed by Berrens (256). Recently, Lehrer and his associates (46,257) started reinvestigating the castor bean allergens. Analytical work on the antibodies against the castor bean allergens was also recently reported by Coombs and coworkers (258).

These efforts supplement the endeavour made by industry for deactivating the allergens in castor pomace. In the following sections a discussion will be made of the aspects outlined above.

4.8.2 Preparation, Purification, and Composition

The castor bean allergen was first prepared by Grabar and Koutseff (259). Spies et al (260) prepared the cotton-seed allergen by what became known as the CS-1 method, and a similar procedure was adopted by them for preparing the castor bean allergen which they coded as CB-1A (219). Starting with defatted castor seeds, they devised a method-ology for extracting CB-1A based on the premise that it was:

a) water soluble

b) stable to boiling water

c) not precipitated by basic lead acetate

d) soluble in 25% ethanol

e) insoluble in 75% ethanol

The procedure is outlined in the flowsheet shown in Fig. 4.10.

Clarke (261) reported a method for preparing small quantities of the allergen. His procedure, like that of Spies et al, Grabar and Koutseff, and Layton et al (262), assumes that the allergen is stable to boiling water and can be precipitated by alcohol. His method is summarized in the flowsheet of Fig.4.11. The presence of ricin in the castor bean made it necessary to boil the allergen preparations so as to destroy the toxin, and hence only the heat-stable allergens were actually investigated. This was pointed out by Lehrer et al (257) who explored the possibility of the existence of heat-labile as well as heat-stable castor bean allergens. They extracted the raw castor beans with phosphate-buffered saline at 24^{o}C and manipulated the extracts to recover the allergens. Earlier, Waller and Negi (98) reported the extraction of ricin and the allergen from the same lot of castor cake, i.e. ricin was not destroyed before the extraction of the allergen. If ricin must be detoxified first, a procedure other than boiling may have to be followed so as to preserve the heat labile allergens.

The chemical and immunological properties of CB-1A were determined (219). The allergen is a glycoprotein, i.e. a protein with an attached carbohydrate moiety. It has a low molecular weight and represents 1.8% of the defatted castor cake. Its nitrogen content is 18.3%, and

Fig 4.10 Procedure for the Isolation of the Castor Bean Allergen (219)

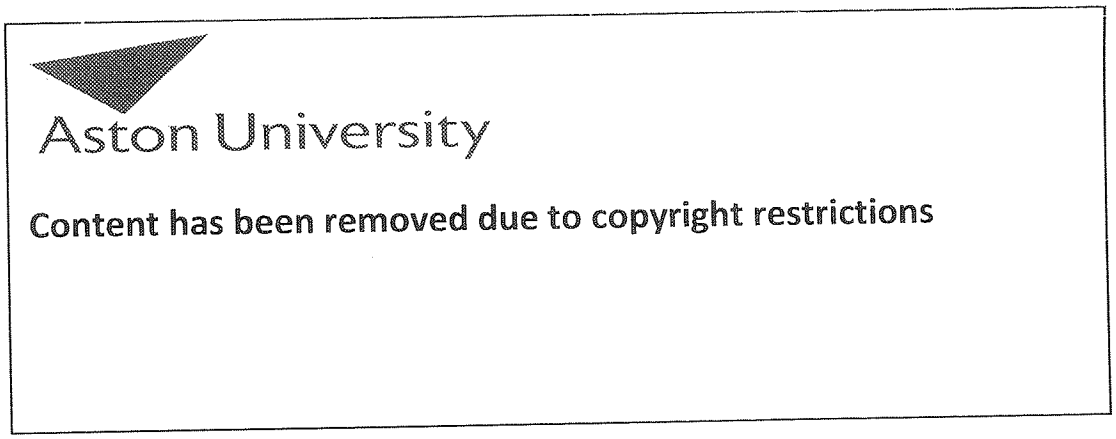


Fig4.11 The Clarke Procedure for Preparing the Castor Bean Allergen (261)

it contains 3.12% carbohydrate. To obtain a carbohydrate-free product, Spies et al (263) used high-voltage electrophoresis. Their intermediate fraction, CB-60C, contained 0.47% carbohydrate and the final fraction had a lower content of 0.11%, and they concluded that the allergenic properties of CB-1A reside in its protein fraction. The role of the carbohydrate portion lies in that it influences the ability of the allergen to induce antibody formation. The amino acid composition of CB-1A is given in Table 4.9.

The allergens isolated from castor beans grown in America and beans imported from Brazil showed close similarity in their chemical properties and had essentially identical immunological characteristics (264). The Brazilian allergen was isolated from the press cake as received, i.e. without solvent extraction, and the yield was 0.45% of the pomace by weight.

Spies and Coulson (219) reported that the castor allergen was non-toxic: guinea pigs were injected with CB-1A in such quantities that the amount given per kilogram of body weight represented about 360 times the lethal dose. The animals were observed for 21 days without ill effects. However, the injection of CB-1A sensitized the animals, which experienced anaphylaxis when challenged with a dose of the allergen. The sensitizing dose of CB-1A nitrogen in pigs was 8.4 µg and the minimum shocking dose was approximately 0.33 µg per guinea pig.

TABLE 4.9

The amino acid composition of the castor bean allergen CB-1A

(% of total nitrogen)

Amino Acid	%
Humin	0.1
Ammonia	13.6
Cystine	5.0
Histidine	1.0
Arginine	26.6
Lysine	3.2
Glutamic acid	8.6
Tyrosine	1.1
Tryptophan	0.0
Mono Amino Fraction	19.3
Dicarboxylic acid Fraction	8.6

The fractionation of CB-1A by column chromatography was reported by Layton et al (265). Antigenic differences between the resulting fractions were detected, and six or more antigenic components were thought to be present. Some of the allergenic factors in CB-1A were found in castor pollen and blossoms. Some of the chromatographic fractions were electrophoretically heterogeneous, and hence electrophoretic fractionation of the antigenic proteins was performed with buffers of different chemical composition, pH values, and ionic strength (262). A sharp resolution of the proteins was obtained with a phosphate buffer at pH 7.4 to 8.0 and an ionic strength of approximately 0.05. The main allergen, CB-1A, was resolved into six or more components at pH 8.0. Each major band was found to exhibit allergic symptons in sensitized guinea pigs, while only five of those bands were shown to be allergenic to humans. These results seem to strengthen the views expressed earlier by Layton et al (265) that allergy to the castor seed proteins may be caused by more than one antigen in the cake.

Lehrer et al (257) proved the existence of heat-labile castor allergens which were of higher molecular weight than the heat-stable allergens. Immunologically, the two types were different, and the heat-stable fraction was the more potent of the two. The authors also raised interesting questions about the relation between ricin and the allergen, since lethality by toxicity was detected in some fractions

of the latter.

4.8.3 Sensitivity to Castor-Bean Dust

The allergenic properties of the castor bean were discovered in the course of studying the toxicity of ricin, and this may explain why some of the early investigators, e.g. Alilaire, attributed the castor allergy to ricin. The reports on the subject range from the effects of ingesting a single bean to the occupational hazards and community illnesses caused by the castor bean dust. Considerable information on the health aspects of the castor bean dust is to be found in the work of Apen et al (42).

Allergy to castor bean dust in humans was first described by Borchardt and Alilaire in 1914 (266), who reported their own experiences of sensitivity to this material. Before that, Schern had already shown the existence of an allergenic substance in castor pomace by demonstrating its effect on guinea pigs (267).

Ratner (268) reviewed the literature on the adverse reactions to the castor bean and the castor pomace. The cases he discussed were mostly concerned with allergy to castor cake, and the individuals affected had a history of working either with the castor bean or its products. However, there were cases where the reaction took place upon ingestion of a single bean (269). Even those who were not immediately sensitized, developed the symptoms

after a long incubation period, may be years. Thus, Ratner describes a case reported by Bernton (270) of an individual who worked in a laboratory of the US Department of Agriculture for three years without a health complaint, but after that time developed symptoms which were later proved to be due to the castor bean. The experience of Snell (271) is also similar, as he handled the castor bean for a long time without ill effects, but was later affected so that a hay fever attack would seize him whenever he came in contact with the beans. Arnold (272) reported the incident when a soldier ate five castor beans and developed acute anaphylactic symptons a few hours later; he died the following day in a coma. Berto and Bassi (273) mentioned the case of an assistant in Jacoby's laboratory who continuously helped Ehrlich in his work on ricin and who exhibited asthmatic symptoms twenty years later whenever he inhaled any castor bean dust.

Allergy to the castor bean lipase was reported by Follweiler and Haley (274), the symptons being those of severe cold with violent sneezing and coughing, together with other complaints. The patient was unaware of the cause of his ailment and his condition steadily deteriorated until his problem was correlated to the castor bean, the removal of which from the work place alleviated his symptons. It is interesting to note that Layton et al (262) fractionated the water-soluble components of castor bean lipase electrophoretically and the resulting electrophoretograms suggested that the lipase was qualitatively very similar to their crude castor protein preparations, each of the various components

of which was found to possess allergenic properties. This may explain why the lipase elicited such strong reactions in the patient mentioned.

4.8.4 Occupational Hazards

The potency of the castor bean allergen makes the pomace a hazardous substance to handle and work with. A large number of reports on cases of asthma in the castor oil industry and their communities were discussed by Apen et al (42). Here, only the major incidents on occupational hazards and community illnesses will be mentioned.

The occupational illnesses reports came from oil mills, fertilizer factories, Castor bean farms, seaports, and railway stations. The frequency of occurrence of casualties in areas where the intact beans were handled is rare compared to cases where the pomace is the commodity in question. Moreover, the pomace from an oil mill where the oil is obtained by mechanical pressure alone is less hazardous from an allergy point of view than the pomace produced by solvent extraction. The main reason is that the dust from the solvent-extracted pomace is very fine and is easily transported by the wind. In contrast, the oil pomace has larger particles and is not so easily dispersed. The importance of particle size in relation to the effects of the pomace has been emphasized by Corwin et al (275).

Berto and Bassi (273) described 16 cases of what they called "ricinus asthma". Skin tests were performed with castor pollen, pomace, and leaves. The patients came from

an area where castor beans were extensively grown. The authors also described an epidemic asthma in 13 individuals living in a region where soap was made out of castor oil. Although the authors consider the reactions as ones of allergy, no allergen was prepared for tests; instead, ricin was prepared and patients were tested with it. No cases of allergy were detected in the factory that produced the oil, a result attributed to the mechanical method which leaves some oil in the cake, thus making it less hazardous.

Bronchial asthma due to castor bean powder was reported by Lucchese (276) in 11 workers in a factory which used solvent extraction to remove the residual oil from castor pomace. Rejsek (277) also described 32 cases of allergy to the castor bean and castor pomace.

Outbreaks of illness among American seaport workers handling castor pomace were described by Cooper et al (278). Based on the reported symptoms, the authors' opinion is that some of the patients had experienced toxic effects from ricin in the pomace shipments. The presence of the toxin was confirmed with bioassays.

Panzani (279) discussed the illness caused among dockworkers in Marseilles, France, as a result of handling castor pomace. 102 patients living in the vicinity of castor oil mills in the city or affected by the pollution by castor dust from the docks were examined. Asthma was the dominant manifestation of allergy, and 25% of the cases

were accompanied by urticaria. Panzani and Layton (143) later described 478 cases in the Marseilles area, again mostly asthmatics. This large number of people affected by castor allergy indicated the need for tightening control measures in loading and unloading the pomace.

Topping et al (280) reported what they described as the first case of castor allergy among the British population. The case studied was that of workers in the upholstery department of a furniture factor where ten out of the fifty six workers complained of persistent symptoms that were suspected to arise from a common offending agent. The materials handled by the workers were screened to find a possible source of the allergy, and the most probable material was thought to be felt. RAST and RAST inhibition assays were made on felt extracts, as well as prick tests. The sera from some of the affected workers gave reactions to extracts from fresh felt different from those made of stored felt. This observation suggested that a contaminant may be responsible for the reactions, and upon enquiry it was found that the felt was prepared partly from sacks that previously contained castor beans. Extracts from the beans gave positive reactions to the patients' sera, thus supporting the diagnosis of a castor allergy.

This case shows the potency of the castor allergen and ease with which it could reach workers not engaged in an industry related to castor. It also shows the value of the

RAST assays for deciding the source of an allergy, but it must be stressed that this depends on making the correct diagnosis.

4.8.5 Community Illness

The Marseilles area allergy reported by Panzani and Layton (156) appears to be the largest of the cases described in the literature. Epidemics of asthma were described, on smaller scale, in Toledo, Ohio (USA), Bauru (Brazil), and other countries (42).

Figley and Elrod (281) in 1928 reported the occurrence of asthma in the residents near a castor oil mill in Toledo, Ohio, USA. The 85 persons affected lived within a mile radius of the mill, and they attributed their symptoms to castor pomace. The 30 individuals examined by the authors experienced the attacks from one to seventeen years after moving into the area. Relief from their complaints occurred when they left the area or when the factory was not emitting dust clouds.

A collective asthma hit the city of Bauru in Sao Paulo, Brazil in 1952. In a few days, 150 cases were registered, with 9 deaths attributed to the illness (282,283). The source of the offending substances was traced to a mill that had started the solvent extraction of the castor oil cake. Cutaneous tests were carried out and the symptoms were

experimentally reproduced. The epidemic declined after the temporary closure of the factory, but it was restored when the factory reopened a month later. With pressure from the population, the factory was ordered to close until it has improved its method of operation. This it did by trapping the allergenic dust in water before venting it to the atmosphere, and after a year from the second closure it was back in operation, apparently with no complaints from the community.

Mendes (282) also reported a similar incident that happened in Ourinhos, Sao Paulo (Brazil) in 1964 and affected approximately 100 persons. Sixty five patients were examined for clinical history and through immunological tests. The castor cake processing responsible for the epidemic was stopped. A follow-up study was undertaken four years later on seventeen persons who continued to live in the same neighbourhood. The result showed that 80% of the asthmatic persons became symptom-free as soon as the industrial process was stopped. This demonstrated the effectiveness of the control measures. Most individuals retained their hypersenstivity to the dust: 70% of the patients gave positive skin tests.

Miskolczy (284) described mass allergy connected with the manufacture of castor oil in Hungary. The 45 cases reported came from the vicinity of a factory where the process employed generated a great amount of dust, and many of the factory workers became ill. More than half the

cases were asthma, a quarter were skin rashes, and the rest other allergic complaints.

In the Sudan, the castor oil factory serving the main cultivation area of the crop in Eastern Sudan was closed in the sixties after outbreaks of allergic reactions in the area. The crop remains to be grown, and cases of allergy are known in the docks in the seaport of Portsudan on the Red Sea. To the author's knowledge, there is no documentation of these cases or of the outbreaks.

4.8.6 Allergy to Castor Oil Products

Reports on allergy to the castor bean were almost exclusively confined to its constituents other than the castor oil. The latter has always been considered non-allergenic, non-toxic, and safe to take (42), as is evident from its use as a cathartic agent. There is only one reported case of sensitivity to castor oil (42), but recently adverse reactions were reported following the administration of intravenous induction drugs containing castor oil. The two drugs that received increased attention in this respect are althesin and propanidid, both solubilized in Cremophor EL (20% polyoxyethylated castor oil). The drugs, especially, althesin, found, good acceptance as reliable anaesthetic agents, but the increased frequency of the reactions to them (45,285,286,287,288) led to investigating the role of the solvent in these phenomena. Thus, Hacker et al (289) found that Cremophor induced alterations in serum proteins, and they questioned its long-term safety.

Glen et al (287) stated that frequent reactions accompany the second administration of Cremophor EL, and they concluded that this solvent was responsible for at least a portion of the reactions associated with the drugs. The possibility of the involvement of Cremophor in these reactions was also mentioned by Clarke et al (45), and the possible reasons for the reactions were discussed by Watkins et al (288,290). There is no mention, however, for tests conducted on the sensitive patients with pure castor oil, nor was there an enquiry into the industrial process by which the polyoxyethylated castor oil was prepared.

Although these reactions do not necessarily lead to the conclusion that castor oil is allergenic, the situation is made more difficult by the findings of Lehrer et al (46) who detected allergens in castor wax. Since the wax was prepared by hydrogenating castor oil, the implication is that the allergens were already in the oil. Again the method by which the oil was produced is not mentioned, and it seems that to obtain conclusive results more careful and widespread checks are needed, especially that no allergens were detected in a deodorant utilizing castor wax (46).

4.8.7 Symptoms

The clinical pattern in individuals sensitized by the castor pomace is characterized by maniifestation of asthma, rhinitis, urticaria and allergic conjunctivitus. Itching and tearing of the eyes and continuous violent sneezing may

precede the actual attack, which itself could develop into dyspnea. The respiratory complaints may or may not be accompanied by the skin reactions. In an outbreak of community illness caused by the pomace, the symptoms may be confused with those of other illnesses (278). The correct diagnosis rests upon suspecting the castor bean as being a factor in the epidemic. Confirmation may then be obtained by cutaneous tests and other procedures (282).

The exposure of non-immunized individuals to heavy clouds of partially detoxified pomace can generate symptoms of ricin poisoning such as chills, fever, headache, abdominal pain, and vomitting (278). These may well be accompanied by symptoms of the allergic response. The main difference between these two types of reactions to castor pomace is that the former is delayed, whereas the latter occurs immediately in sensitized individuals (268).

4.8.8 Detection and Assay

The presence of the castor bean allergen can be determined qualitatively and quantitatively by in vivo or in vitro techniques. Animal experiments fall in the first category; to prove the presence of castor antigens, the animals are sensitized with test material, allowed a suitable incubation period, and are later challenged with the same material. Allergic responses to the second injection mean that the antigen was contained in the original material. In detoxification experiments, this approach could be adopted to establish the process conditions which would inactivate the pomace and

thus make the allergens unable to elicit shock in the test animals (232,248).

In humans, cutaneous tests on patients sensitive, or suspected of being sensitive to the castor bean are applied diagnostically in clinical investigations to prove the source of an allergic reaction. They can also furnish information on the level of residual allergens in treated pomace samples. In preparing extracts for these tests, every precaution should be taken to eliminate ricin from the injected dose. Panzani (279) has pointed out the difficulty in obtaining commercial castor bean extracts that are ricin-free, and hence tests for the presence of the toxin are necessary. Further vigilance is essential in choosing the dilution level, which should be very high, owing to the potency of the castor allergen. Intracutaneous tests have been reported (282) but there is a warning against intradermal tests because of the risks involved. Panzani (279) and Panzani and Layton (156) employed scratch tests and did not recommend intradermal tests. A dilution of 1:2,000,000 was reported by Layton et al (42) in their initial trials. Spies and Coulson (219) reported positive cutaneous tests on sensitive subjects with dilutions of $1:10^6$.

A passive transfer test known as the Prausnitz-Küstner (PK) test was employed by some investigators for demonstrating allergy in humans. It is performed by obtaining serum containing lgE molecules specific for a given allergen from

an allegenic individual and injecting it into the skin of a person who is not sensitive to that allergen. The lgE will thus fix to the skin after a certain incubation period, usually 12-24 hours. If at the end of that period the allergen is injected into the antibody site, a reaction takes place, characterized by a wheal and flare. This technique is infrequently chosen as a test method due to the risk of transferring serum infection. Spies et al (291) applied the PK test to the study of castor pomace deactivation, and discussed the quantitative relationship between the quantity of allergen injected and the wheal-producing capacity of the reagins present.

The PK test is a local application of the Passive Cutaneous Anaphylaxis (PCA) phenomenon. The PCA technique was demonstrated in animals by Layton et al (292) who proved that the serum from humans sensitive to the castor bean could sensitize the skin of certain species of Philippinese monkeys. This reaction was also reproduced in guinea pigs by Layton et al (293) and Mottola et al (294) in their study of the deactivation of castor pomace allergens.

The PCA test is carried out as follows (293). The test animal is sensitized by injecting rabbit anticastor serum in marked sites on its abdomen. After 2½-3 hours, the animal is injected with a dye (Evans blue dye) plus the material to be tested for castor allergens. A blueing of the antigen-antibody site indicates a positive result. As low as 1.0 µg of antigenic protein was detected in this

manner when injected intravenously in guinea pigs.

The PCA technique is usually performed via intravenous (IV) injections, and the feasibility of employing the intradermal (ID) rate was investigated by Mottola et al (294). They concluded that the ID route was superior to the IV route for the following reasons:

a) The ID method has less variability than the IV method, and is thus more precise. Fewer animals will therefore be needed to obtain a certain level of precision.
b) The ID route is more sensitive; some antigens that failed to elicit a response in animals by IV injections were detected by the ID method.
c) When an animal is injected intravenously the reaction is systemic, involving the whole body, hence the animal can be used only once. When the ID route is followed, the animal may be repeatedly challenged and thus fewer animals would be needed for obtaining the same amount of information.

In spite of the enhanced sensitivity of the PCA technique with ID injections, Mottola et al noted that it is somewhat slow, and that a quicker method of assay is needed. The ID method was applied to the analysis of the pilot-plant scale treatment of castor cake with steam (97), lime (295), and ammonia (296).

A serological method was developed, based on the precipitin reaction between antigen and antibody, for the measurement of allergens in castor pomace (96). The

procedure involves precipitating a pomace extract with CB-1A antiserum and comparing the precipitate with that obtained by adding CB-1A solution to a normal (i.e. non-immunized) rabbit serum. The two disadvantages of the method are that it is subjective because it employs visual comparison, and that the serial dilution employed causes an error in one tube to be propagated to the rest of the tubes.

Coulson et al (297) determined the allergen content of castor beans and castor pomace by utilizing the quantitative precipitin method of Heidelberger and Kendall (298) in which the antigens are neutralized by specific antibodies raised against them in animals. The reaction mixture is centrifuged, the supernatant is decanted, and the remaining precipitate is analysed for nitrogen using the Kjeldahl method. A plot is made of the nitrogen precipitated against the antigens added (from cake or seeds) and the allergen content can thus be estimated.

Precipitation in gels is well suited to the determination of antigens, but there is no widespread application for it in assaying castor allergens. The analysis of antigen E of the ragweed pollen by single radioimmunodiffusion (the Mancini technique) has been mentioned by Gutman (299). Immunoelectrophoretic analysis (IEA) was employed by Daussant et al (300) in their study of the proteins of germinating castor beans. Gel immunodiffusion, immunoelectrophoresis, and radioimmunoelectrophoresis were among the methods applied by Mendes (282) in the study of castor bean hypersensitivity.

Other immunological procedures using labelled antigens or antibodies may equally serve for this purpose, such as immunofluorescence, radioimmunoassay (e.g. RAST, PRIST), and enzyme-immunoassay (e.g. ELISA). The radioallergosorbent test (RAST) has found acceptance as a diagnostic tool (258), and the paper radio-immuosorbent test (PRIST) is said to be the most commonly used method for measuring low levels of IgE (176). The enzyme-linked immunosorbentassay (ELISA) was developed to avoid the use of radioisotopes inherent in RAST and PRIST. These methods were briefly described in Chapter three of this thesis.

A passive haemagglutination procedure for detecting antibodies to castor allergen was described by Coombs et al (301), and it may be possible to modify the technique to enable the estimation of castor allergens in the pomace (302). Comparison of this system with solid-phase radio-immunoassay was made by Scott et al (258).

The Schultz-Dale technique is an in vitro method for detecting the castor allergens. It consists of measuring (arbitrarily) the degree of contraction of the uterine from a sensitized female guinea pig, a response caused by the histamine released upon challenging the sensitized organ with allergens (303). The amount of histamine released from other organs was employed by some workers in an attempt to quantify certain allergens, but the method is not considered promising (304).

The variety of available techniques for assaying allergens provides a chance for developing a procedure to measure quickly and accurately the level of allergens in the pomace and to be of such a design as to make it useful to process

engineers in the oilseed industry.

4.9 THE INACTIVATION OF THE CASTOR-BEAN ALLERGENS

4.9.1 Introduction

The problem of rendering the castor pomace harmless was not completely solved by the detoxification of ricin because the allergenic factor was still active. Before it was established that the allergy to castor cake was not due to the presence of ricin, the efforts for inactivating the toxin were thought adequate for the safe utilization of the pomace, but after the pioneering work of Spies and his coworkers on seed allergens, new processes were proposed for the deallergenization of castor pomace.

The unusual stability of the main castor bean allergen, CB-1A, to heat and strong acids may have been one of the factors that made its total removal a difficult task, especially that preserving the nutritive quality of the pomace called for the adoption of somewhat mild treatment conditions. Although ricin is more hazardous than the allergen in that it is very toxic, the elimination of the allergen remains essential because of the community and occupational illnesses it can cause. The various trials undertaken for deactivating the allergen will be given in the rest of this section.

4.9.2 Physical Methods

The dry heating of the pomace at high temperatures (205^{o}C) inactivated the allergen but resulted in a charred

product (96). Heating with steam at 175°C for 1 hour reduced the allergen content to low levels (22). When the pomace was treated with 15 psi steam for 100 minutes, the allergens were destroyed and the resulting material was unable to elicit shock in sensitized animals (232). Corwin (309) autoclaved the allergen with 15 psi steam and found that 30 minutes was the minimum treatment time to obtain satisfactory results.

The steam treatment of castor pomace on a pilot-plant scale was reported. The first set of experiments (305) was conducted with steam pressures in the range 15 to 120 psig and exposure times of 1 to 80 hours. Each batch was made up of three kolograms of castor meal added to water at a liquid:solid ratio of 1:1. The results of these exploratory treatments were applied to the design of the second set of experiments where the steam pressure was varied from 10 to 80 psig and the residence time varied from 15 to 60 minutes. A 1:2 liquid:solid ratio was adopted. The analysis of the results indicates the significance of the steam pressure and residence time on the deactivation of the pomace. Although considerable reduction in the allergen content may have been achieved, the loss of some heat-labile amino acids, especially lysine, was pronounced. Steam dealler-genization may be accomplished at 10 psig for 60 minutes at a liquid:solid ratio of 1:2 (97).

Corwin et al (306) attempted the deactivation of castor bean solutions with ultraviolet radiation. They proved

that UV light can destroy the biological activity of the allergen, but that the results are affected by the energy input and the actual amount absorbed by the allergen. The variables they investigated included the wavelength of the radiation, the output of the UV lamp, the distance of the test solution from the lamp, and the length of exposure. Thus, a 0.05% solution exposed for one hour to radiation from a 0.033 watt lamp situated 4 inches away, was over 99% deactivated as proved by a mouse assay. When the distance between the lamp and the solution was increased to 14 inches, no change in the physiological activity was noted even after an exposure of six and a half hours to the radiation. Lamps with higher output proved more efficient in destroying the allergen's activity.

4.9.3 Chemical Methods

4.9.3.1 Inorganic Chemicals

Gardner et al (96) investigated the effects of a number of chemical treatments for castor meats and meals. Alkaline cooking employing sodium hydroxide, potassium hydroxide, or calcium hydroxide was carried out before the solvent extraction steps. It was found that sodium hydroxide was the most effective of the three alkalis when tested by moist cooking at 20 psig pressure. Ammoniation of partially deallergenized pomaces had no apparent effect in further reduction of allergen content. Chemical treatment of pomaces produced by alkaline or non-alkaline cooking of the meats was attempted with sodium hypochlorite and formaldehyde (separately and mixed), and potassium permanganate.

The formaldehyde treatment of meats (with the addition of sodium hydroxide or hydrochloric acid) reduced the allergen content to practically 0% as tested by the precipitin method and the Schultz-Dale method. Cooking the meats with sodium hydroxide, and adding formaldehyde or formaldehyde plus sodium hypochlorite to the pomace also resulted in the elimination of the allergen. The addition of sodium hypochlorite to the pomace was only effective if the meats were cooked with sodium hydroxide. Potassium permanganate added to the pomace produced by non-alkaline cooking was not effective in eliminating the allergen. The authors give the five most promising deallergenization treatments and the accompanying percentage reduction of allergen content as measured by the precipitin method in the following:

Dry heating of pomace to 205^{o}C , 100%
Moist cooking of flaked meats with 2% NaOH and 10%HCHO, possibly 100%
Moist cooking with 0.9% HCl and 3%HCHO, possibly 100%
Moist cooking with 2% NaOH at 20 psig pressure, possibly 100%
Moist cooking with 1% NaOH, 98.4%

Layton et al (293) found that extracts of castor meal which had been cooked for 32 min. at 100^{o}C with calcium hydroxide solution at pH 12.4 elicited no passive cutaneous anaphylaxis, an indication that the allergen has lost its antigenicity. Spies et al (291) investigated various levels of temperature, time and pH for deactivating allergens by heating with aqueous solutions of calcium hydroxide.

The effect of heating the castor bean allergen in solutions of pH 4 to 10 had already been determined for various time intervals (307). Thus, at pH9, the precipitating capacity was abolished in less than one hour at 130°C, while the reagin-neutralizing capacity at pH9 was destroyed in less than one hour at 115°C and in four hours at 100°C. In the presence of calcium hydroxide, the reagin-neutralizing capacity was abolished in one hour at 100°C and pH 12, while for the same conditions of time and temperature, the immune-precipitating property was destroyed at pH 10.8.

An ammoniation process for the deallergenization of castor pomace was patented by Layton and Greene (308). It comprises heating a mixture of ammonium hydroxide and pomace under pressure. The water content is critical, and there should always be at least one part of water per part of pomace. The temperature may be varied from 100° to 150°C, and the molar ammonium hydroxide concentration should at least be unity. The residence time will vary according to the temperature and ammonium hydroxide concentration.

The effect of sodium hypochlorite solutions on the castor bean allergen was investigated by Corwin et al (309). They added 500 mg of chlorox (a stabilized 5.25% solution of sodium hypochlorite) to 100 mg of CB-1A and stirred with a glass rod. After 20 minutes, the solution was diluted with saline and injected into sensitized mice, and severe symptoms of shock were observed after 10 minutes, and hence a ratio of chlorox:allergen of 5:1 was not sufficient for

deallergenization. To avoid the toxic effects of chlorox if higher ratios were employed, it was decided to remove the excess hypochlorite by the action of heat and acid. The ratio of chlorox to allergen was increased to 50:1 and the excess oxidant was removed by heating in HCl (pH 2.5) at 55°-60°C for six hours at 60°C for a further four hours. The end product raised no sumptoms of shock when injected into mice, which meant that the allergen was oxidized. The experiment was repeated with the same ratio of chlorox: allergen but the excess hypochlorite was removed under milder conditions by adding 5 c.c. of 10% urea. A mouse assay proved that the allergen was oxidized.

In the experiments of Gardner et al (96), sodium hypochlorite (added at a level of 1% of the charge) was not effective in deallergenizing the castor pomace when heated with it at 170°F for 15 min., but it reduced the allergen level to 0.8% when it was added (0.84%) under the same conditions to the pomace produced from alkali-cooked castor flakes.

The deallergenization methods already mentioned in this section were carried out on a bench scale, and it was therefore necessary to obtain an idea about their industrial potential by conducting them on a pilot-plant scale.

The pilot-plant trials of the lime process were reported by Mottola et al (295,310). Calcium hydroxide was stated to be a suitable base for deactivation (293); moreover, the

calcium phosphate produced in the neutralization step can supply the calcium and phosphate requirements of the livestock (310). In their first report, Mottola et al (310) employed a biological assay that resulted in highly variable responses, and thus they could not determine optimum process conditions. However, they found that treating the pomace with 8% lime at a 3:1 liquid:solid ratio was effective in deactivating the allergen when the mixture was heated to 140°C for 60 minutes. After a more sensitive biological assay was adopted, the results were re-evaluated and optimal deallergenization could be obtained by heating the pomace at 120°C for 15 minutes in 4% lime. It was noticed that the essential amino acids were considerably decreased by the lime treatment, a factor that may limit the outlets for the product of this process to certain areas in the feeding industry.

The ammonium hydroxide process was investigated on a pilot plant scale by Mottola et al (296). The process variables studied were the ammonia concentration, the process temperature, the quantity of the liquid in the slurry, and the process time, the temperature ranging from 20° to 120°C, and the time from 0.75 to 4.5 hours. Ammonia molarities of 1,2,4,6, and 10 were studied, and the liquid:solid ratios were varied between 0.25:1.0 and 0.60:1.0. The runs were conducted in a steam-jacketed reactor equipped with a ribbon-type agitator.

Analysing the results revealed a significant temperature effect. It was shown that deactivation could be achieved in

45 minutes with a 0.25:1.0 liquid:solid ratio, using 6M ammonia at 80°C. Losses of some essential amino acids were also reported.

4.9.3.2 Organic Chemicals

Corwin and coworkers (311) found formaldehyde unsuitable as a laboratory reagent for the deactivation of the allergen. They applied the regime which destroyed over 99% of ricin's activity to the treatment of the allergen, and it was noted that the latter lost about 75% of its activity. Ricin preparations acted upon with formaldehyde also showed definite residual allergenic power. Gardner et al (96) stated that "formaldehyde definitely reacts with the allergenic component in some unexplained way to render it undetectable", but it should be noted that their results are not directly comparable to those of Corwin et al because of the different strategies adopted. Thus, Gardner and his coworkers heated the pomace and formaldehyde with or without added chemicals; the meats from which the pomace was produced were cooked with a variety of chemicals. Since some of those chemicals were reported by Gardner to deactivate the allergen, and since formaldehyde alone could not abolish the allergen's activity, it may be reasonable to assume that the action of formaldehyde is enhanced or 'catalysed' by the added chemicals, or that some of those chemicals has already achieved partial deallergenization.

Urea was found unsuitable for deallergenizing the pomace when heated at 170°F for 15 minutes. A 5% urea solution was used.

4.9.4 Biochemical Methods

The experimental work reported on the deactivation of castor allergens by utilizing biochemical methods is very scanty. The allergenic principle in the castor beans was resistant to the enzyme pepsin, but was destroyed by trypsin (265). Gardner et al (96) obtained a 98.4% reduction in the allergen's content by tryptic digestion in an aqueous alkaline medium of partially deallergenized pomace that had been prepared from alkali-cooked flaked meats. Aerobic fermentation, howevever, was not successful in deallergenizing the pomace.

4.10 THE NUTRITIVE VALUE OF THE CASTOR CAKE

Detoxified pomace, as has been explained before, has a potential for use in animal feeds. The reports on feeding trials including the pomace do not always elaborate on the details of the experiments, and hence direct comparison between their results is not possible. However, the more recent reports are more quantitative and descriptive, thus allowing an appraisal of the techniques employed and the results achieved.

In his review of the castor bean proteins, Jones (312) remarked that only a few cases are recorded on the feeding of castor cake to farm animals. He presented two cases, one in which birds died and others lost their weight when fed

castor cake at a level of 16% of their diet, and the other where it was found safe to include castor cake in bird feed at a level of up to 30% if it was fed progressively and carefully. Kodras (229) conducted biological tests to determine the feeding value of the detoxified pomace. His results indicated that the protein was deficient in tryptophan and hence could not support well the growth of rats and chicks. Young (230) extended Kodras' work by carrying out more feeding experiments, but he arrived at essentially the same conclusion. He also found that the supplementation of the detoxified cake with Lysine and methionine markedly improved its biological value. The nutritive value of castor cake that was treated in various ways was assessed by Ambekar and Dole (243). A level of 17% of pomace in the meal, together with peanut proteins, gave normal growth rates in rats, but higher levels resulted in lower gains in the weights of the animals. The results were attribted to the lack of certain essential amino acids. Raymond (313) gave an account of a Brazillian product, SANBRA, which is a brand of detoxified castor meal that was reported by its producers to be cheap and of good value. The material was satisfactory when it formed about 2½ to 10 per cent of chicken feed but caused retarded growth to poultry at the 15 per cent level. Weiss (4) provided a summary of data from feeding trials in Texas, USA, employing detoxified pomace in cattle rations. The operation is a large-scale one and the results are apparently good.

The castor cake contains a toxic alkaloid, ricinine, whose growth-depressing effects on young chicken were indicated by Japanese workers (314), and American workers have confirmed these findings at high levels of pomace in the feed. The recommended level is 10-15% of the ration Vilhjalmsdottir and Fisher (53) improved the growth response in chicken by feeding them on pomace extracted with hot water, the treatment apparently resulting in removing the growth-depressing factor without changing the protein or amino-acid content. Excellent growth rates were obtained upon supplementing the extracted meal with lysine and tryptophan, the first and second limiting amino acids in the pomace respectively.

A review of the castor bean by-products in beef cattle rations indicated the feasibility of adding the detoxified castor cake in such feeds (315). At the level of 5.71% of the diet, no ill effects were noted; the cattle showed good weight gains, produced high quality beef, and exhibited no symptoms of toxicity. The digestibility of the castor meal was equal to that of the cottonseed meal, but its energy value was less, a difference that could possibly be made up with the addition of a small amount of castor oil.

The feeding of detoxified castor cake to cattle and laying hens does not produce harmful effects on their meat, milk, or eggs, provided the percentage added is within the limits (48,314).

Castor protein isolates were prepared and reported to be non toxic and non allergenic, although a deficiency in the essential amino acids lysine and methionine was noted (49). Feeding tests on rats indicated good net protein utilization and the figures were even better when lysine and methionine were added to the meal.

4.11 OTHER UNDESIRABLE COMPONENTS OF THE CASTOR BEAN

The presence of undesirable substances in the castor bean, apart from ricin and the allergens, has been reported. The two main components in this category are ricinine and chlorogenic acid. An enzyme, lipase, also is present in the bean and its effect will be discussed later.

Ricinine, $C_8H_8N_2O_2$, is a moderately toxic alkaloid derived from pyridine, and is present at a level of 0.3% in the pomace (22). It is reported to have adverse effects on the growth of young chicken (314), but this occors only at high castor levels in the diet. As a result, it is advised that the castor meal should be limited to about 10-15% of the total ration. Ricinine was affected when the castor cake was treated with ammonia, but the toxicity of the reaction products was not reported (341).

Chlorogenic acid (3-caffeoylquinic acid) is commonly found in fruits and plants, including oilseeds. Its interaction with proteins gives rise to quinonoid oxidation products that affect the nutritive value of the proteins (23). Siddiqi and Freedman (342) reported the identification of the chlorogenic acid in castor beans. The suggestion by

Freedman et al (343) that chlorogenic acid is one of the castor allergens was vigorously opposed by Layton and his group, and a discussion of this topic was given by Berrens (256).

The castor lipase belongs to a group of enzymes which hydrolyse the glycerol esters of long-chain fatty acids. If the favourable conditions for its action exist, it may seriously affect the oil content of the castor seed, but fortunately it is active only within a small pH range (24). The water-soluble component of the active castor lipase were resolved into eight component bands by Layton et al (262). An ether extract of castor beans was used to hydrolyse raw animal fat, cottonseed oil and sunflower oils to glycerol and fatty acids at 40^{o}C (344). The colour of the fatty acids prepared in this way was better than that of the acids obtained by hydrolysis at 25 atm and 200-225^{o}C. No mention is made as to whether the fatty acids prepared by enzymatic hydrolysis exhibited any allergenic properties.

Chapter Five

EXPERIMENTAL INVESTIGATION

5.1 INTRODUCTION

The experimental work was carried out in stages as follows:

Hull removal

Work on toxins and allergens

Size reduction

Solvent extraction, including the choice of solvent.

The seeds were received in 25 kilogram lots and each lot was sampled on receipt, the samples being transferred to plastic bags which were sealed and stored until they were later analysed.

5.2 SEED CHARACTERISTICS AND COMPOSITION

5.2.1 Sampling

The seeds were supplied by Croda Premier Oils Ltd., Ann Watson St., Stoneferry, Hull HU8 OBJ, England. The shipments were made in jute or plastic bags of the thick type. The jute bags were more dusty than the plastic bags, but the latter had to be sent inside one or more of its kind to ensure that they will sustain the seed weight. It was necessary to put on dust protection equipment when emptying the jute bags. The sampling was done with a sampling stick, overall length 900 mm., diameter 25 mm., with three apertures, supplied by Gallenkamp.

5.2.2 Characteristics and Composition

The shape was usually oval, with a carnucle at the thin end, but some of the seeds were elliptical or cylindrical. The oval seeds were convex on one side and slightly concave on the other. The colours were varied, with a mottling effect. The sizes varied from 5 x 10 mm. to 14 x 20 mm.

Hull Content

The hulls were manually removed with long-nose pliers, care being taken to put on a pair of gloves. The average hull content was 23.24%. Weiss (4) gives the typical value as 20% but it may rise to 33% in some cases.

Moisture Content

The moisture content was determined according to the American Oil Chemists Society (AOCS) Official Method No. AC 2-52 revised in April 1956 (316). This oven method was also used for determining the moisture content of the seeds whose oil content was to be determined.

The average moisture content of the seeds was 6.4%.

Weight per hundred seeds

This varied from 46.38g for 8.0mm seeds to 78.58g for 11.2 seeds.

Oil Content

The oil content of the whole beans was determined with the soxhlet method. The AOCS official method No. AC 3-52 (revised April 1956) was adopted, with some modifications. Thus, isomantles were preferred to electric hot plates because they apply heat uniformly around the extraction flasks, and support them well. Hexane was the extraction solvent but some runs were made with carbon tetrachloride (317) which proved to be a good solvent. Anti-bumping granules were placed in the flasks before the solvent was poured in. The seeds were extracted for 24 hours and all the runs were made in a fume cupboard. The average oil content for the whole castor seeds was 50.38%.

5.3 CLEANING AND SIEVING

The seeds were hand picked to separate the intact beans from the rest. All foreign matter was removed and stored until disposed of later as chemical waste. Broken or immature seeds were also rejected, so that the sound beans were the only ones that proceeded to the later processing stages.

Dry sieving was carried out with a set of sieves (11.2, 9.5, 8.0, and 6.7 mm.) on a shaker. The sieves were supplied by Endecotts (Filters) Ltd., London, S.W.19. Trial runs were made to establish the optimum combination of seed charge and sieving time that will give reproducible results. It was found that a charge of about 230 g and

sieving time of five minutes was a satisfactory combination. The clean, graded seeds were stored in labelled plastic bags in locked cupboards, together with those awaiting cleaning or sorting.

5.4 ELECTRON MICROSCOPY

5.4.1 Transmission Electron Microscopy

The ultrastructure of the castor bean kernel was studied with the aid of the transmission electron microscope (TEM). To view specimens under the TEM, they must first be fixed in order to preserve the cell structure from distortion, and they should then be embedded in resin, from which sections are cut on a microtome. These sections are mounted on special grids and are then viewed under the microscope. The materials used in the specimen preparation are given in Appendix 3.

The procedure adopted was as follows. Thin sections, approximately 1 mm^3, of dry dormant castor seed kernels were cut with a sharp razor blade and were immediately transferred into the glutaraldehyde-buffer fixative for two hours. The sections were next transferred to a sucrose-buffer solution and left there for four hours, were washed again with the sucrose solution and allowed to stand in it overnight. This process was meant to adjust osmolality and prevent interaction between the primary and secondary fixatives. The tissues were next fixed in osmium tetroxide-buffer for at least four hours. Dehydration was performed with a series of washes in ethanol of

varying concentrations (40%, 50%, 70%, 80%, 90% alcohol by volume), each once for 15 minutes, then twice in 100% ethanol, each for 15 minutes. The embedding resin was prepared, and the dehydrated specimens were placed in it and left overnight. They were then transferred to embedding capsules which were filled with the resin and cured in an oven for 16 hours at about 50°C. When the capsules were dry, sections were cut on a microtome {LKB, Type 4801A}, placed on copper grids, and examined under the microscope {AEIM 6B}. The micrographs are shown in Figs. 5.1 and 5.2.

If desired, the sections could be stained before viewing them. Some were stained in uranyl acetate followed by lead citrate. The micrographs presented are for unstained sections.

Safety in the TEM is of major importance because of the high toxicity of most of the chemicals employed in the fixation and staining processes. Thus, overalls, goggles, and gloves were always put on in the laboratory. Osmium tetroxide was handled in a fume cupboard and all its discarded solutions were placed in a special bottle in the fume cupboard until later removed for reprocessing. Pasteur pipettes were always used for transferring solutions. Care was taken not to allow drops of liquids to reach the working benches; special paper towels were placed on the bench, and at the end of the day the working area was thoroughly cleaned. All the bottles were clearly labelled.

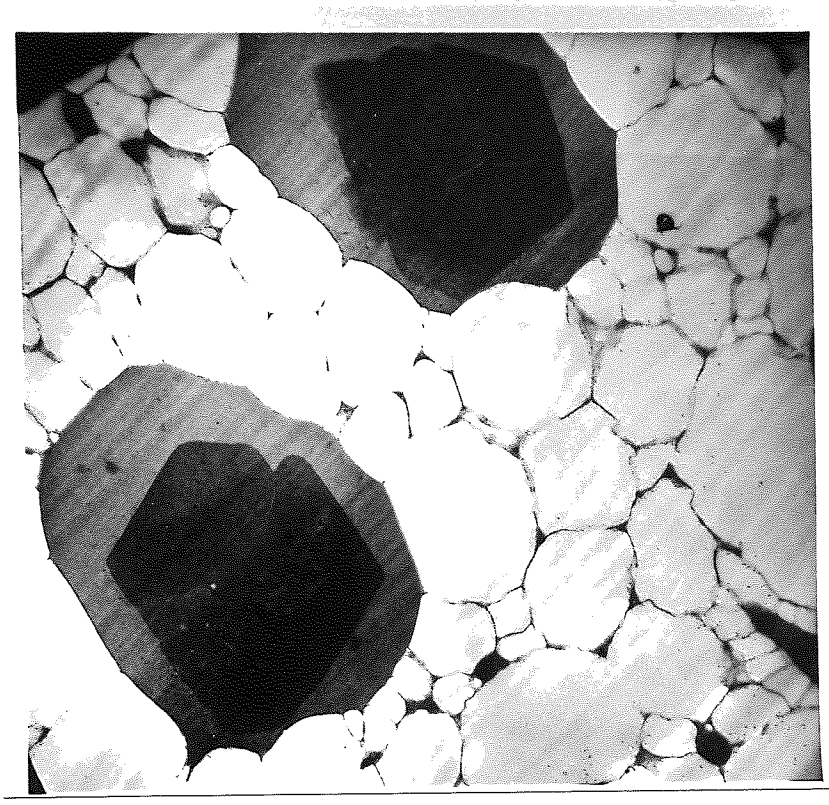


Fig. 5.1 TRANSMISSION ELECTRON MICROGRAPH FOR A SECTION OF DORMANT CASTOR SEED SHOWING ITS ULTRASTRUCTURE.

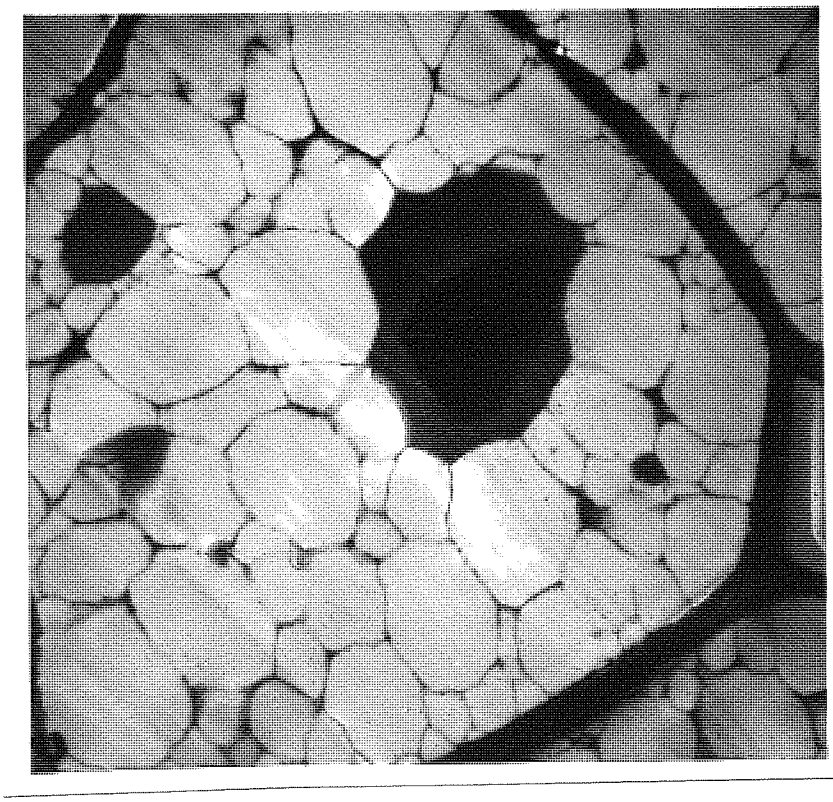


Fig. 5.2 TRANSMISSION ELECTRON MICROGRAPH (5,000 MAGNIFICATION) OF THE CASTOR SEED SECTION SHOWING THE CELL WALL.

Key to Figs. 5.1 and 5.2

C : Crystalloid Proteins

M : Matrix Proteins

L : Lipids

P : Protein Bodies

CW : Cell Wall

5.4.2 Scanning Electron Microscopy

The SEM was applied for examining the surface structure of the castor seed husks and to determine their thickness. The difference between the SEM and the TEM is that the former yields information about the external characteristics of a specimen, while the latter allows an examination of the internal structure. Therefore the TEM accepts thin sections only, a restriction not met with in the SEM.

Preparing the castor husks for SEM was made as follows. The husks were glued to the metallic studs of the SEM and coated to make them conductive. Three different types of coatings were applied, with varying degrees of success. Thus, carbon coating gave good detail under the SEM, but it caused charging and charring of the specimens at high magnifications because it could not absorb a substantial portion of the heat generated by the electrons bombarding the specimens. Gold coating solved the problem of overheating, but gave good detail only when it was about 1.5 Å thick. The aerosol coating was the least satisfactory of the three and therefore had to be supplemented by gold coating. The specimens were coated under vacuum in a special counting machine. The coated specimens were placed in a dry seal dessicator and were usually evacuated with a vacuum pump one hour prior to the microscopic examination. The specimens were examined

under a Cambridge 150 SEM, photographs were taken, and are shown in Figs. 5.3 through 5.8.

5.5 DECORTICATION

The equipment in which decortication was carried out is shown in Fig.5.9 and consists of a pressure vessel connected to a compressed air source and a vacuum line. The vessel was fabricated from a 3" steam pipe to which was welded a 3"-2" steam reducer. Two 1/4" steam valves were fitted for introducing and releasing air into and out of the vessel, which was also fitted with a 0-400 psi pressure gauge. A 2" gate valve was screwed to a short piece of pipe welded to the side of the vessel for introduction and removal of seeds, and a 2" plug was usually screwed to the valve after it was closed. The vessel had a capacity for 280-300 g of seeds, depending on their size. A hydraulic test was made on the bomb twice and it was shown to stand a pressure of 380 psig.

The compressed air was supplied from an air cylinder (2000 psig) which was fitted with a two-stage air regulator (0-600 psi) from the British Oxygen Co.Ltd., Deer Park Road, London SW19. A special air hose connected the regulator to the pressure vessel; Ermeto pressure fittings were used in these connections. The main vacuum line of the pilot plant was connected to the pressure vessel with a high-pressure hose and fittings. A steel chamber, tested at 100 psig, was placed between the bomb and the

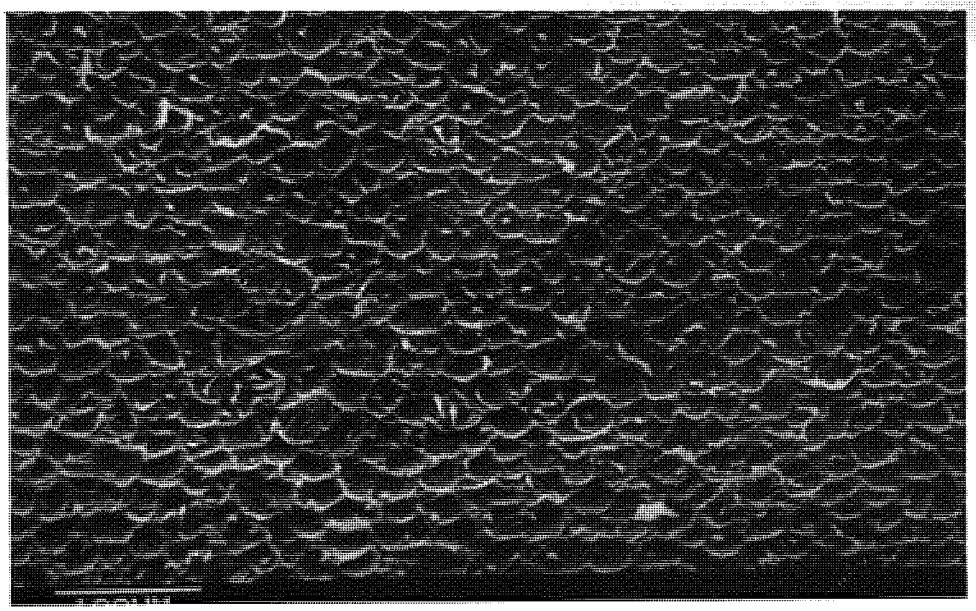


FIG. 5.3 SCANNING ELECTRON MICROGRAPH FOR THE HUSK OF AN 8.0 mm CASTOR SEED

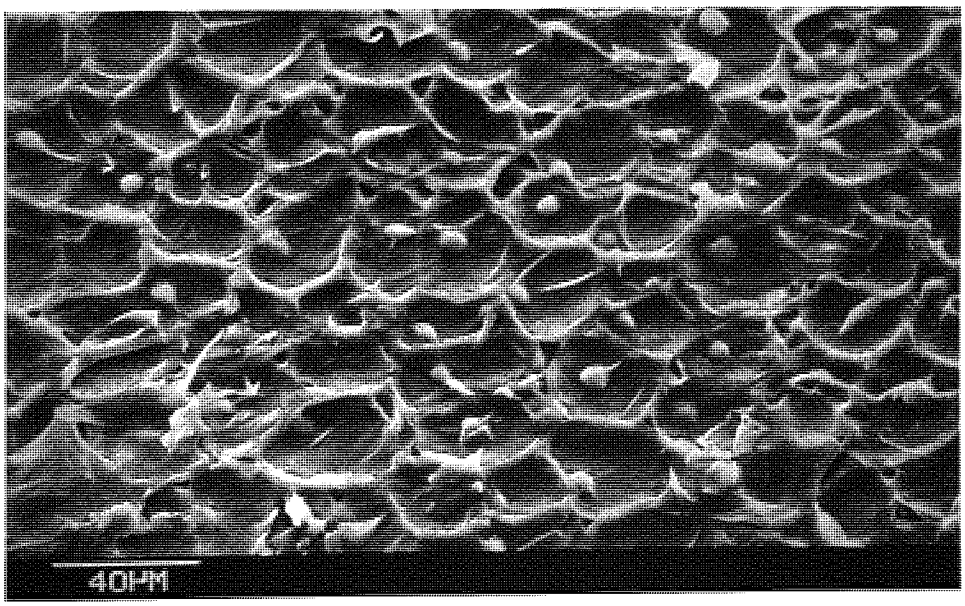


FIG. 5.4 SCANNING ELECTRON MICROGRAPH (HIGHER MAGNIFICATION) FOR THE HUSK OF AN 8.0 mm CASTOR SEED

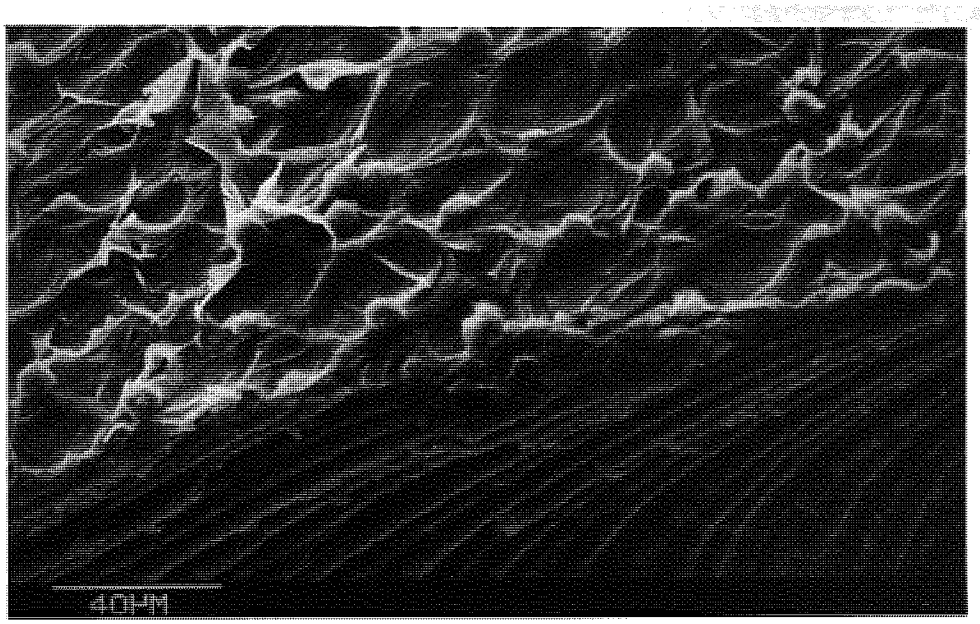


FIG. 5.5 SCANNING ELECTRON MICROGRAPH SHOWING THE HUSK OF A 9.5 mm CASTOR SEED AND PART OF ITS EDGE

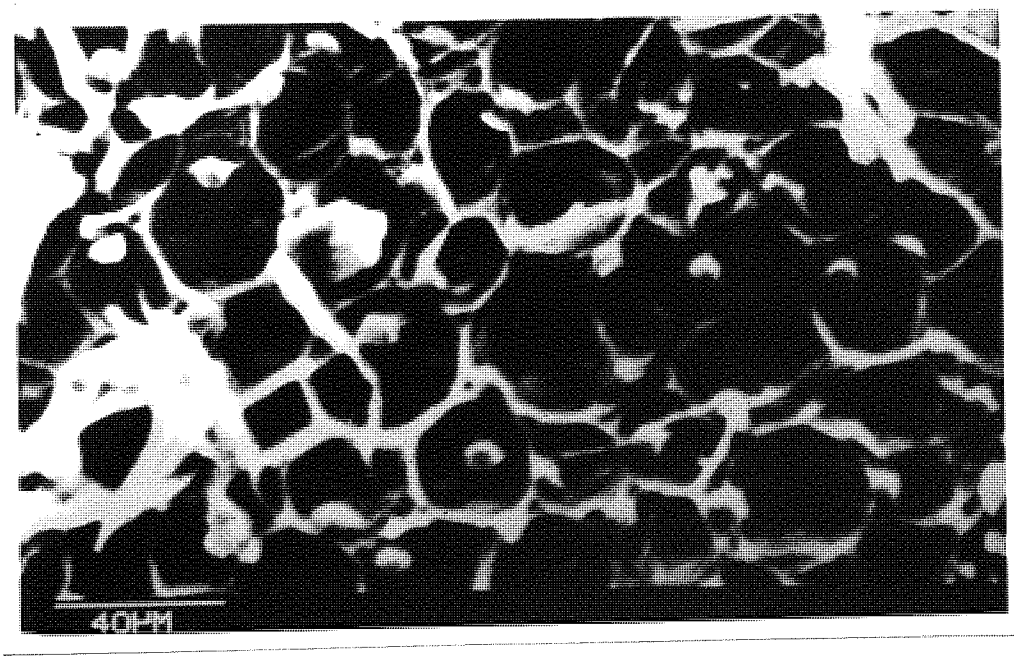


FIG. 5.6 SCANNING ELECTRON MICROGRAPH SHOWING THE HUSK STRUCTURE OF AN 11.2 mm CASTOR SEED

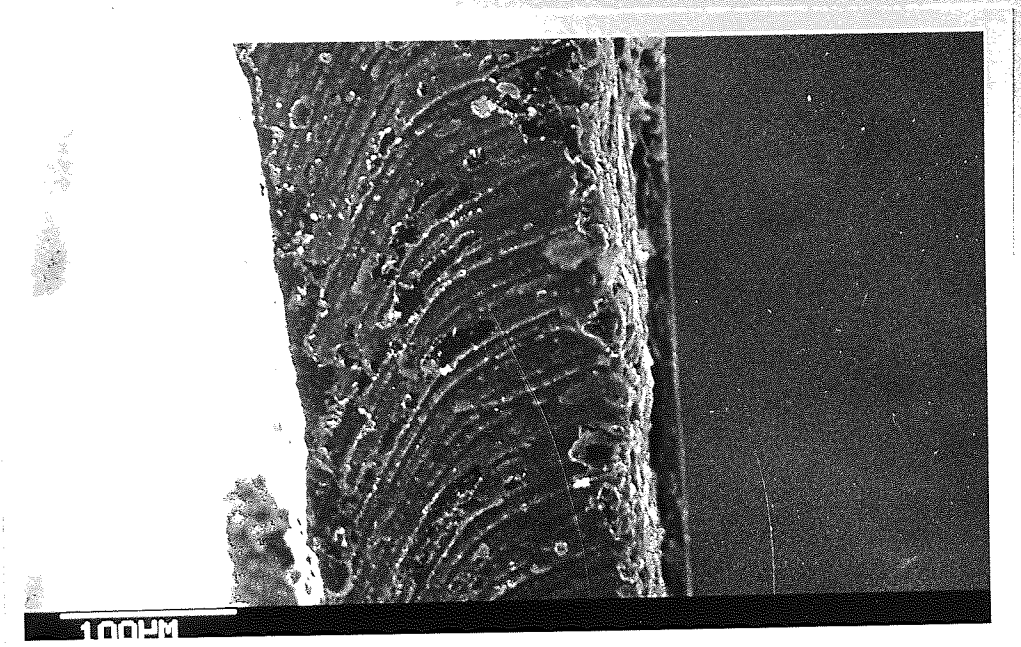


FIG. 5.7 SCANNING ELECTRON MICROGRAPH OF THE EDGE OF A 9.5 mm (OVAL) CASTOR SEED SHOWING ITS THICKNESS

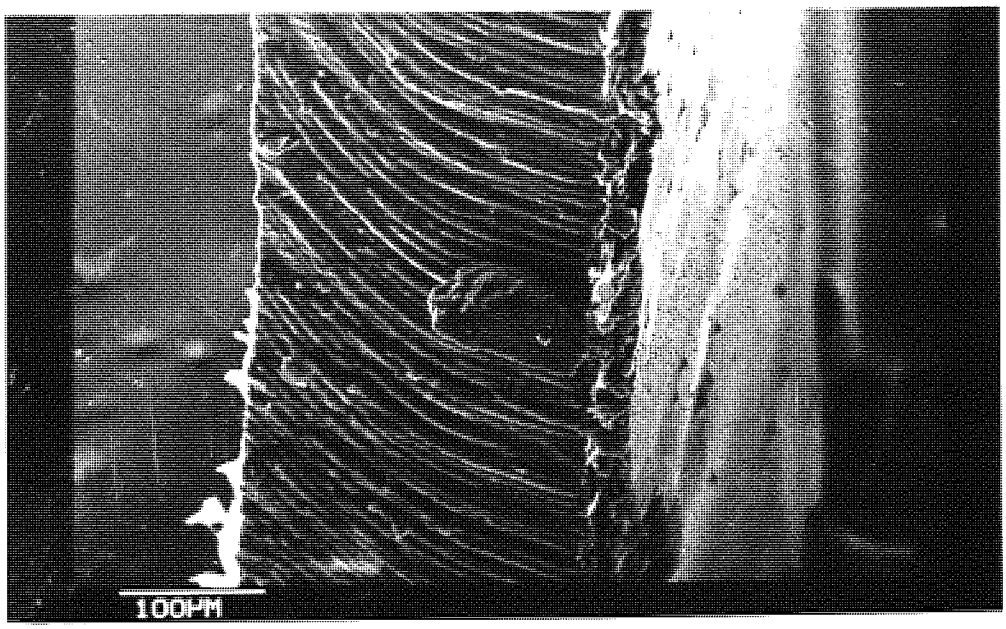
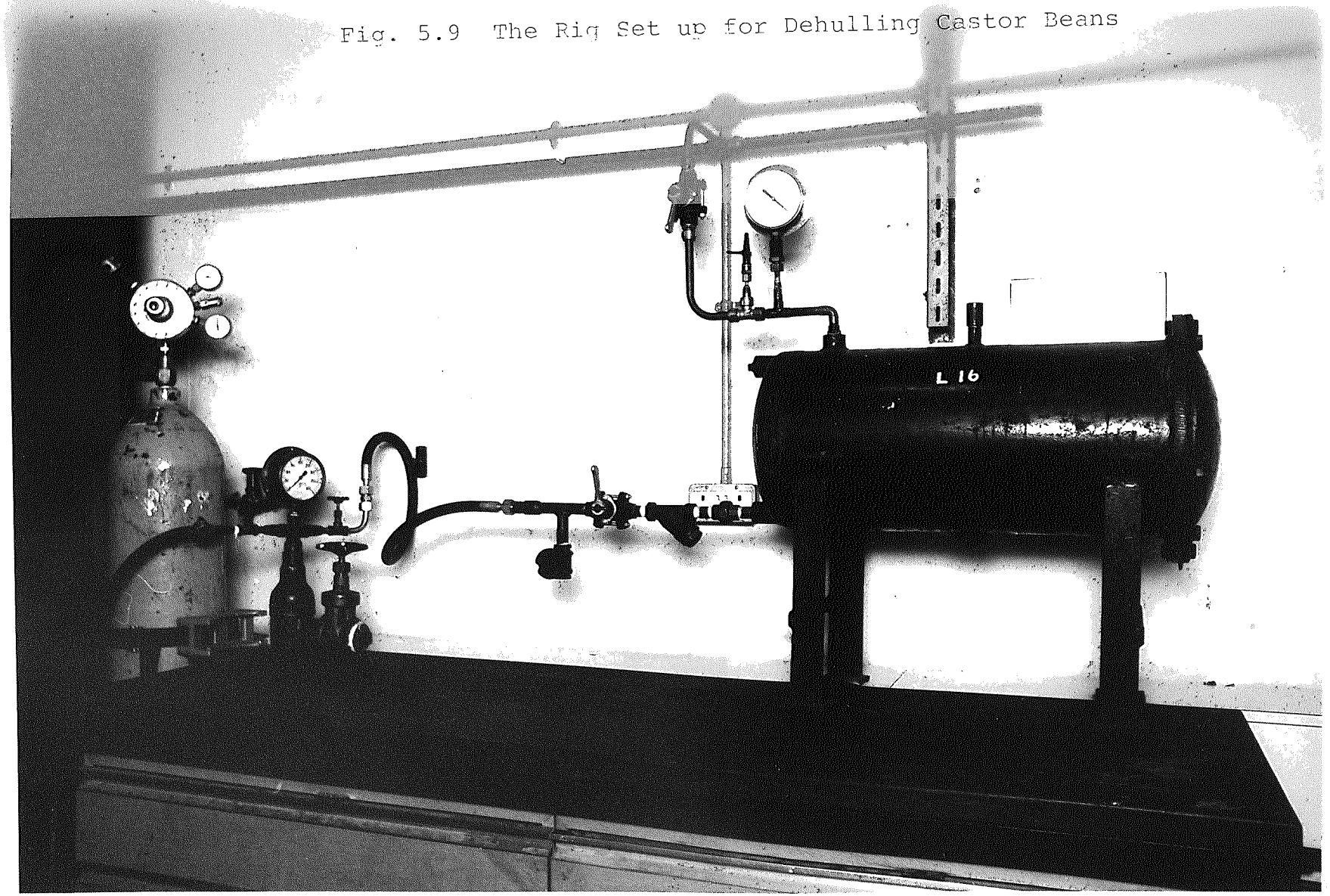


FIG. 5.8 SCANNING ELECTRON MICROGRAPH OF THE EDGE OF A (CYLINDRICAL) 9.5 mm CASTOR SEED SHOWING ITS THICKNESS

Fig. 5.9 The Rig Set up for Dehulling Castor Beans



vacuum line to absorb the effects of the sudden pressure release. A 1" quick release ball valve was installed to bring about the sudden release into vacuum.

Operating this rig was usually carried out as follows. It was first ensured that the main valve on the air cylinder was closed and that the regulator was off. The required amount of seeds were weighed out and placed in the bomb, the gate valve was closed, and the plug was screwed on. The main cylinder valve was opened and the regulator manipulated until the desired pressure level was attained, whereupon the regulator was turned off and the air cylinder was closed. In this way, the bomb was isolated from the air cylinder, but it still maintained its pressure. The quick release valve was then suddenly opened and was closed about 10 seconds later. A 1" Saunders valve, situated between the ball valve and the bomb, was used to release any residual air in the line and was kept closed after that. The beans were recovered in a plastic bag after the plug was removed and the gate valve opened, and the contents were reweighed before analysing them.

The analysis was done by separating the intact beans from the rest by hand picking. The beans affected by the treatment were either completely decorticated - and these were termed "shattered" - or only partially dehulled, and were termed "cracked". Each fraction was weighed and recorded. The data is presented in Appendix No.1. Graphs were plotted

for the percentage of seed affected against the applied pressure, with the seed size as parameter, and these are shown in Figs. 5.10 and 5.11.

For some batches, the dehulling process was repeated more than once. Thus, after the quick release valve and the Saunders valve have both been opened and closed, the cycle was repeated on the same batch the desired number of times, and the rest of the procedure was as described before. Plots were made for the percent of seeds remaining intact against the number of cycles executed. The results are shown in Fig. 5.12. Rose Cocoa beans were dehulled in the same way as the castor beans, but the results could not be presented because the insufficient amount of beans did not allow a full analysis to be made.

5.6 WORK ON TOXINS AND ALLERGENS

Having developed a method for the dehulling of castor seeds, attention was next focussed on the deleterious components in the seeds, especially ricin and the allergens. A study was made for the methods employed in assaying these substances, and it was found that the feasible techniques are biological, involving animal experiments, or immunological. The latter use animals for raising the antisera against the antigens to be quantified, while the former methods test the direct action of the substances on animals. Whichever technique would have been adopted, the procedure demanded the existence of certain facilities and these were

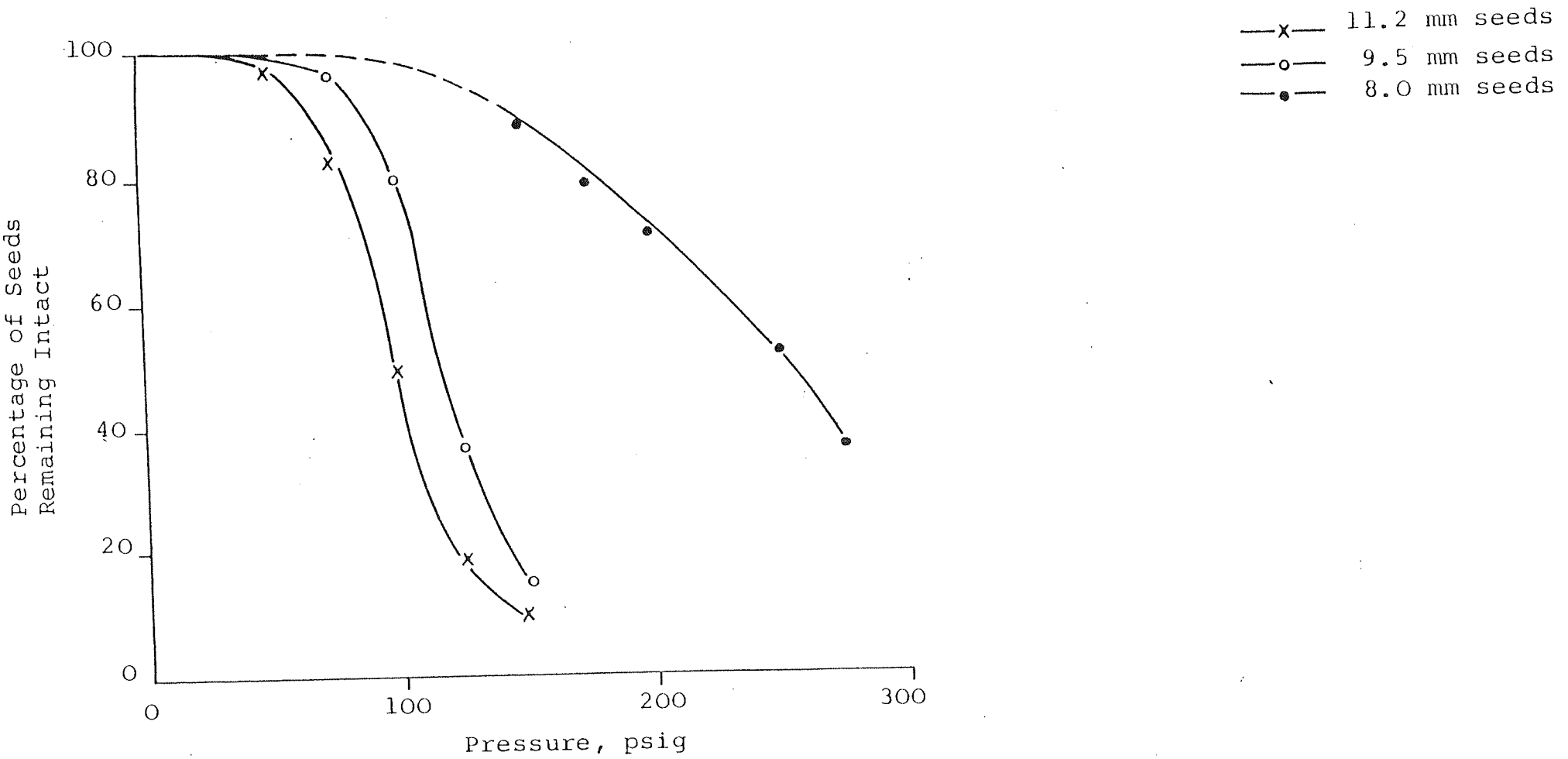


Fig.5.10 Percentage of intact seeds as a function of pressure and bean size

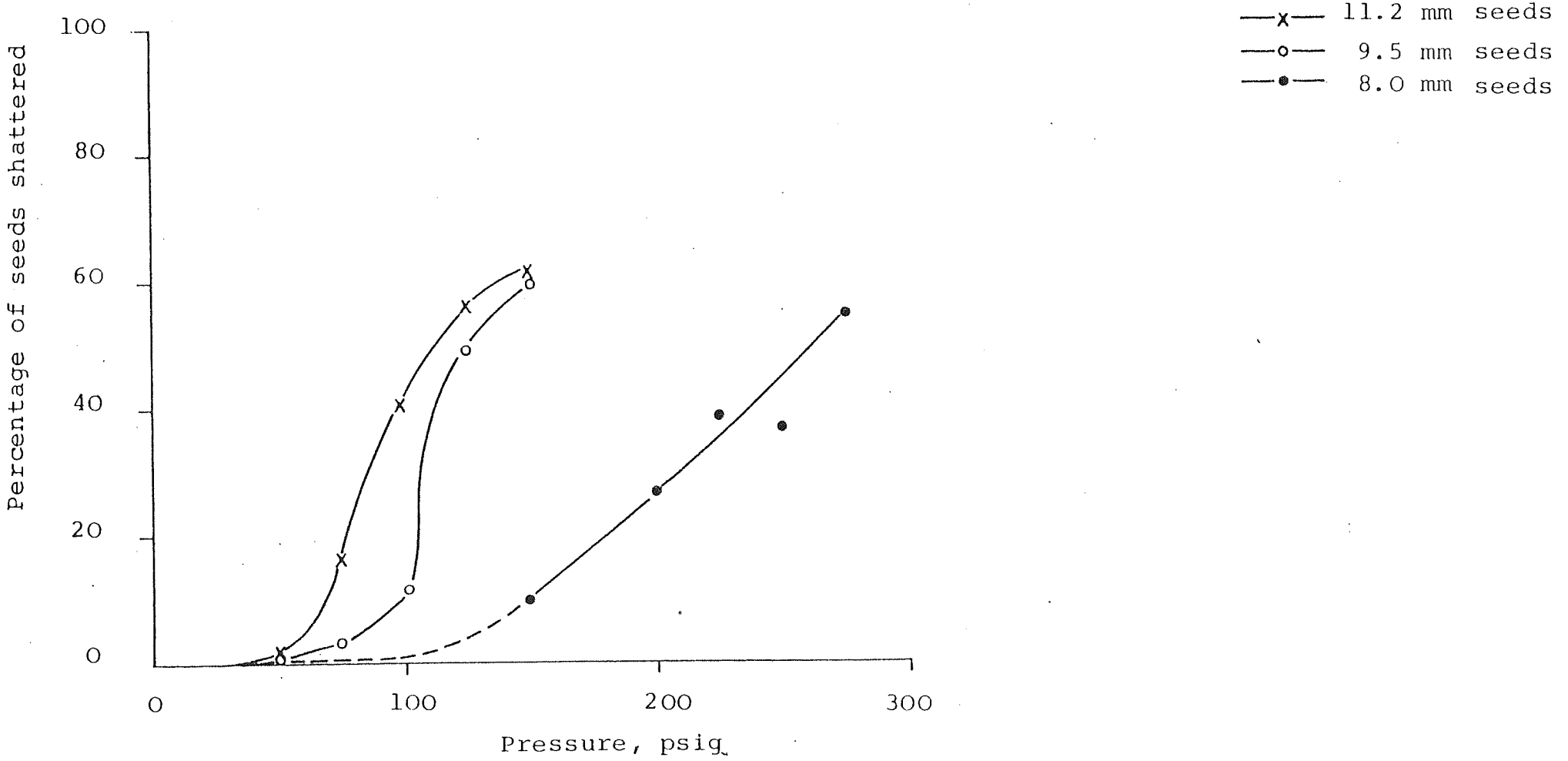


Fig. 5.11 Percentage of shattered seeds as a function or pressure and bean size

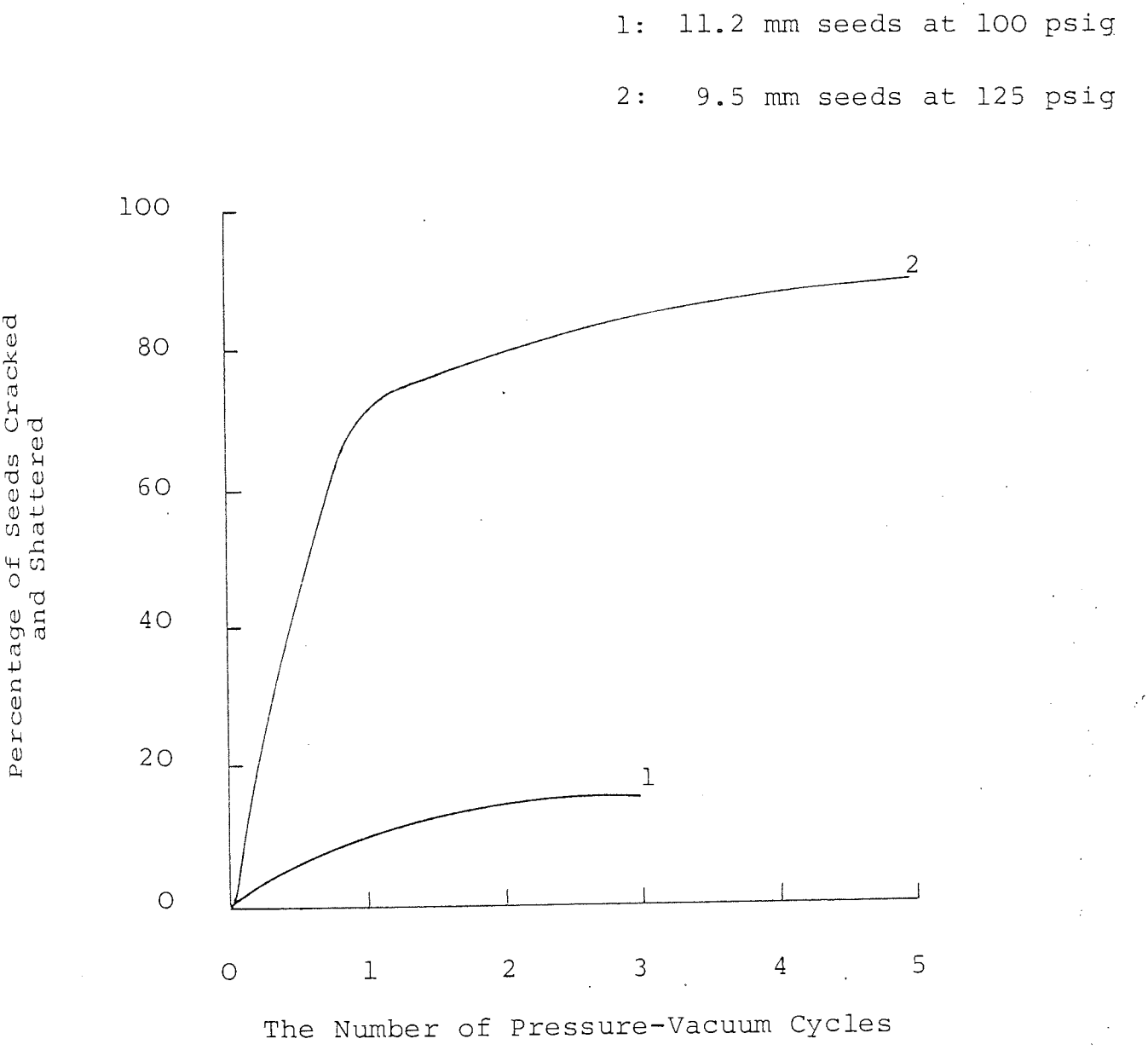


Fig.5.12The Effect of Repeated Operation on the Degree of Decortication

thought to be part of the Biological Sciences Department, who were contacted for assistance. The sound nature of the method of assay chosen was confirmed and the necessary steps were thought to be as follows:

a) obtaining the toxins and allergens in a reasonably pure state

b) raising the antisera against the toxin and allergens and setting up the assay procedure

c) testing the assay methods with pomace deactivated according to the conventional methods and to the methods suggested for this research.

To procure ricin and the allergens, contacts were made with workers in these fields, but it was discovered that locating a supplier would be difficult, and therefore a decision was made to prepare the castor bean allergen according to the Spies et al (219) procedure. However, the results were not encouraging (318) since the yield was only one tenth of the expected value, and therefore this was not proceeded with. No such attempt was made regarding ricin; instead, the search for a commercial supplier was again pursued. Finally, the castor bean allergen was obtained from Allergon AB (Välinge 55, S-262 00, Engelholm, Sweden) who also directed us to Sigma London Chemical Company Ltd. (Fancy Road, Poole, Dorset BH17 7NH England) for a supply or ricin. However, when these materials were received, the antisera could not be raised locally because the departments concerned thought the risk

was beyond the capabilities of their existing facilities, and therefore another source for the antiserum had to be found. Contacts were made with a number of organizations, and in the end the job was taken up by Miles Research Laboratories, Inc. (Miles Research Products, Elkhart, Indiana 46515, U.S.A.). The unusual toxicity of ricin manifested itself in delaying the delivery of the antisera from 12 to 52 weeks; the final schedule employed was as follows (RCA-60 is a code for the ricin preparation used) (319)

A. Antiserum to RCA-60

Solution of antigen was heated for five minutes at 80°C in a water bath before use.

0- Immunization of rabbits intradermally with emulsion made of: 1 part of saline solution of antigen with 10 µg protein

1½ part complete Freund's adjuvant

0.1 ml vaccine (Bordettela Pertussis)

Each rabbit received a volume of about 2 ml emulsion of the above composition.

30 days Boosting injection as above but with 50 µg antigen and with no addition of vaccine.

60 days Boosting injection as above.

73 days Test bleeding

Serum was separated. Merthiolate was added to 0.01% final concentration and antiserum was checked by

Ouchterlony test for reaction against immunogen heated and not heated as well as for cross reactivity towards castor bean allergen.

B. Antiserum to Castor Bean Allergen

0- Rabbits were immunized each with 0.1 mg antigen in an emulsion similar to that previously described.

30 days Boosting injection with 0.5 mg per rabbit of antigen without vaccine.

60 days Second boosting injection with 1 mg per rabbit was given as above.

84 days Third boosting injection with 2 mg per rabbit.

98 days Fourth boosting injection with 5 mg per rabbit.

105 days Test bleeding by Ouchterlony test with immunogen and Ricin-60.

Processing of this antiserum was done exactly as with the anti RCA-60. The results of the immunological tests conducted by the firm for both antisera are given in Tables 4.10 and 4.11.

When the antisera were received, they were placed in a deep freeze at -20°C. The unused antigens were returned with the antisera and were locked in a special poisons cabinet in the stores; the keys were kept with the Chief Technician who would open the cabinet only on authorization from the Departmental Safety Officer.

The next stage in the assay procedure involved the use

TABLE 4.10

Analysis of ricin antiserum

Product: Anti RCA-60

Produced In: Rabbit

Immunoelectrophoretic pattern of product:

By Ouchterlony test:

Versus RCA-60 (2 mg/ml solution) - positive

Versus castor bean allergen (5 mg/ml solution - negative

Preserved by: 0.01% merthiolate

Storage: For long storage keep at -20°C. For continuous use keep at 4°C. Avoid repeated freeze-thaw.

TABLE 4.11

Analysis of Castor allergen antiserum

Product: Anticastor Bean Allergen

Produced in: Rabbit

Immunoelectrophoretic pattern of product:

By Ouchterlony test

Versus Castor bean allergen (5 mg/ml solution) - positive

Versus RCA-60 (2 mg/ml solution) - negative

Preserved by: 0.01% merthiolate

Storage: For long storage keep at -20°C. For continued use keep at 4°C. Avoid repeated freeze-thaw.

active ricin and allergen preparations as standards on the same plates set up for determining the level of these substances in castor cake. The handling of the hazardous proteins proved to be an insurmountable obstacle as no agreement could be reached on the matter with the parties concerned. Since the detoxification of the pomace is vital for raising its value and making the end products safe to handle, it was decided to carry on with the effort of quantifying ricin and the allergen by establishing a special laboratory in this Department capable of meeting the relevant health and safety regulations. After consulting with HM Inspector of Factories, a cabinet of the type shown in Fig.3.2 was taken as the basic unit of the analytical facility; the rest of the suggested operational detail is given elsewhere in this thesis. The cost and time scale involved did not enable the unit to be built in time for the results to be presented here.

5.7 SIZE REDUCTION

The size reduction of the castor seeds was carried out by freezing in a suitable medium prior to grinding. Whole or decorticated seeds could be fed to the process, and the freezing agents could be solid, liquid, or a mixture of the solid in a suitable organic solvent. Solid carbon dioxide (Cardice) was employed in most of the experiments, and in others it was substituted by liquid nitrogen. Organic solvents, such as ethanol and acetone, were added to the

cardice in some of the runs.

The procedure adopted was to crush the cardice in a steel mortar and then add the beans. To obtain intimate contact between the cardice and the castor seeds, the freezing in later experiments was carried out in a plastic container fitted with a cover, which was manually rotated. The cool carbon dioxide gas generated was an added factor in improving the freezing process.

When an organic liquid was added to the cardice, the operation was performed in the same steel mortor, but some of the runs were conducted in a round-bottomed flask immersed in the freezing mixture and connected to the rotating mechanism of a rotary evaporator unit. Some cardice was placed in the flask with the beans, and the set up proved satisfactory.

The liquid nitrogen runs were carried out in the steel mortar or a small Dewar flask. No ordinary glass apparatus could be used because of the intense cooling action of liquid nitrogen. The beans solidified almost instantly when they came in contact with the liquid, but with the cardice a certain period was allowed before solidification occurred, and the time required depended on the temperature of the medium and the rate of agitation of the contents. The solidified beans could be broken with a hammer without flattening, and it was found that this could be achieved by treating the beans with cardice for ten minutes.

The frozen beans were then comminuted in a domestic coffee grinder, with a rating of 180 watts, and which was fitted with a transparent plastic cover. To avoid thawing as a result of grinding, cardice was added to the beans in the grinder, and the seed particles were recovered by allowing the dry ice to sublime.

5.8 SOLVENT EXTRACTION

5.8.1 Phase Equilibria

Cyclohexane, ethanol, and acetone were investigated as potential solvents for castor oil extraction. Solubility data were determined for the systems castor oil-water-cyclohexane, castor oil-water-ethanol, and castor oil-water-acetone by the titration method (320) at 25°C. The apparatus was made up of:

i) A constant-temperature water bath

ii) Narrow glass measuring cylinders, capacity 50 cm^3, fitted with glass stoppers.

iii) Pipettes, 10 and 25 ml., and automatic pipette fillers

iv) Bottles, 100 ml and 200 ml capacity

v) Conical flasks 500 ml

vi) Electrical balance capable of weighing milligrams (0.001 g)

Stock solutions of castor oil in each of the solvents were used for preparing solutions of various concentrations in the measuring cylinders. Aqueous solutions of ethanol and acetone were also made up to be titrated with the castor oil. The stock solutions were prepared on the day of the

experiment to avoid prolonged storage.

The procedure was as follows. The water bath was switched on and allowed to reach the set temperature. The solutions to be titrated were placed in the bath, together with the stock solutions. The preweighed measuring cylinders contained known weights of the solutions to be titrated. A drop of distilled water was added to a castor oil-solvent solution and the cylinder was vigorously shaken and returned to the bath. This was repeated until the solution became turbid. The cylinder was then thoroughly dried with paper towel and weighed. A series of similar titrations for various castor oil-solvent concentrations produced the extract side of the binodal curve. Titrating aqueous solutions of ethanol and acetone with castor oil yielded information on the raffinate side of the curve. In the case of cyclohexane, only one side could be obtained because both castor oil and solvent are water immiscible. The results were presented on triangular diagrams and are shown in Figs. 5.13-5.15.

5.8.2 Solvent Extraction

The solvent extraction of the castor seed particles was attempted with liquid ammonia and liquid butane. Both extractions were carried out in the rig designed by Biray (321) for investigating the equilibrium relationships in ternary systems using liquid ammonia as a solvent. The process to be developed was for the separation of aromatic

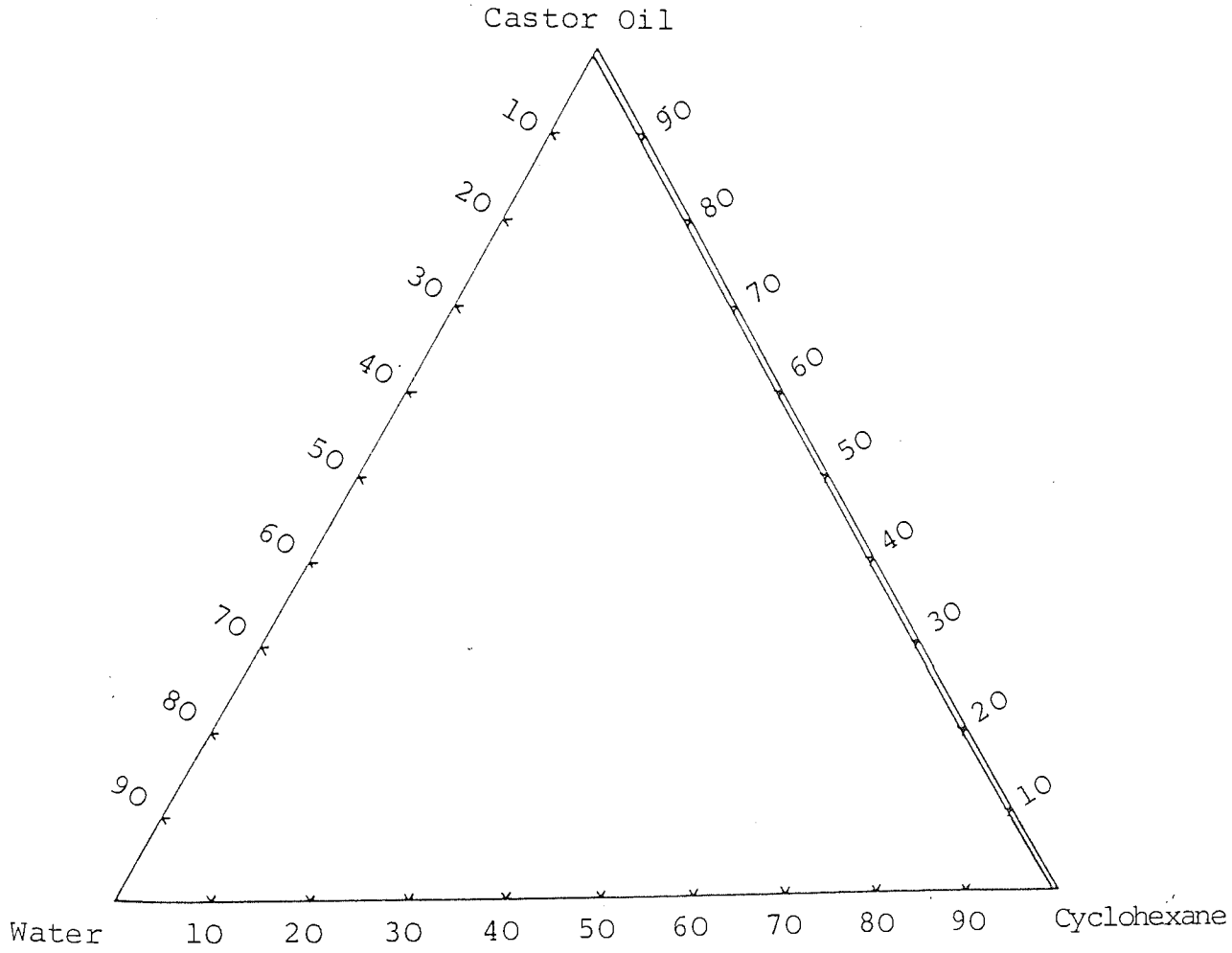


Fig.5.13 The Solubility Curve for the System Cyclohexane-Castor Oil-Water at 25°C

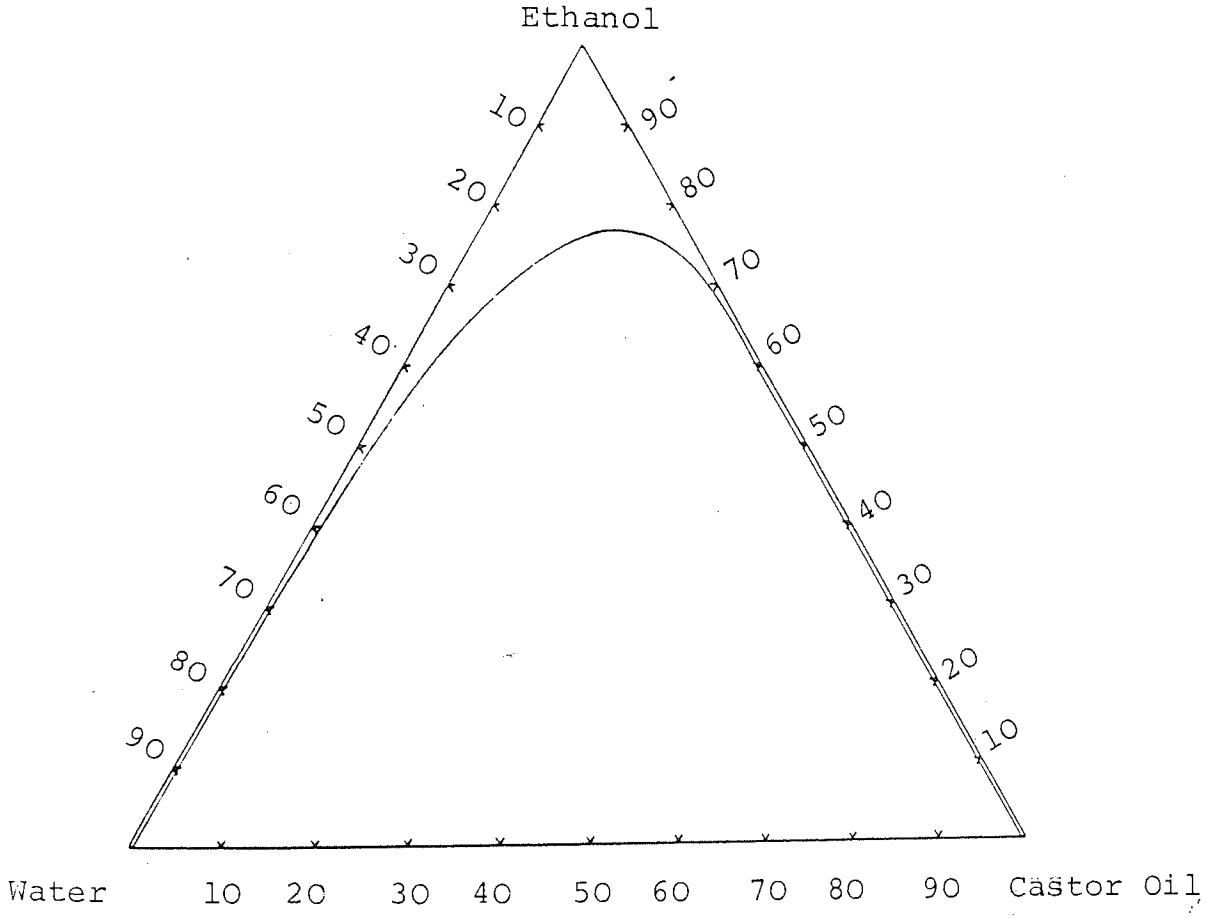


Fig.5.14The Solubility Curve for the System Ethanol-Castor Oil-Water at 25°C

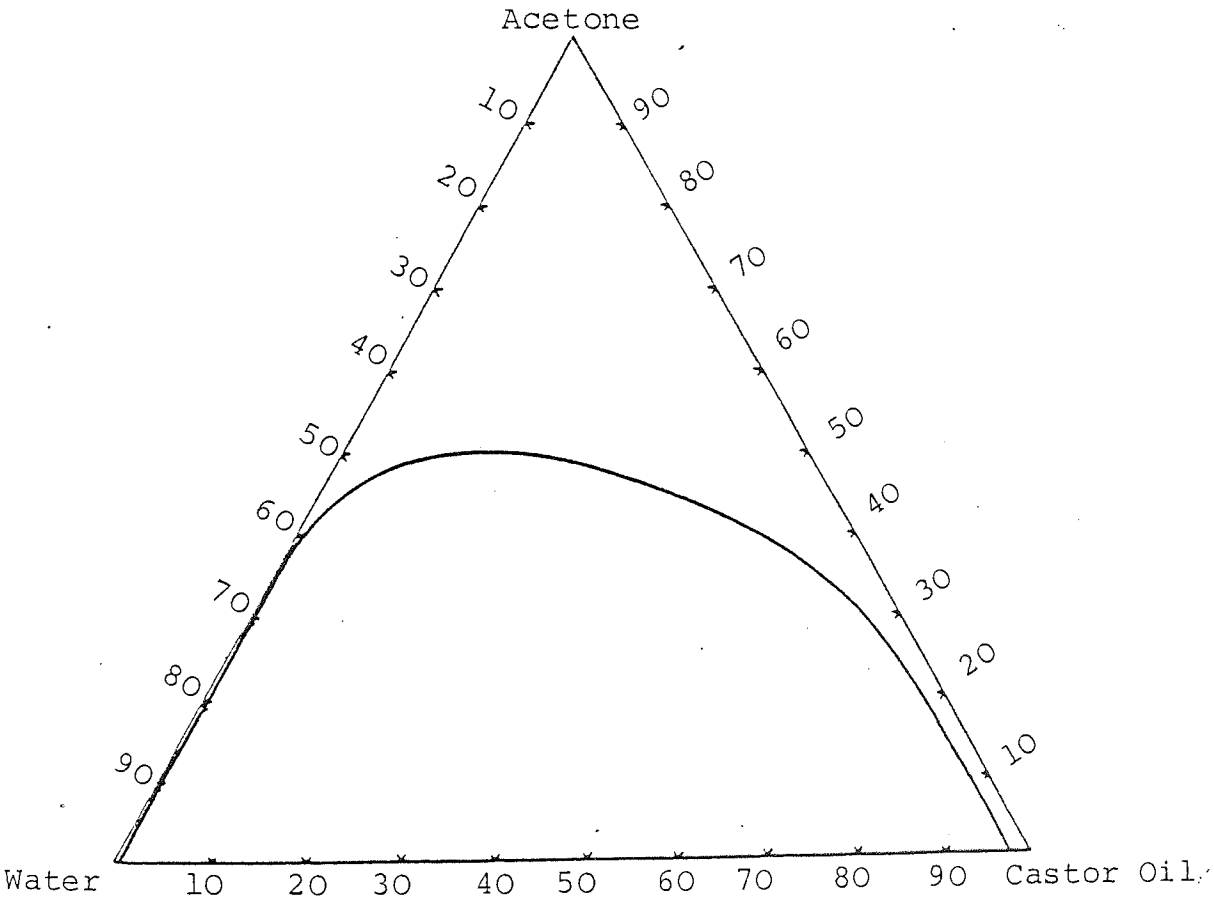


Fig.5.15 The Solubility Curve for the System Acetone-Castor Oil-Water at 25°C

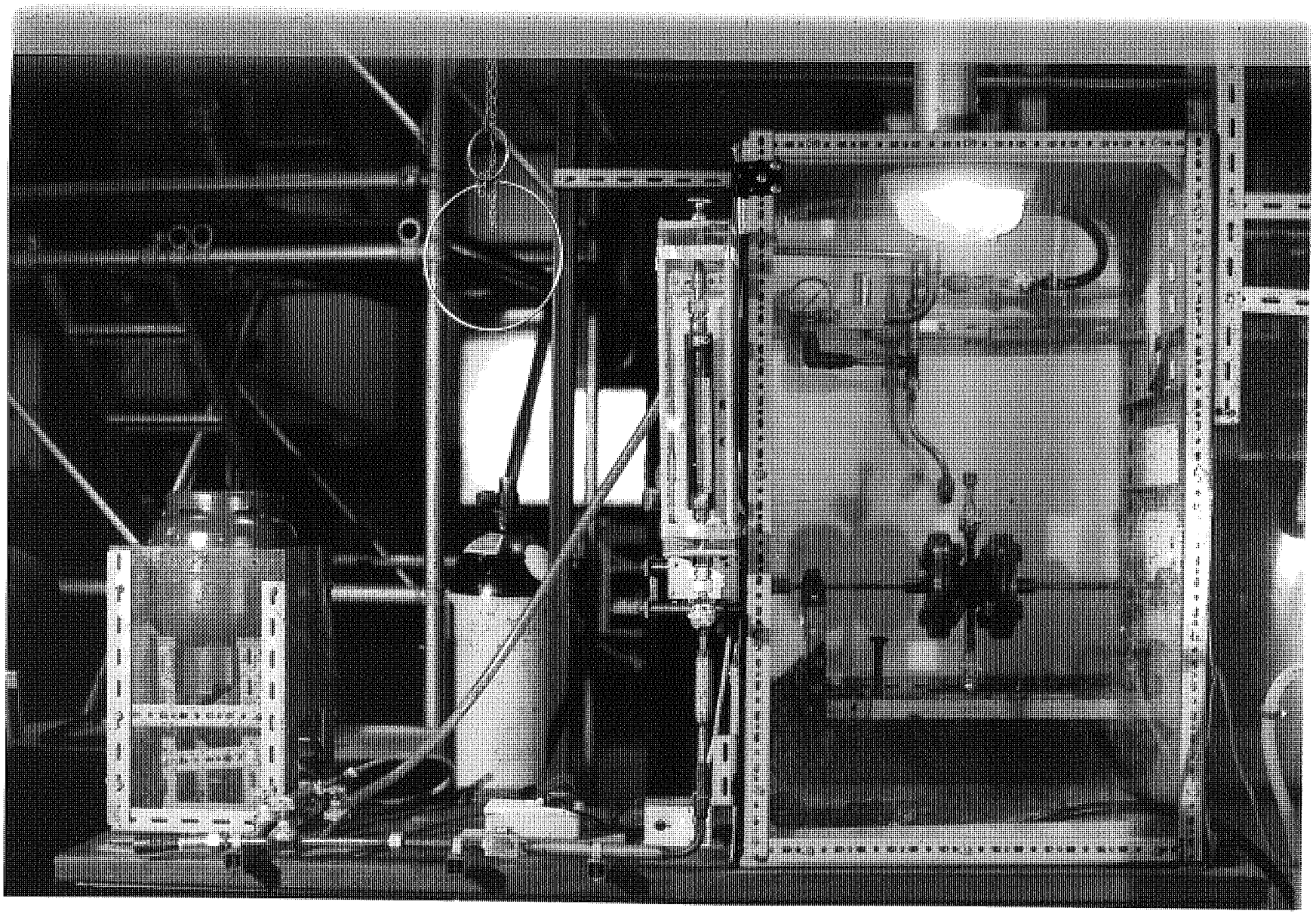
hydrocarbons from mixtures with paraffins.

Modifications were made in the rig relating to the type of pressure vessel employed, the piping system for charging the liquid in the vessel, and the mode of mixing its contents. Fig.5.16 shows a close-up view of the rig.

A flanged pressure vessel constructed of a mild steel pipe, 2 inches in diameter and 2.5 inches long, replaced the original bomb which was designed for liquid-liquid extraction studies. Two 6.5" flanges were welded to the ends of the pipes and their faces were fitted with connections to attach them to two shafts, one connected to the driving motor used for rotating the vessel and the other supported on a journal bearing; the driving shaft also passed through a journal bearing. The bomb was tested hydraulically at 300 psig, and the piping and joints were tested with nitrogen at 150 psig. In addition, whenever a flange was removed for introducing a specimen, the assembled bomb was tested for leaks with nitrogen.

The piping was modified to allow charging the vessel and mixing its contents to be carried out without changing its position; the technique elaborated by Biray (321) had two positions for the bomb: a filling position and a rocking position. The rocking motion was replaced by a rotary motion, mainly because of the mechanical problems associated with the old system. The speed or rotation was limited to 63 rpm to ensure that the contents were intimately mixed.

Fig 5.16 A Close up of the Solvent Extraction Rig



A timer capable of timing up to 60 minutes was installed.

The rig was housed in a cabinet made of handy angles and reinforced perspex, fitted with a door, and connected to an exhaust fan. A nitrogen cylinder was connected to the rig for leak tests as mentioned before.

The liquid ammonia was supplied from a cylinder (ICI, Billingham) placed on a specially designed stand inclined at 5^{o} to the horizontal. A special hose for liquid ammonia service was connected to the cylinder by Ermeto pressure coupling. For the liquid butane supply, a calor gas bottle obtained from a local dealer was inverted to facilitate liquid withdrawal. The bottle was placed inside a reinforced perspex box in a handy angle frame fixed to the working bench. A special hose for high pressure butane service was obtained from the same supplier to connect the cylinder to the rig.

Operating Procedure (see Fig. 5.17)

The bomb was dismantled, cleaned with a detergent solution, and allowed to dry. A known weight of castor seed particles was placed in an extraction thimble and covered with filter paper in such a way that the particles were kept inside the thimble during rotation. The pressure vessel was securely closed and tested with nitrogen at 150 psig, and when it was ensured to be leak-proof the nitrogen supply was disconnected and the bomb depressurized. The

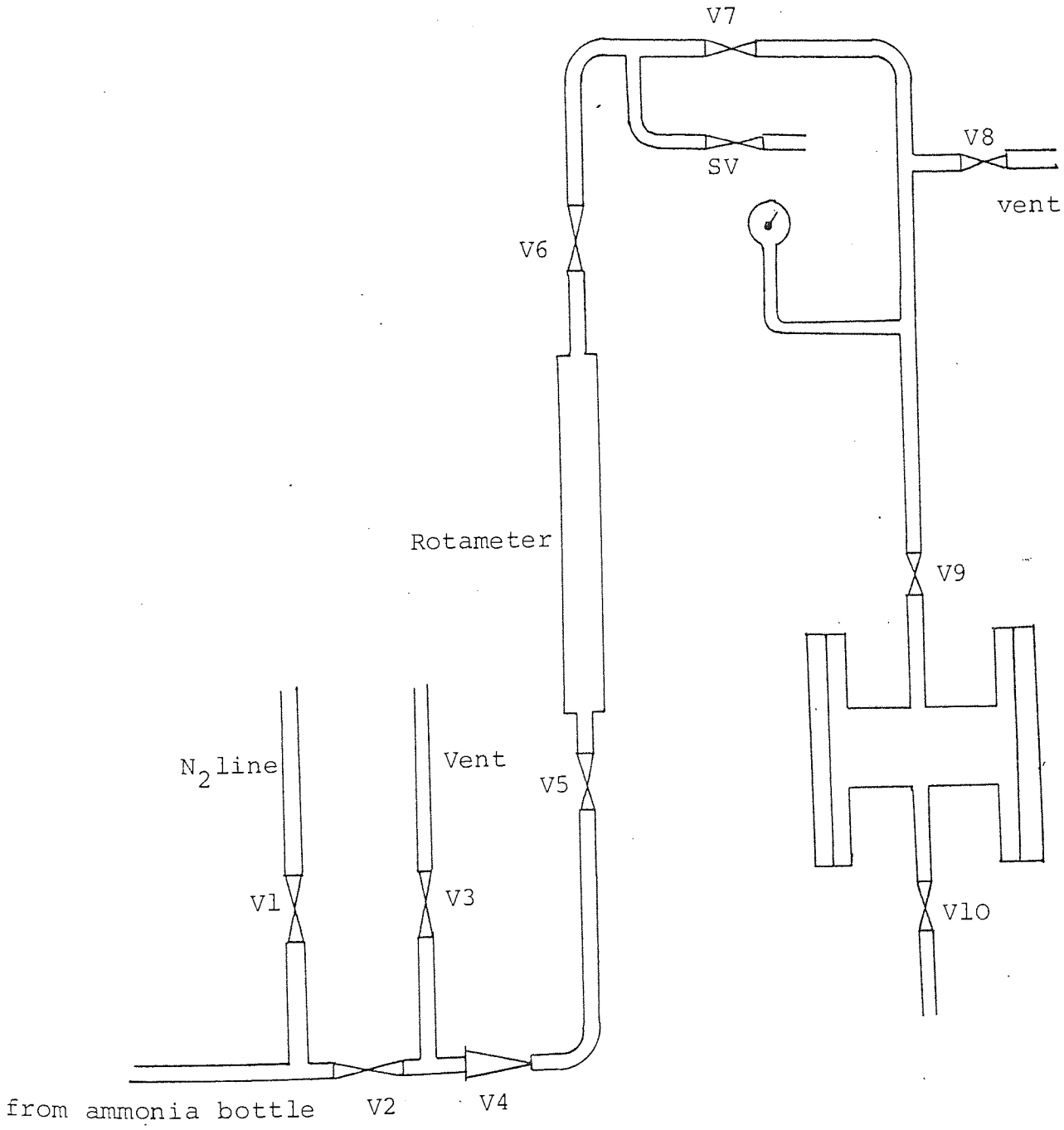


Fig.5.17 Piping Arrangement During Liquid Ammonia Extraction

liquid ammonia was charged in the following sequence. After making sure that the ammonia cylinder valve was closed, the exhaust fan was turned on. To purge all non-condensibles from the charge line, valve V8 was opened. Keeping V1 and V3 closed, V2, V5, V6, and V7 were opened. The cylinder valve was then very slowly and carefully opened to allow ammonia gas to vent to the atmosphere. This was continued until liquid ammonia appeared in the sight glass, at which point valve V8 was closed; the ammonia continued to flow until the pressure gauge read 150 psig, whereupon the valve leading to the bomb, V9, was opened. Having charged the liquid into the bomb, V9 was closed and the ammonia cylinder valve was also closed.

Before disconnecting the piping to allow the bomb to rotate, the liquid ammonia had to be vented. The portion between V4 and the ammonia cylinder was vented by opening V3, and when the ammonia flow ceased V8 was opened to drive off the rest of the liquid. The coupling above V9 was disconnected and the bomb was rotated by switching on the timer. The bomb automatically stopped when the period set on the timer had elapsed. The piping was reconnected to vent the bomb to the atmosphere by closing V7 and opening V8 and V9. After the vessel was completely vented, the thimble was removed and left in the cabinet to dry and was then reweighed.

To prepare the rig for the butane extraction, the ammonia hose was disconnected and the line was capped. The butane cylinder was placed in position and the regulator and hose were both connected to it. After setting up the bomb and testing with nitrogen, the nitrogen line was disconnected and the butane hose was connected to valve V1. Because of the relatively low pressure of the butane cylinder compared to the ammonia cylinder, it was necessary to evacuate the bomb to enable more butane to flow in it. For this purpose, a small vacuum pump was connected to V10. The cylinder valve, the cylinder regulator Valve V3 and V8 were closed and the rest of the valves were opened to evacuate the line. V9 was then closed and the bomb was furhter evacuated. After that V10 was closed and the vacuum pump stopped. To charge the butane, the cylinder valve was opened and then the regulator was turned on; when the liquid appeared in the rotameter, V9 was opened. After the flow has stopped, the butane cylinder was disconnected by closing the main valve and turning off the regulator. V9 was closed and the bomb disconnected. The venting was done as for the liquid ammonia with the exception that V3 was kept closed, i.e. all the venting was made via V8. After mixing the vessel's content for periods of 30 and 60 minutes, as was the case with the liquid ammonia, the bomb was vented and its contents reweighed. For both extractants there was no appreciable extraction as judged by the differences in weight before and after the runs were made.

The hazards associated with liquid ammonia and butane dictated adherence to strict safety precautions. For work with the former solvent, a plastic overall with a head cover was always worn, together with a safety helmet and plastic gloves. A visor attached to an air cannister (VIT-AIR cannister type H, The Leyland and Birmingham RPR Co. Ltd., Leyland Lancs.) was worn from the start of the experiment until the venting was completed, and was also put on when opening or closing valves inside the cabinet at any time during the experiment. A mobile oxygen mask was always brought near the rig and tested before starting the work. Warning signs were displayed around the area, and the emergency exit was ensured usable before the test was started. Since the rig was in the pilot plant, no work was carried out on it whenever undergraduates were conducting experiments in there. The hoses and valves used in the rig were those recommended for liquid ammonia service. As for the liquid butane, the main hazard was its flammability and tendency to form explosive mixtures with air. Hence, the appropriate warning sign was used, the apparatus was carefully checked for leaks, and it was ensured that there was no welding or naked flame within a five metre radius. The warning devices installed in the pilot plant to detect ammonia leaks were discussed by Biray (321).

Chapter Six

RESULTS AND DISCUSSION

6.1 SEED CHARACTERISTICS

Transmission electron microscopy proved to be a useful tool in elucidating the ultrastructure of the castor seeds. The Photomicrographs, Figs. 5.1 and 5.2, were similar to those published by other investigators (14,20,322) and they reveal the large number of oil bodies in a typical cell. The packing of the oil bodies and the intimate contact between them and the protein bodies are noteworthy features of the micrographs.

The micrographs also show the protein bodies to be made up of a crystalloid and a matrix, and there is evidence to suggest that ricin is located in the crystalloid portion (20,322) whereas the allergen is contained in the matrix portion (13). It should be noted that there is no membrane or a similar barrier between the matrix and the crystalloid. Because castor oil is not harmful, it has always been suggested that the toxin and the allergen are insoluble in it. However, the close contiguity between the oil bodies and the protein bodies indicates the need for treating the process of oil extraction with the necessary care it deserves.

The results obtained through the use of the microscope

demonstrate the effectiveness of the fixation procedure adopted and the materials employed. Thus, the choice of the cacadyolate buffer and the two-stage fixation with glutaraldehyde and osmium tetroxide proved successful in producing clear micrographs. Other procedures and chemicals were reported for obtaining information about specific aspects, e.g. the components of the protein bodies.

6.2 DECORTICATION

6.2.1 Analysis of the Results

The procedure developed in this research for hulling castor seeds was successful in overcoming many of the shortcomings of the conventional processes in this field. It was simple to operate and control, and the results demonstrate its potential for industrial application. The analysis of the results will be followed by discussing the problems met with in the course of investigating this process.

Appendix 1 contains the results of the decortication experiments, and it will be seen that the seeds affected by the pressure-vacuum sequence were described as "cracked" and "shattered". The former term denotes those seeds which lost no more than a third of their hulls, or those in which the hull was opened but the two halves still adhered firmly to the seed. The seeds which are graded as "shattered" were those which were completely hulled or those whose adhering

could be removed with the minimum of handling. The former type was not significantly affected when placed on a vibrating table, but the latter readily gave up any clinging hulls upon such treatment.

Figs. 5.10 and 5.11 show the degree of hulling as a function of pressure, the parameter being the seed size: 11.2, 9.5, and 8.0 mm. The percentage of seeds not affected by the decortication process is shown in Fig 5.10, and it can be seen that at a given pressure this percentage increases with decrease of the bean size. Below 50 psig pressure, the beans are not significantly affected, but above that pressure the rate of dehulling starts to pick up so that less than 50% of a charge of 11.2 mm seeds remains intact at 100 psig. For the 9.5 mm size, most of the dehulling takes place between 100 and 150 psig. Less than 10% and 15% of 11.2 and 9.5 mm seeds respectively remains intact at 150 psig. An interesting feature of the figure is the sigmoid shape of the curve for the 11.2 and 9.5 mm sizes.

The 8.0 mm seeds behaved in a markedly different way from the larger seeds. The shape of the curve for this size of seed is flat, and at 150 psig about 88% of a typical charge was still intact. Also much higher pressures had to be applied to the 8.0 mm seeds to obtain a reasonable percentage of hulled seeds. Thus, the rig was operated at the maximum pressure of 275 psig and at that pressure

the intact seeds were 37% of the charge. The change in the percentage of intact seeds for an increment in pressure was much smaller than the corresponding value for 11.2 or 9.5 mm seeds. As the pressure was increased this incremental change also increased but was still less than that of the other two sizes.

The percentage of the seeds shattered is plotted in Fig. 5.11 against the applied pressure for the three seed sizes. For any seed size at a given pressure the total of the ordinates of the two figures, when subtracted from 100%, gives the percentage of that seed which was cracked at the particular pressure. The percentage of seed shattered is the portion of the charge that requires only the removal of hulls, and the kernels can then proceed to the next stage in the process. The cracked and intact seeds must be separated and the latter recycled for dehulling. In this figure there is also the clear difference between the curve for the 8.0 mm seeds and the larger sizes.

The construction of the operational curves shown in Figs. 5.10 and 5.11 can provide useful information about designing and operating equipment for the dehulling of castor seeds. Thus, they can be used to determine the pressure required to achieve a certain degree of dehulling, or for determining the percentage of seed dehulled at a certain pressure. Depending on the maximum degree of hulling, the equipment will be designed to operate at a

certain maximum pressure. It is best to design for the smallest size of seed expected to be processed, as the smaller sizes require higher pressures than the larger sizes.

Although the bulk of the affected seeds seemed to explode on the application of vacuum to the pressurized charge, some of the seeds exploded during the pressurization. It is not known whether those seeds were shattered or whether they only cracked, but the latter possibility is thought more likely because this behaviour was noticed with batches of large seeds, and it occurred at low pressures. Most probably, those seeds partially collapsed or "caved in", and evidence for this was found when the seeds were examined at the end of some runs. The seeds which were completely decorticated were broken at their seam into two identical halves; in some of them the kernel was still intact, but in others the two cotyledons came apart.

At the end of a run, the seeds were recovered from the bomb in a plastic bag and laid out on a clean sheet of paper in an isolated working place for examination. After a number of runs were analysed, tiny spots of oil were observed on the sheet of paper. Since the beans were gently handled, the spots of oil could not be due to squeezing. A possible explanation was later derived from the work of Merma (324) on the theory of the mechanical

extraction of oilseeds. The particular aspect of his work that is relevant to this discussion is what he termed undrained compression, i.e. the pressure above which the oil starts issuing from the seeds. It may be that in some of the runs the undrained compression pressure for the castor seeds was exceeded and the oil spots started to appear. Merma believes that his results are not limited to the rapeseeds and the cashew nuts with which he experimented, and it will be interesting to find out how far the castor beans fit into the suggested pattern.

6.2.2 Difficult-to-hull seeds

The seeds used in these experiments were received at various times, and it soon became clear that the variation in the percentage of unhulled seeds for the same size under the same conditions was significantly different than the average. The reason behind these variations was eventually traced to the batches in which the majority of the seeds were of the difficult-to-hull (dh) type. Most of the dh seeds were cylindrical in shape, as opposed to the ordinary oval shape of the castor seeds, and they were mostly found in the 9.5 and 8.0 sizes. The batches that contained high proportions of the dh seeds yielded fractions of intact seeds that were higher than those deduced from the operational curves for the same conditions of bean size and applied pressure. To prove that the shape factor was one of the reasons for the variations in the results, beans of

the same size were sorted according to their shape and decorticated. The results showed that the cylindrical type was more difficult to hull. Further tests to explain the different responses of the two types of beans included surface studies using the scanning electron microscope, measurement of hull thickness, and determination of compressive strength.

The husks from the various bean sizes and shapes were prepared for scanning electron microscopy as described in the chapter on experimental work, and the micrographs are shown in Figs. 5.3-5.8. The examination of the micrographs reveals no apparent differences between the various husks to which the differences in the results can be attributed. Therefore, the surface characteristics of the beans do not seem to offer an explanation for their behaviour in the decortication process, or provide criteria for sorting the dh seeds in a batch. The importance of this conclusion is that it does away with the need for microscopic analyses of seed lots before processing to classify them according to some surface property. The only classification necessary will be the segregation of the beans according to size.

The honeycomb structure of the husk can be seen in Fig. 5.3, and the micrographs for the thickness of the hull, Figs. 5.7 and 5.8 , suggest that multiple layers exist. The photographs also show the pores that exist in the husk. An illustration for the hull of the sunflower seed

presented by Knowles (325) reveals some similar aspects to those encountered in the castor seed.

Measurements for the thickness of the husk were made from the micrographs and by using a micrometer, and the two sets of results showed reasonable agreement. The average thickness for the ordinary oval type was 0.263 mm, and for the cylindrical type it was 0.392 mm. Thus, the dh seeds appear to have thicker husks than the ordinary type, and this may be one of the factors that contribute to their extra strength.

The investigations on the mechanical strength of the castor seeds were carried out on equipment used for determining the compressive strength of metals in the Metallurgy Department. The intact seeds were placed, one at a time, in the machine in such a way that the load was applied on the seam of each bean. A chart recorder was connected to the machine to draw the compression curve versus time. The load was gradually applied until the bean cracked, whereupon the machine was stopped and the load read. The results could not give a conclusive inference on the compressive strength of the dh beans compared to that of the others. A similar situation, relating to peanut kernels, was reported by Yatsu (326) after our experiments were completed. He also employed microscopy and mechanical strength tests, but these tests did not enable a final interpretation to be made.

In spite of the difficulties reported with this process, the results based on bean sizes are expected to represent the average behaviour of castor beans. It was not possible to relate the differences between the beans to varietal or geographical factors because no information was available about either. Better understanding in this respect could be gained by processing beans of different varieties from a number of countries. The potential of this process is not thought to be impaired by the existence of the dh seeds because the same difficulty would have been faced by the conventional mechanical decortication methods. The work of Yatsu (326), cited earlier, proves this point, as the process he reported involved the hydraulic pressing of peanut kernels and yet some of the lots were more difficult to press than others.

6.3.3 Other Factors

A feature of the developed decortication process is the correlation between the bean size and the fraction of the seeds hulled. In the batches where seeds of various sizes were decorticated there was no apparent relation between the applied pressure and the percentage of seeds that remained intact, but upon sieving the seeds a relation was obtained, the parameter being the bean size, Fig. 5.10 and 5.11. The advantage of working with seed lots of definite sizes is that the fraction of hulled seeds can be controlled by setting the pressure to the required level

as determined from the operational curves.

The size classification of oilseeds does not seem to be practised in industry; thus, there are no reports that castor seeds are sieved before decortication, and in many sunflower crushing plants the seeds are not segregated according to size before dehulling (327). It was suggested (328) that sizing, combined with well-adjusted and maintained separators and cyclones, could prevent loss of oil beyond the 1.9% normally present in the hull and would reduce the cellulose content of the meal to a minimum. The size classification of castor seeds may well prove to be an advantage in the later step of size reduction.

Experiments were carried out to find the number of pressure-vacuum cycles required to reduce the percentage of intact seeds to equilibrium values. It was found that the performance of the process was improved for the oval type of seeds by repeating the cycles on the same batch a number of times. For the dh seeds, the improvement with this technique was only slight. Fig.5.12 shows that for the 11.2 mm seeds, the decrease in percentage of intact seeds with the number of operations at 100 psig pressure is much smaller and happens more slowly than for 9.5 mm seeds at 125 psig. If repetitive decortication did not increase the hulled fraction, the pressure must be raised before any improvement in performance can take place. The alternative would be to separate the intact beans and recycle them for

re-processing, but the former option is preferred, being simpler and more effective. Thus, a central control unit, e.g. a microprocessor, can easily handle the opening and closing of valves for repeating the operation any desired number of times; however, the choice of the actual unit would involve technical and economic factors.

The effect of the residence time on the degree of decortication was also investigated. The beans were allowed to stand under pressure for periods varying between ten minutes and thirty minutes before the sudden release to vacuum. There is no indication from the results of a benefit to be gained by prolonging the pressurization time, and hence it is advisable to release the pressure as soon as its desired level has been attained. This should bring about a considerable simplification to the process and an increase in its capacity.

The decortication experiments were carried out at ambient temperatures. It is possible to operate at higher temperatures in order to inactivate ricin, but this approach was not adopted for a number of reasons. Thus, for the process to be effective in deactivating ricin in the intact seeds high temperatures, of the order of several hundred degrees centigrade, must be employed. This calls for a special design of the processing equipment which was not possible in the circumstances of this research. Also, for the oil and protein quality not to be destroyed the

residence time must be as short as possible, in fact fractions of a minute, but the actual time is governed by the effect of the treatment on the toxin. Furthermore, it is doubtful whether the allergen will be considerably, if at all, affected by such a procedure. It is essential to ascertain the impact of the treatment on the allergen, for if it was not destroyed a second treatment is mandatory, and this may raise questions about the justification of the original treatment.

The moisture content of the seeds in these experiments was about 6.5%. The only other moisture level investigated was 3.5%, but there was no clear correlation between the moisture content and the fraction of seeds exploded. It is possible that the lack of correlation be due to the variations discussed earlier. The possibility of continuous operation was investigated, and it was concluded that there is a potential for such a mode of operation for this process.

6.2.4 Application to Other Oilseeds

The successful extension of the decortication technique to oilseeds other than the castor is desirable for enhancing its industrial potential. An attempt was therefore made to find out how some of the important oilseeds would respond

to the pressure-vacuum treatment. However, the choice of the seeds to be tested was limited to those which were readily available, and thus the experiments were carried out on peanuts, cocoa beans, and sunflower seeds. These oilseeds are similar to castor in possessing hulls that do not fit tightly around their kernels, as opposed to seeds such as rapeseeds and soya beans whose husks closely adhere to their kernels. The former class of oilseeds should be easier to dehull with the technique being discussed, while the latter would be expected to need higher pressures, so that they will be weakened by the alternating pressure levels imposed. This point will be further discussed in connection with the mechanism of the process.

The oilseeds to be dehulled were examined to ensure that only the sound ones were processed. Rose cocoa beans, size 8.0 mm, were successfully decorticated at 150 psig. Peanuts and sunflower seeds, however, could not be dehulled even at 275 psig. Since the peanuts used were at least 8 mm in size, the reason for failing to dehull them was not attributed to smallness of size, as in the case with the sunflowers, but rather to a

surface property. It was argued that the peanut shells had a porous structure of such a nature as not to allow them to retain the pressure built inside them long enough, after the pressure release, to make them explode. Thus, if they could be treated, while still under pressure, in such a manner as to enable them to hold the pressure, they would be likely to shatter. The technique chosen for this purpose was carried out as follows. The unshelled peanuts were placed in the bomb together with pieces of polyethylene-glycol distearate wax. The pressure was raised to 150 psig and the bomb was brought in contact with hot water at a temperature that would melt the wax without raising the pressure above a certain level, in this case 175 psig. Sufficient time was allowed to melt the wax, after which the bomb was removed from the hot water bath and was cooled by running cold tap water to solidify the wax. The pressure was released and it was found that the batch was partially dehulled, an indication that the original hypothesis was correct. Although the solidified wax seems to have sealed the pressurized peanuts, the procedure was slow, and an attempt was therefore made to speed it by coating the peanut with substances that may modify its porosity. Trials were made with an aerosol coating spray and with a suspension of high alumina cement in water, but no success was achieved with either agent. The search for alternative materials for this purpose should be guided by the criteria laid down for additives to foods to ensure that no harmful effects are introduced into the oil or cake through the

extraneous agents added.

6.2.5 Mechanism of the Process

The mechanism suggested for explaining the results of the decortication process will be outlined in this section and the supporting evidence will be presented. A sound knowledge of how the process works helps in improving its operation on castor seeds and extending its usefulness to other oilseeds.

Each castor seed has an air pocket between its kernel and hull. Before applying the pressure, the seeds are in equilibrium with the surroundings and the air gap is at atmospheric pressure. When the compressed air is introduced into the bomb the pressure rises in the air gap to the same level as in the bomb. Releasing to vacuum causes the pressure in the bomb to drop; the pressure built in the bean must also drop, but there is a very brief interval in which there is high pressure in the bean's shell and subatmospheric pressure in the surrounding space. It is this pressure difference that explodes the beans in the process of equalizing the pressures inside and outside their shells.

From the above, it can be seen that there are two requirements for the successful implementation of this technique; the first is that the bean should be able to hold the pressure built in its shell and the second is that the force generated by the sudden pressure release should be sufficient to explode the pressurized bean. The

castor seed fulfills the first requirement, and the second depends on the operating conditions and the resistance of the bean to breaking.

There are a number of observations that point to the validity of the suggested mechanism. Thus, when the castor beans were placed in the bomb at atmospheric pressure and suddenly released to vacuum, no dehulling occurred, obviously because the pressure drop was too low to affect them. Applying a 150 psig pressure and releasing to the atmosphere caused only a tiny fraction of the beans, about 1%, to explode, an indication that the pressure difference was not sufficiently high to explode a higher percentage of seeds. When the high pressure was released to vacuum, the performance depended on the pressure level and the size of the beans as was explained earlier.

In the course of some investigation the beans were placed in a hot C_{10} hydrocarbon solvent (Clarasol) and it was found that some of them burst. This may have been due to the expansion of the air inside the bean's shell. When the beans were boiled in water, none of them exploded, maybe because the water, which has a small molecular weight compared to the hydrocarbon solvent, quickly replaced the air inside the bean's shell. The experiments with the peanuts also confirm the importance of the pressure drop as the source of the breaking force. Rapeseeds and soya-beans would not be expected to respond to this process favourably, and they actually behaved as predicted for no decortication was achieved with them.

6.3 WORK ON TOXINS AND ALLERGENS

6.3.1 Control Measures

The literature survey conducted on the toxins and allergens of the castor seed clearly showed the seriousness of these hazards occupationally and environmentally. It was therefore necessary to devote considerable time and effort to the question of rendering the castor cake harmless, and to understand the properties of these materials and the way in which they exert their harmful effects so as to be able to suggest methods for their detoxification and assay. The difficulties met with in setting up the assay procedures were mentioned earlier, and they confirm the need for improving on the existing techniques. An appraisal for the detoxification-deallergenization methods reported in the literature will be made in the next section. In the rest of this section the safety and control measures adopted for this research will be evaluated.

The effectiveness of these control measures becomes clear on knowing that no cases of ricin intoxication or allergy to castor seed or castor dust were observed in the Department during the whole period of this research. The basis of the control policy was restricting access to the seeds and the cake; in fact, no one other than the author handled these materials. The seeds were kept in locked containers and they were manipulated in isolated working spaces. Moreover, extractions were carried out

in a fume cupboard. Any residues which were to be discharged were placed in plastic bags that were securely sealed, clearly labelled, and properly disposed of as chemical waste. Glassware was washed with dilute sodium hypochlorite before further cleaning. The equipment used for grinding the seeds was chosen with the criterion of being leak-proof to prevent any dust from escaping.

These results prove that it is possible to obtain reasonable protection from the harmful components of the castor seeds by adopting the proper safety precautions. In large-scale production, these considerations apply, in addition to the screening of personnel to make sure that none have allergy complaints.

6.3.2 Appraisal of the Reported Detoxification - Deallergenization Methods

In spite of the large number of reports on the deactivation of ricin, the castor allergens, or castor pomace, not all of the proposed treatments would be of practical value. The first observation regarding these processes is that most of them were not designed for the total removal of the harmful constituents in the castor cake. Thus, the primary emphasis was on ricin detoxification, but it soon became evident that the cake was still a hazardous material because of its allergen content, and new measures were suggested for dealing with this problem. It would have been expected that the castor pomace be the test material for the detoxification trials; instead, it

is found that a substantial number of the reports in the literature describe work with pure ricin (and later allergen) preparations with no subsequent application of the results to the commercial product. Also the scale of the treatment was usually bench-scale, and only in the last decade have pilot plant experiments been undertaken on castor cake deallergenization. On the industrial scale, only two cases are reported of detoxification processes whose products were meant for utilization as animal feed. It is not stated whether the allergens have been destroyed or not.

Three types of processes have been described:physical methods, chemical methods (organic and inorganic chemicals), and microbial and enzymatic methods.

The physical methods employed either dry heat, steam, UV radiation, or Ultrasonic waves; all these agents are non-toxic and they do not constitute occupational or environmental hazards, but the treated material loses part, or all, of its nutritive value. It has always been realized that high temperatures are desirable for abolishing the biological activity of the toxic factors in the cake, but a balance must be made between the extent of deactivation and the damage done to the heat-labile amino acids in the proteins. Low residence times are desirable because they increase the capacity of the process, but a minimum time must elapse before the cake is rendered harmless. There is an obvious

objection to the use of dry heat because of the high temperatures involved (205°C) and the charring that occurs in the cake. The steam treatment is more practical and can be incorporated in existing plants, but it has the limitations already mentioned.

There is only one reference to the attempted detoxification of ricin by ultrasonic waves (329) but no details are given on the extent of the detoxification, the frequency used, or the material treated. The implementation of this technique requires that the waves be propagated in solution, and it may be possible to soak the castor seed particles in a solvent before extraction, or apply the waves to a suspension of cake in water. The former option is preferred if its safety is proved; the other option requires the additional step of drying the treated product.

UV radiation was used for deactivating ricin and allergen preparations. The results depend on the concentration of the hazardous material in the treated specimen and on the energy it absorbs. Thus, the UV sources have to be fairly powerful and reasonably close to the material being deactivated. Since the UV treatment was not extended to the castor cake, it is not possible to know the effect of the rays on the nutritive value of the cake.

The chemical methods utilized organic or inorganic chemicals for destroying the activity of ricin and the

castor allergens. Most of the chemicals were used as liquids, but some were in the gaseous form, and in the latter case the test material was placed in solution. Although some of these methods achieved rapid detoxification, they were implemented only on a bench scale and their application on a larger scale may prove to be difficult. An example is potassium permanganate which produced brownish black precipitates of a flocculent nature when it was added to ricin solutions. When concentrated solutions of both ricin and the permanganate were employed, the precipitates were very heavy and resulted in gel-like masses (224). The handling of such material would be troublesome, especially that the cake must be freed of its permanganate content before it can be put to any further use. Moreover, the biological value of the product was not determined, so it is not clear whether the treatment has damaged the protein or not. Also the potassium permanganate was found unsuitable for deallergenizing castor cake (96), and hence a second treatment is necessary for this purpose.

The properties of some of the chemicals investigated in these processes may also limit their application. Thus, chlorine gas detoxified ricin (224) but the toxicity of the gas calls for special care in its handling and storage, and this applies to chlorinated compounds such as sodium hypochlorite. Others, like formaldehyde and hydrogen peroxide, are irritating to the eyes and the nasal passages.

The tendency of hydrogen peroxide to detonate if a certain concentration limit is exceeded is an additional source of hazard that must be carefully looked into. The toxicity and other health-endangering properties of detoxifying agents pose obvious restrictions on the levels at which they are included in the treated charge. The end use of the detoxified product will also dictate restrictions on the choice of the detoxifying agents, especially if it will be incorporated in animal feed.

There are conflicting reports on the effect of enzymes on ricin, and the reports on the allergen are very few indeed. The detoxification of castor cake with proteolytic enzymes was patented (253). With the advent of a new era in biotechnology as a result of the successful commercial-scale extraction and purification of a large number of enzymes, it may be worthwhile applying this technology to castor cake detoxification. There are numerous applications for enzymes technology in the food industry and a special area of interest is in the processing of oilseed proteins. This discussion will be limited to the detoxifying enzymes. A review of the subject was presented by Liener (157).

Petersen (330) highlighted the benefits of the enzyme hydrolysis of protein-containing raw materials, and his arguments can be summarized as follows.

1. It has been found that the hydrolysis of proteins in such raw materials may increase yields in recovery processes, improve functional properties or improve process methodology e.g. with regards to providing a better means of process control.

2. The hydrolysis of non-proteinaceous materials accompanying the proteins may have a beneficial impact both on yields and on ease of recovery.

3. The mild conditions characteristic of enzymic processes enable a protein to retain its nutritive value better than in the traditional acidic or alkaline hydrolysis.

The hazards associated with enzyme engineering arise mainly from the fact that all enzymes are proteins and hence may cause allergy to certain individuals. Some proteolytic enzymes have negative effects on humans; in some instances such effects may be due to a toxin produced by the source of the enzyme. In such cases, the enzyme concerned may be banned from food-grade products, an example being the castor bean lipase which is virtually useless to the food industry due to the toxic and allergenic factors in the castor bean.

Work has been reported on the detoxification of gossypol in cottonseed meal with a suspension of spores from a fungus (10), and on the detoxification of jojoba meal with bactobacilli (58), but the most extensive research

relating to enzymic detoxification was carried out on the seeds of the cruciferous plants, e.g. rapeseed and mustard (8,157,331). Enzyme processing with detoxification as the primary objective is at present on a rather small scale but is likely to grow progressively as consumers become increasingly concerned with food safety and nutritional value (331).

The physical methods of deactivation, especially the steam process, are thought to offer a reasonable compromise between the various factors involved in the treatment of castor pomace. The chemical methods may present acceptable alternatives if the process employed safe chemicals that would deactivate the cake efficiently at economic prices. Although at present in a state of development, the biochemical detoxification of castor cake may find more extensive future applications.

The effect of the deactivation procedures on ricin and the castor allergens was monitored by the various assays referred to in the relevant parts of this thesis, where their nature and limitations were pointed out. Misleading results have been obtained because of the adoption of techniques inappropriate for the job in hand. Thus, tests for agglutinating activity failed to correlate with the toxic properties of castor cake samples because the two properties, i.e. toxicity and agglutinating power, are not related (278). The biological value of the cake was

assessed with feeding experiments, and in some instances different conclusions were arrived at.

The two main objectives of the deactivation processes are to make the pomace a safe article of commerce and to sell it at the best possible price. In spite of this, the reported methods do not fully discuss the relevant safety aspects in handling the castor seed and cake. It is felt that such a discussion has the important function of creating the awareness of the need for systematic procedures in this area. Since the castor seed and cake are traded in world wide, all parties concerned must adhere to some common safety regulations and quality control checks in an effort to minimize occupational and environmental hazards. As for the economic aspects, there is practically no evaluation, in the published literature, for the cost of alternative treatments or for the effect of changing the process parameters on the cost factors. There is also no discussion of the relation between the detoxification costs and those of the overall process. A possible reason for the lack of such information may be that the reported deactivation techniques are not practised on an industrial scale, but then this type of analysis is notably absent from the pilot plant investigations.

6.4 SIZE REDUCTION

6.4.1 The Method

Freezing the beans before size reduction provided a useful method for handling them during grinding without affecting their oil content. The beans solidified in the treatment and as a result the oil was not released during comminution. The freezing agents employed were solid carbon dioxide (Cardice), Cardice plus an organic solvent, and liquid nitrogen.

The Cardice was a satisfactory freezing agent as it was readily available in blocks or pellets at reasonable prices. Its handling and storage did not pose special problems, and in operation it had distinct advantages over the other freezing agents. Thus, it sublimes to the gas phase without leaving liquid behind, and the cold carbon dioxide gas evolved further helps in the freezing process. It was noticed that the castor particles from seeds frozen by Cardice retained their white colour, unlike those from seeds treated with cardice in an organic solvent; the latter category yellowed after a time. Furthermore, the cardice could be added to the beans in the grinder, an advantage not shared by either of the other two freezing agents. This last point could be industrially utilized by adding the cardice to the beans in the material handling devices to freeze them during transport, as well as adding

it during the milling process.

The addition of organic liquids to the cardice lowered the temperature of the mixture but caused other problems in that the comminuted seeds were wet and became grey on storage. The liquid nitrogen had the lowest temperatures among the three freezing media, but it was expensive and required special precautions in handling and storage.

Because of the low temperatures used, the process could be applied to whole or decorticated seeds, thus offering a possibility for extracting the oil from undecorticated seeds if this course was preferred by a processor for any particular reason. The castor bean kernels thrown into liquid nitrogen developed visible cracks, and this was taken as an indication of the severe stresses set up within them.

It was noticed that the castor bean kernels became soft and spongy when allowed to thaw after cardice freezing. This observation appears to suggest that the oil cells were shattered by the alternate freezing and thawing, thus causing the oil to be released. A similar observation was made during the investigation of the possibility of flaking the castor seeds on a microtome. The procedure employed was to place the kernels in a mould and pour molten wax into it. After the wax has solidified, the block was removed from the mould and sections were cut and frozen in a cardice-acetone mixture before being mounted on

the microtome. However, when the sections were removed from the freezing cell, exposure to the atmosphere caused the oil to flow out of the cells, and as a result the section disintegrated. To avoid oil loss, the freezing-grinding sequence should be performed immediately before oil extraction.

The frozen beans were ground in a domestic coffee grinder. This method of size reduction was adopted after experimenting with other techniques such as mincing, and was preferred to them because of its ease and safety. The grinder had a tight fitting cover, a feature thought to be essential for confining the dust to the processing equipment, and the design was such that it would not operate unless the cover was securely in position. It was found that cooling the grinder with cardice before charging the beans made it unnecessary to add the cardice during pulverization, but the routine procedure was to add the beans together with the cardice before grinding. The uniformity of the size distribution of the particles was easier to achieve by feeding beans of the same size in each batch, and this shows the value of grading the seeds according to size in the earlier step of decortication.

6.4.2 Application to Other Oilseeds

The freezing process was applied to a number of oilseeds, namely soybeans, peanuts (shelled and unshelled),

cocoa beans, and rapeseeds. It was hoped that the differential contraction of the seed's shell and kernel would cause the former to fall off in the case of small oilseeds, rapeseeds and soybeans. The cocoa beans used were of two types; the Rose Cocoa beans were similar to castor seeds; the other type, kindly supplied by Gill and Duffus Co., St. Dunstan's House, 201 Borough High Street, London, SE1 1HW, comprised samples of African and West Indian cocoa beans, and differed from the first type in that their hulls were not closed surfaces surrounding the kernels. The castor seeds, the Rose Cocoa beans, and the peanuts were not dehulled by freezing; however, the skin on the peanut kernels could be removed by freezing and rubbing between two rough surfaces. This prompted trying the same technique with soybeans, but the latter needed longer freezing times than did the peanuts. Hence the freezing was carried out in a freeze dryer for periods varying between 8 hours and 25 hours. At the end of 8 hours, the soybeans were completely decorticated, and the skin was removed from the shelled and unshelled peanuts. The castor seeds, cocoa beans, and rapeseeds were not affected. This process may prove useful for dehulling the small oilseeds if the treatment time could be shortened and its efficiency improved.

6.4.3 Economics - Advantages

The economic factors are of equal importance to the technical considerations for this process. Since refrigeration is an essential operation in the food industry, the

cost of freezing prior to grinding can be reduced by applying some of the more recent developments in low-temperature engineering. Thus, liquid carbon dioxide is being increasingly specified for operations requiring rapid chilling because of its ease of application and relative cheapness (332). Temperatures as low as -80°C have been obtained with liquid carbon dioxide, either by direct injection or by using the liquid to chill circulating refrigerant liquids. During the size reduction of the beans it may be more practical to incorporate solid carbon dioxide rather than the liquified gas. An important consideration that improves the overall economics of a solvent extraction process employing this freeze-grind technique is the elimination of heavy flaking rolls. Although these rolls do not usually form part of a castor oil processing facility, they are widely used for reducing the size of other oilseeds to which the freezing process can equally be applied. The extra cost of pre-grinding freezing may be offset by the reduction made in oil losses as a result of implementing this technique.

Freeze drying is practised on a commercial scale in the food industry, and its success for dehulling the small oilseeds will be largely dependent on having a better economic performance than the existing dehulling processes for those seeds. In addition to the merits shared with the ordinary freezing process, freeze-drying has the additional advantage of the cell rupture that accompanies it,

and it may prove to be a viable future alternative to the present techniques in this area.

The advantages of the size reduction technique developed can be summarized as follows.

1. The oil losses are reduced to the minimum.
2. It can be applied to oilseeds other than the castor seed
3. It eliminates the need for flakers.
4. It has the potential of application for the dehulling of small seeds.
5. The cell rupture that occurs facilitates oil removal.
6. It offers the possibility of reducing the number of units in the process by allowing more than one operation to be carried out on the same piece of equipment.

6.5 SOLVENT EXTRACTION

6.5.1 Phase Equilibria

The short-chain hydrocarbons, especially n-hexane, have found very extensive application as industrial solvents for vegetable oils; however, their extreme flammability is a serious drawback, and efforts have always been made to replace them with safer solvents (333). Since a complete switch from hydrocarbons is unlikely in the near future, a solvent from this class of chemical compounds, cyclohexane, was studied in this research. In addition, ethanol and

acetone were investigated as solvents for castor oil.

Cyclohexane is similar to n-hexane in many respects, but differences also exist. Thus, cyclohexane is less volatile then hexane but it dissolves castor oil at room temperature, unlike the other solvent, but is equally non-toxic. The extraction of castor oil with cyclohexane has not been reported before, but where a comparison was made between these two solvents, their performance was comparable. An example is the extraction of rapeseed oil, for which the only suitable solvents found were n-hexane and cyclohexane (102). The American oilseed industry does not utilize cyclohexane as widely as its European counterpart, mainly because of the nature of the composition of their crude oils (66). The ternary diagram for the system cyclohexane-castor oil-water (Fig.5.13) shows that the miscibility between castor oil and cyclohexane exists in the whole concentration range, thus indicating the suitability of this solvent for extracting the oil. Also, the immiscibility of both castor oil and cyclohexane in water means that the solvent will extract the oil, leaving the moisture in the cake, and hence the solvent will be recovered from the extract phase only.

Ethanol is a good solvent for castor oil because of its polarity, and was reported by a number of investigators. Although its boiling point (78.4^{o}C) is comparable to that of cyclohexane (80-81^{o}C), ethanol has the distinct advantage

of being inflammable, but its miscibility with water means that it will distribute itself between the oil and the moisture in the seeds, hence these must be as dry as possible before they enter the extractor. One of the disadvantages reported for ethanol is that it dissolves the non-fatty components of oilseeds, such as phosphatides and pigments, but since these components do not dissolve in the oil they can later be separated from it.

The cost is a major factor in specifying ethyl alcohol as an industrial solvent for castor oil. With the current interest in using renewable resources for energy production, considerable work is being undertaken for the conversion of substances such as biomass into alcohol for blending with gasoline to obtain the fuel known as gasohol. These processes also depend for their viability on the price of alcohol, and a number of methods have been studied for producing it. These efforts may succeed in making this solvent available at competitive prices. Countries which do not produce oil, like the Sudan, will find it both difficult and expensive to secure the delivery of hydrocarbon solvents and hence ethanol will be an attractive alternative for them.

The triangular diagram for the system ethanol-castor oil-water (Fig.5.14) shows a type one system with a very large area of heterogenity, an indication of the good solvency of ethanol for castor oil. The figure also

shows that the castor oil - ethanol miscella is sensitive to the addition of as low as 0.5% water, and thus the moisture content of the seeds and the percentage of water in the solvent must be carefully controlled.

Like ethanol, acetone dissolves castor oil at room temperature, but reference to Fig. 5.15 for the ternary system acetone - castor oil - water shows that acetone is less effective than ethanol in extracting castor oil. However, acetone has more tolerance for the presence of water than has the alcohol but it is more toxic and is much more volatile than the alcohol. The high volatility of acetone poses problems of losses by evaporation, and the need for cooler water in the condensers than is neccesary with ethanol or cyclohexane.

Other castor oil solvents reported in the literature such as benzene, toluene, carbon disulphide were not investigated because of their hazardous properties like toxicity.

6.5.2 The Extraction Process

The solvent extraction of the castor seed particles was attempted with liquid ammonia and liquid butane, at room temperature. The use of liquefied gases has the advantage of simplifying the solvent recovery process. Liquid ammonia is polar and it dissolves glycerol which possesses hydroxyl groups in its structure; it was therefore expected to dissolve castor oil. As for liquid butane, it was chosen with the knowledge that straight chain hydrocarbons which are liquid at room temperature, e.g. hexane and heptane, dissolve

castor oil at about 30°C, and it was therefore expected that liquid butane would dissolve the oil at a lower temperature.

Under the conditions employed, neither of these two solvents was effective in extracting castor oil. One of the possible reasons is that the temperature was not high enough to enable the removal of castor oil. To raise the temperature substantially above room temperature would lead to increasing the pressure inside the vessel, especially with liquid ammonia, but the equipment was designed for a working pressure of 150 psi, and therefore a higher temperature could not be investigated.

The solvent removal was effected by decreasing the pressure and allowing the vapours into the exhaust system. The ammonia rig had a recovery system whereby it could be liquefied and re-used, but this was not attempted in this case because of the small amounts of solvent handled.

6.6 THE SUGGESTED PROCESS

Based on the results of this research it is possible to suggest a process for the direct solvent extraction of castor seeds. Although the actual extraction step was not fully investigated, solutions were found for the two problems that were obstacles in the way of preparing the seeds for extraction, namely hull removal and size reduction.

In the envisaged process, the seeds will first be cleaned to remove foreign matter, especially metallic

objects. The clean seeds are then sieved and the various sizes separately stored. The next step is to dehull the batches of beans of the same size with the required number of pressure-vacuum cycles as deduced from the operational curves. After separating the husk from the rest of the batch, the intact seeds are recycled to the decorticator and the kernels are sent to the size reduction unit where they are frozen with cardice or liquid carbon dioxide and comminuted in the presence of cardice. The seed particles will be transported to the extractors; after removing the oil, the exhausted particles are sent to the desolventizers and the oil will proceed for refining, bleaching and deodorization. If some or all of these treatments are not required, the oil can be sent directly to storage. The desolventized cake will be sent to the deactivation section for ensuring the removal of ricin and the castor allergens.

There are a number of industrial systems for cleaning oilseeds and for husk separation from batches of dehulled seeds. Therefore the choice of these process items will be made from the available brands according to the working capacity. An important consideration in this section of the process is to ensure the efficient containment and proper discharge of dust in order to guarantee the safety of the employees and the public at large.

The decorticator will be a pressure vessel with connections to a compressed air source and a vacuum line. Techniques for the quick opening and closure of large vessels

have been developed in industry (338) and are valuable for saving process time. The equipment can be connected to a control unit that will monitor the charging and discharging of seeds, together with the sequence and number of pressure-vacuum cycles.

The frozen kernals can be ground in a device similar to the one adopted for milling cottonseeds in the liquid cyclone press (LCP); the seeds in the LCP are dry-milled in a sieveless, wide-chamber, impact stud mill (339). It may also be possible to consider freezing the beans in this equipment, and this will reduce the number of units in the process.

The direct solvent extraction of castor and other high oil-content seeds is not practiced on a commercial scale but was reported on a bench-scale using the filtration-extraction process (88,340), and the oil produced was No.1 in quality. This method may therefore be attempted for the pilot-plant and large-scale direct solvent extraction of castor seeds. Although the commercial filtration-extraction plants, e.g. those processing cottonseeds, use hexane as a solvent, cyclohexane or ethanol can be introduced in the suggested castor oil process; the only possible limiting factor to the use of cyclohexane maybe the process economics.

Liquid gases such as liquid ammonia and liquid butane may be further investigated as castor oil solvents at temperatures higher than the ambient in suitable equipment. Liquid carbon dioxide is finding increased application in the

extraction of natural substances, and it may prove useful to look into the possibility of utilizing it for leaching castor seeds. The advantages of using this type of solvent were covered in chapter three of the present work.

In the suggested process, the detoxification-deallergenization step is to be carried out on the desolventized cake. The cooking of the castor seeds was considered by some investigators as a means of detoxification, but since this technique leaves residual allergicity in the cake, it is considered better to defer the treatment to the last stages of the process and carry it out in a single operation. Cooking the seeds as a technique for rupturing the oil cells was not presented in this discussion because the pretreatment suggested in the present process, i.e. freeze-grinding, is belived to achieve this goal.

The economics of the process are significantly influenced by the nature of the castor beans and the unique characteristics of castor oil, and the effect of this appears in the choice of processing equipment and the specification of seed storage and transport systems. Thus, the beans are best stored in bags because although bulk storage is possible, it involves complications (69,334). Also, the normal conveying methods cannot be directly used as they cause seed breakage. The selection of processing equipment is affected by the soft bean kernel, hard shell, and high oil content.

An important factor in the economic performance of a castor bean processing facility is that it is strongly

recommended not to crush the beans in a factory that will produce other vegetable oils, especially edible oils, in order to avoid contamination by toxins and allergens. If the castor seeds must be processed with other seeds in the same equipment, then extensive safety and cleaning procedures must be detailed and strictly applied, and this will add to the cost of the process. The location of the plant can also have an influence on its economics; thus, if it is decided to install the castor oil plant away from urban areas for safety reasons, the transportation costs must be included as an extra factor to be considered.

In addition to the usual safety measures in a solvent extraction plant, castor oil extraction requires an efficient system for confining dust and treating it before discharge to the atmosphere. The detoxification of the cake should be an integral part of the process, so that it leaves only after tests were made to ensure its being safe. These factors add extra costs to the process, and it is therefore essential that the various process stages be conducted as efficiently and cheaply as possible. The methods suggested in this research were intended for this purpose, and it is hoped that their implementation will be a contribution towards improving the efficiency and safety of castor bean utilization.

Chapter Seven

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

7.1 CONCLUSIONS

1. A pressure-vacuum technique for dehulling castor seeds was developed. It marks an improvement over the conventional methods in this area, and is simple to operate and has a potential for industrial application.

2. The dehulling technique developed was successfully applied to cocoa beans, and to peanuts with some modification. The equipment used operated satisfactorily but improvements in design and control are possible.

3. The size reduction of castor beans is the second major problem, after dehulling, in the preparation of the seeds for solvent extraction. Freezing the castor beans in cardice and milling them in a bean grinder provided a new and convenient solution. The procedure is simple but efficient; its industrial application is technically feasible, and it is not limited to castor seeds.

4. The dehulling of soybeans was possible with an extension of the freezing process employed for castor seeds.

5. Cyclohexane and ethanol can serve as industrial castor oil solvents if their economic feasibility is proved. From a safety point of view ethanol is superior to cyclohexane, but it has the disadvantage in that its use entails a close control over its moisture content and an

additional processing of the oil-solvent solution.

6. Liquid ammonia and liquid butane were not effective as castor oil solvents under the conditions tested, but they may prove useful under different conditions.

7. The toxins and allergens in the castor seeds and in the resulting cake are serious hazards that require adopting special precautions during and after seed processing. It is necessary to improve the existing deactivation techniques and to look into the assay methods for ricin and the castor allergens with a view to simplifying them and enhancing their sensitivity.

8. Since the major problems in preparing castor seeds for solvent extraction have been tackled, it is now possible to develop a process for their direct solvent extraction.

7.2 RECOMMENDATIONS FOR FURTHER WORK

1. To develop a continuous process for the dehulling operation suggested in this research.

2. To extend the scale and scope of the dehulling operation by increasing the equipment size and investigating the major oilseeds, especially cottonseeds, sunflower, and safflower.

3. To study the effect of temperature and that of the seeds' moisture content on the dehulling operation.

4. To develop a continuous freeze-grinding process.

5. To study the leaching of castor seeds with cyclohexane, ethanol, and mixtures of the two solvents at various temperatures, and to investigate the variations in extraction rates and solution hold-up for the different solvents.

6. To determine the extraction conditions that will minimize the total process costs when the various solvents are used. This requires expressing the cost as a function of the process variables and minimizing that function with a suitable minimization subroutine.

LIST OF REFERENCES

1. Eckey, E.W. Vegetable Fats and Oils, Reinhold Publishing Corp., New York, 1954.

2. Zimmerman, L.H. Castorbeans: A New Oil Crop for Mechanized Production. Advances in Agronomy 10, 257-288, 1958.

3. Salih, S.H. Genetic Variability and Correlation Studies in Castor (Ricinus Communis, L.). A Master of Science Thesis (Agric.). Faculty of Agriculture, University of Khartoum, Khartoum, Sudan, April 1973.

4. Weiss, E.A. Castor, Sesame & Safflower, Leonard Hill, London 1971.

5. Watt, J.M. and M. Breyer-Brandwijk (eds.). Medicinal and Poisonous Plants of Southern and Eastern Africa, second edition, E.S. Livingstone Ltd., Edinburgh and London, 1962.

6. Formo, M.W. "Fats in the Diet," in Bailey's Industrial Oil and Fat Products, Swern, D. (ed.), Chapter 4, pp.233-270, Wiley-Interscience, New York, 1979.

7. Tookey, H.L., C.H. Van Etten, and M.E. Daxenbichler, "Glucosinolates," in Toxic Constituents of Plant Foodstuffs, Liener, I.E. (ed.), Chapter 4, pp.103-142, Academic Press, New York, 2nd ed. 1980.

8. Ohlson, R. and R. Sepp, "Rapeseed and Other Crucifers," in Food Protein Sources, Pirie, N.W. (ed.), Chapter 9, pp.65-78, Cambridge University Press, 1975.

9. Knowles, P.F. Modification of Quantity and Quality of Safflower Oil Through Plant Breeding, J. Amer. Oil Chemists' Soc., 46, 130-132, 1969.

10. Berardi, L.C. and L.A. Goldblat, "Gossypol," in Toxic Constituents of Plant Foodstuffs, Liener, I.E. (ed.), Chapter 7, pp.184-238, Academic Press, New York, 2nd ed. 1980.

11. Miller, P.A. "Genetic Basis and Breeding Procedures for Glandless seeded Cotton" in Glandless Cotton: Its Significance, Status, and Prospects, ARS, US Dept. of Agric. BARC-West, Bld. 005, Room 224, Beltsville, Md. 20705, USA Feb. 1978.

12. Balint, G.A. Ricin: The Toxic Protein of Castor Oil Seeds. Toxicology, 2, 77-102, 1974.

13. Youle, R.J. and A.H.C. Huang, Evidence that the Castor Bean Allergens are the Albumin Storage Proteins in the Protein Bodies of the Castor Bean, Plant Physiol., 61, 1040-1042, 1978.

14. Ory, R.L., L.Y. Yatsu, and H.W. Kircher, Association of Lipase Activity with the Spherosomes of Ricinus Communis. Arch.Biochem.Biophys., 123, 255-264, 1968.

15. Hensarling, T.P., L.Y. Yatsu, and T.J. Jacks, Extraction of Lipids from Cottonseed Tissue, II, Ultrastructural Effects of Lipid Extraction, J. Amer. Oil Chemists' Soc., 47, 224-225, 1970.

16. Jacks, T.J., L.Y. Yatsu, and A.M. Altschul, Isolation and Characterization of Peanut Spherosomes, Plant Physiol., 42, 585-597, 1967.

17. Tombs, M.P. Protein Studies of the Soybean, Plant Physiol., 42, 797-813, 1967.

18. Muller, L.L., T.P. Hensarling, and T.J. Jacks, Cellular Ultrastructure of Jojoba seeds, J. Amer. Oil Chemists' Soc., 52 (5), 164-165, 1975.

19. Appelqvist, L.A. and R. Ohlson (eds.), Rapeseeds, Elsevier, Amsterdam, 1972.

20. Tully, R.E. and H. Beevers, Protein Bodies of Castor Bean Endosperm: Isolation, Fractionation, and Characterization of Protein Components, Plant Physiol., 58, 710-716, 1976.

21. Hussein, A.M., Green Valley Co., Khartoum, Sudan, Private Communication.

22. Rao, T.H. Toxic Factors and their Detoxification in Castor, J.Food Sci.Technol., 7 (6), 77-82, 1970.

23. Davies, A.M.C., V.K. Newby, and R.L.M. Synge, Bound Quinic Acid as a Measure of Coupling of Leaf and Sunflower-seed Proteins with Chlorogenic Acid Congeners: Loss of Availability of Lysine, J.Sci.Fd.Agric., 29, 33-41, 1978.

24. Kulkarni, L.G. Castor, Indian Central Oilseeds Committee, Hyderabad, Examiner Press, Bombay, India, 1959.

25. Hunt, A.S. and C.C. Edgar (eds.). The Oil Monopoly of Ptolemy, in Select Papyri, Vol.2, pp.10-35, William Heineman Ltd., London, 1934.

26. Bewley, J.D. and M. Black, Physiology and Biochemistry of seeds in Relation to Germination, Vol. 1: Development, Germination, and Growth, Springer, Berlin 1975.

27. Canvin, D.T. Formation of Oil in the Seed of Ricinus Communis, L. Can. J. Biochem. Physiol., 41, 1879-1885, 1963.

28. Beevers, H. "Organelles from Castor Bean Seedings: Biochemical Role in Gluconegenesis and Phospholipids Biosynthesis," in Recent Advances in the Chemistry and Biochemistry of Plant Lipids, Galliard, T. and E.I. Mercer (eds.), Chapter , pp.287-300, Academic Press, London, 1975.

29. Skau, E.L., F.C. Magne, R.R. Mod, and R.L. Durr, General Density Equation for Glycerides Oil-Solvent Mixtures. Calculation of Density-Composition-Temperature Data from Oil and Solvent Densities, Ind.Eng.Chem.,47(5), 1043-1049 1955.

30. Formo, M.W. "Physical Properties of Fats and Fatty Acids" in Bailey's Industrial Oil and Fat Products, Swern, D.(ed.), Chapter 3, pp.177-232, 4th ed., Interscience-Wiley, New York, 1979.

31. Kudchadker, A.P., S.A. Kudchadker, and D.K. Singh, "Castor Oil Cracking Products," in Encyclopedia of Chemical Processing and Design, Vol. 6, pp.401-420, Marcel-Dekker, Inc., 1978.

32. Markley, K.S., "Identification of Fatty Acids", in Markley, K.S. (ed.) Fatty Acids, their Chemistry, Properties, Production, and Uses, Part 4, Ch.XXI, pp. 2481-2582, 2nd ed., Interscience Publishers, New York, 1968

33. Singleton, W.S., "Properties of the Liquid State," in Fatty Acids: Their Chemistry, Properties, Production, and Uses, Markley, K.S. (ed.), 2nd ed., Ch.VI-A, Part 5, Interscience Publishers, New York, 1968.

34. Schweitzer, M.K. Continuous Processing of Fats, Leonard Hill Ltd., London, 1951.

35. Naughton, F.C. Production, Chemistry, and Applications of Various Chemicals from Castor Oil, J.Amer.Oil Chemists' Soc., 51 (3), 65-71, 1974.

36. Naughton, F.C., F. Duneczley, C.R. Swenson, T. Kroplinski, and M.C. Cooperman, "Castor Oil," in Kirk-Othmer Encyclopedia of Chemical Technology, Vol.5, pp.1-15, Wiley and Sons, Inc., New York, 3rd ed., 1979.

37. Sonntag, N.O.V., "Reactions of Fatty Acids," in Bailey's Industrial Oil and Fat Products, Swern, D. (ed.), Chapter 2, pp.99-176, 4th ed., Interscience-Wiley New York, 1979.

38. Sonntag, N.O.V., "Sources, Utilization, and Classification of Oils and Fats," in Bailey's Industrial Oil and Fat Products, Swern, D. (ed.), Chapter 5, pp.271-288, 4th ed., Interscience-Wiley, New York, 1979.

39. Anon. Vegetable Oil as Diesel Fuel? J. Amer.Oil Chemists' Soc., 57, (11), 805A-819A, 1980.

40. Bartholomew, D., Vegetable Oil Fuel, J. Amer.Oil Chemists' Soc., 58 (4), 286A-288A, 1981.

41. Fang, Ken Shou, Vegetable Oils as Diesel Fuel for China. M.Sc. Thesis, Department of Agricultural Engineering, University of Nebraska, 1949.

42. Apen, E.M., Jr., W.C. Clark, R.J.M. Horton, and L.D. Scheel, Health Aspects of Castor Bean Dust. Review and Bibliography, U.S. Department of Health, Education, and Welfare, Public Health Service, Bureau of Disease Prevention and Environmental Control, Cincinnati, Ohio, 1967. PHS Publications No. 999-AP-36.

43. Blank, P. Sensitivity to the Oral Administration of Castor Oil, Ann.Allergy, 3, 297, 1945. Cited in Apen,E.M. Jr., et al. Health Aspects of Castor Bean Dust, U.S. Dept. of Health, Education and Welfare, Cincinnati, Ohio, 1967.

44. Horton, J.N. Adverse Reactions to Althesin. Anaesthesia, 28, 182-183, 1973.

45. Clarke, R.S.J., J.W. Dundee, R.T. Garrett, G.K. McArdle, and J.A. Sutton, Adverse Reactions to Intravenous Anaesthetics. A Survey of 100 Reports, Br.J.Anaesth., 47, 575-585, 1975.

46. Lehrer, S.B., R.M. Karr, D.J.G. Müller, and J.E. Salvaggio, Detection of Castor Allergens in Castor Wax, Clinical Allergy, 10, 33-41, 1980.

47. Murti, K.S. and K.T. Achaya, Cottonseed: Chemistry and Technology, Publications and Information Directorate, No.598, CSIR, New Delhi, India, 1975.

48. Betschart, A.A., C.K. Lyon, and G.O. Kohler, "Sunflower, Safflower, Sesame, and Castor Protein," in Food Protein Sources, Pirie, N.W. (ed.), Chapter 10, pp.79-104, Cambridge University Press, 1975.

49. Polit, P.F. and V.C. Sgarbieri, Some Physicochemical and Nutritional Properties of Castor Bean (Ricinus Communis) Protein.J.Agric. Food Chem., 24 (4), 795-798, 1976.

50. A. Sattar, M.F.H., M. Nurul Huda, and M.A. Sattar, Studies on the Utilization of Castor Cake. Part II. Separation and Identification of Amino Acids in Castor Protein by Thin Layer Chromatography and their Quantitative Estimation by Spectrophotometric Method, Bangladesh J.Sci.Ind.Res., XIV(3-4), 325-333, 1979.

51. Circle, S.J. and A.K. Smith, "Soybeans: Processing and Products," in Food Protein Sources, Pirie, N.W. (ed.), Chapter 8, pp.47-64, Cambridge University Press, 1975.

52. Dendy, D.A.V., "Protein Products from Coconuts," in Food Protein Sources, Pirie, N.W. (ed.), Chapter 7, pp.43-46, Cambridge University Press, 1975.

53. Vilhjalmsdottir, L. and H. Fisher, Castor Bean Meal as a Protein Source for Chicken: Detoxification and Determination of Limiting Amino Acids, J. Nutrition, 101 (9), 1185-1192, 1971.

54. Sonntag, N.O.V., "Composition and Characteristics of Individual Fats and Oils," in Bailey's Industrial Oil and Fat Products, Swern, D. (ed.), Chapter 6, pp.289-478, 4th ed. Interscience-Wiley, New York, 1979.

55. Parekh, H.V. Solvent Extraction of Vegetable Oils, Indian Central Oilseeds Committee, Hyderabad. Examiner Press, Bombay, India, 2nd ed., 1964.

56. Spies, J.R., E.J. Coulson, D.C. Chambers, H.S. Bernton, H. Stevens, and J.H. Shimp, The Chemistry of Allergens. XI. Properties and Composition of Natural Proteoses Isolated from Oilseeds and Nuts by the CS-1A Procedure, J.Am.Chem.Sco., 73, 3995-4001, 1951.

57. Bose, P.K. Detoxification of Argemone Oil, J.Amer. Oil Chemists' Soc., 54, 322, 1977.

58. Verbiscar, A.J., T.F. Banigan, C.W. Weber, B.L. Reid, R.S. Swingle, J.E. Trei, and E.A. Nelson, Detoxification of Jojoba Meal by Lactobacilli, J.Agric.Food Chem. 29, 296-302, 1981.

59. Olsnes, S. Toxic Proteins Inhibiting Protein Synthesis. Naturwissenschaften, 59, 497-502, 1972 (English).

60. Sudan Year Book of Agricultural Statistics, 1974.

61. Current Agricultural Statistics (CAS), Volume 1, Number 2, June 1976.

62. Langstraat, A. Characteristics and Composition of Vegetable Oil-Bearing Material, J.Amer.Oil Chemists' Soc., 53 (6), 241-247, 1976.

63. Rickles, R.N. Liquid-Solid Extraction, Chemical Engineering (N.Y.), 157-172, March 15, 1965.

64. Norris, F.A., "Handling, Storage, and Grading of Oils and Oil-Bearing Materials," in Bailey's Industrial Oil and Fat Products, Swern, D. (ed.), Chapter 7, pp.479-510, 4th ed., Interscience-Weiley, New York, 1979.

65. Bredeson, D.Y. Mechanical Extraction, J.Amer. Oil Chemists' Soc., 55 (11, 762-764, 1978.

66. Norris, F.A. "Extraction of Fats and Oils," in Bailey's Industrial Oil and Fat Products, Swern, D. (ed.), Chapter 15, pp.637-718, 3rd ed. Interscience Publishers, New York, 1964.

67. Alderks, O.H. "Cooking of Meats and Recovery of the Oil", in Cottonseed and Cottonseed Products, Bailey, A.E. (ed.), Chapter 15, pp.615-650, Interscience, New York, 1948.

68. Shenouda, W.M. The Effect of Cooking Conditions on the Extraction Efficiency and Quality of Oil from Undecorticated Sudanese Black Cottonseed. Project. Oct. 1975. Thesis for th Degree of M.Sc. in Food Engineering, Procter Dept. of Food and Leather Sciences, University of Leeds, Leeds, England.

69. Cracknell, D., Simon-Rosedowns Ltd., Hull, England. Private Communication (23.11.76).

70. Sharder, J.H. and A.C. Goetz, Operation of the Gainsville Castor Oil Plant, Chem.Met.Eng., 22, 833-837, 1920.

71. Dunning, J.W. Processing of Castor Beans, J.Amer.Oil Chemists' Soc., 31, 290-291, 1954.

72. Anderson, R.T. Expelling Oil from Vegetable or Animal Material such as Copra, Cottonseed, Castor Beans, ... etc. US Patent No. 2,065,848, 1937. Chem. Abstr. 31: 1239 (1937).

73. Ward, J.A., Processing High Oil-Content Seeds in Continuous Screw Presses, J.Amer. Oil Chemists' Soc., 53, (6), 261-264, 1976.

74. Boucher, D.F., J.C. Brier, and J.O. Osburn, Extraction of Oil from a Porous Solid, Trans.Amer.Inst. Chem. Engrs., 38, 967-993, 1942.

75. Osburn, J.C. and D.L. Kotz, Structure as a Variable in the Application of Diffusion Theory to Extraction, Trans.Amer.Inst.Chem. Engrs., 40, 511-531, 1944.

76. King, C.O., D.L. Katz, and J.C. Brier, The Solvent Extraction of Soybean Flakes, Trans. Amer.Inst. Chem. Engrs., 40, 533-566, 1944.

77. Fan, H.P., J.C. Morris, and H. Wakeham, Diffusion Phenomena in Solvent Extraction of Peanut Oil. Effect of Cellular Structure, Ind. Eng. Chem., 40, 195-199, 1948.

78. Othmer, D.F. and J.C. Agarwal, Extraction of Soybeans. Theory and Mechanism, Chem. Eng. Prog., 51, (8), 372-378, 1955.

79. Othmer, D.F. and W. Jaatinen, Extraction of Soybeans. Mechanism with Various Solvents, Ind.Eng. Chem., 51, (4), 543-546, 195.

80. Karnofsky, G., The Theory of Solvent Extraction. J.Amer. Oil Chemists' Soc., 26, 564-569, 1949.

81. Coats, H.B. and G. Karnofsky, Solvent Extraction, II. The Soaking Theory of Extraction, J.Amer.Oil Chemists' Soc., 27, 51-53, 1950.

82. D'Aquin, E.L., H.L.E. Vix, J.J. Spadaro, A.V. Graci,Jr., P.H. Eaves, et al, Filtration-Extraction of Cottonseed, Ind. Eng. Chem., 45 (1), 247-254, 1953.

83. Becker, W., Solvent Extraction of Soybeans, J. Amer.Oil Chemists' Soc., 55, (11), 754-761, 1978.

84. Milligan, E.D., Survey of Current Solvent Extraction Equipment, J.Amer.Oil Chemists' Soc., 53 (6), 286-290, 1976.

85. Critchfield, C.E.M., Safety in Solvent Extraction Plants - Europe, J.Amer.Oil Chemists' Soc., 53 (6), 1976.

86. Karnofsky, G., The Mechanics of Solvent Extraction, J.Amer.Oil Chemists' Soc., 26, 570-574, 1949.

87. Schwartzberg,H., Continuous Countercurrent Extraction in the Food Industry, Chem.Eng.Prog., 76, 67-85, 1980.

88. D'Aquin, E.L., J. Pominiski, H.L.E. Vix, N.B.Knoepfler, B.S. Kulkarni, and E.A. Gastrock, Direct Solvent Extraction of Castor Beans Yields High Grade Oil, J.Amer.Oil Chemists' Soc., 37, (2), 93-97, 1960.

89. Goss, W.H., Solvent Extraction of Oilseeds, J.Amer.Oil Chemists' Soc., 23, 348-354, 1946.

90. Crockin, J.M., Water-Injection Streamlines Miscella Clarification, Chemical Engineering, 57, (11), 160-162, 1950.

91. Hutchins, R.P., Continuous Solvent Extraction of Soybeans and Cottonseed, J.Amer.Oil Chemists' Soc., 53, (6), 279-282, 1976.

92. ASTM D-1539-60 (1974). Taken from "Castor Oil" G.J. Hutzler, pp.524-534, Kirk-Othmer, Encyclopedia of Chemical Technology, 2nd ed. 1964.

93. Wingard, M.R., Extraction Methods for Drying Oils, J.Amer.Oil Chemists' Soc., 36, (10, 483-490, 1959.

94. Ayers, A.L. and J.J. Dooley, Laboratory Extraction of Cottonseed with Various Petroleum Hydrocarbons, J.Amer.Oil Chemists' Soc., 25, 372-379, 1948.

95. Good, R.D., Theory of Soybean Extraction. Paper presented at the 45th Annual Convention of the Tri-State Oil Mill Superintendents Association, June 1970.

96. Gardner, H.K., Jr., E.L. D'Aquin, S.P. Koltun, E.J. McCourtney, H.L.E. Vix and E.A. Gastrock, Detoxification and Deallergenization of Castor Beans. J.Amer.Oil Chemists' Soc., 37, 142-148, 1960.

97. Mottola, A.C., B. Mackey, and V. Herring, Castor Meal Antigen Deactivation - Pilot-Plant Steam Process, J.Amer.Oil Chemists' Soc., 48, (9), 510-513, 1971.

98. Waller, G.R. and S.S. Negi, Isolation of Ricin, Ricinine, and the Allergenic Fraction from Castor Seed Pomace from Two Different Sources, J.Amer.Oil Chemists' Soc., 35, (8), 409-412, 1958.

99. Eaves, P.H. L.J. Molaison, and J. Spadaro, Specifications for a Bench-Scale Reaction Vessel and Cooker, Ind.Eng.Chem., 48, (10), 45A-46A, 1956.

100. Elamin, A.E.,Oil Production from Sudanese Cottonseed by Solvent Extraction, Ph.D. Thesis, Proctor Dept. of Food and Leather Sciences, University of Leeds, Leeds, England, January 1977.

101. Arnold, L.K. and R. Choudhury, Ethanol Extraction of Soybean Oil, J.Amer.Oil Chemists' Soc., 39, 372-380, 1962.

102. Loury, M. and H. Feng, Comparison of Different Solvents for the Extraction of Colza and Linseed, Rev.fran.Corps gras, 5, 83-90, 1958, Chem.Abstr. 52:8593 (1958).

103. Temple, S., 1,1,2-Trichloro-1,1,2-Trifluroethane for Oilseed Extractions, J.Amer.Oil Chemists' Soc., 53, (1), 32-35, 1976.

104. Temple, S. and R.E. Sullivan, 1,1,2-Trichloro-1,1,2-Trifluoroethane for Oilseed Extractions: Demonstration of a Viscosity Effect, JAOCS, 55, (7), 587-90, 1978.

105. Rao, R.K. and L.K. Arnold, Alcoholic Extraction of Vegetable Oils, Part I. J.Amer.Oil Chemists' Soc., 32, (7), 420-423, 1955.

106. Rao, R.K. and L.K. Arnold, Alcoholic Extraction of Vegetable Oils, Part II. J.Amer.Oil Chemists' Soc., 33, (2), 82-84, 1956.

107. Rao, R.K. and L.K. Arnold, Alcoholic Extraction of Vegetable Oils, Part III, J.Amer.Oil Chemists' Soc., 33, (9), 389-391, 1956.

108. Rao, R.K. and L.K. Arnold, Alcoholic Extraction of Vegetable Oils in Aqueous 2-Propanol, J.Amer.Oil Chemists' Soc., 34, (8), 401-404, 1957.

109. Kaparthi, R. and K.S. Chari, Solubilities of Vegetable Oils in Aqueous Ethanol and Ethanol-Hexane Mixtures, J.Amer. Oil Chemists' Soc., 36, (2), 77-80, 1959.

110. Kaparthi, R. and K.S. Chari, Laboratory Investigations on the Extraction of Oil from Vegetable Oil Cakes with Ethanol, J.Amer.Oil Chemists' Soc., 36, (2), 81-83, 1959.

111. Arnold, L.K. and R. Choudhury, Hexane and Ethanol as Peanut Oil Solvents, J.Amer.Oil Chemists' Soc., 39, (6), 296-297, 1962.

112. Ayers, A.L. and C.R. Scott, A Study of Extraction for Cottonseed and Soybean Flakes Using n-Hexane and Various Alcohol-Hexane Mixtures, J.Amer.Oil Chemists' Soc., 29, (6), 213-218, 1952.

113. Frampton,V.L. and A.B. Pepperman, Jr., On the Extraction of Oil from Raw Comminuted Cottonseed Kernels with the Acetone-Hexane-Water Azeotrope. J.Amer.Oil Chemists' Soc., 44, (7), 455-456, 1967.

114. Cross, D.E., D.T. Hopkins, E.L. D'Aquin and E.A. Gastrock, Experiments with Solvent Extraction of Glandless Cottonseed and Glanded Cottonseed, J.Amer.Oil Chemists' Soc., 47, 4A, 1970.

115. Pons, W.A., Jr., and P.H. Eaves, Aqueous Acetone Extraction of Cottonseed, J. Amer. Oil Chemists' Soc., 44, (7), 460-464, 1967.

116. Andre, E., The Industrial Production of Castor Oil, Bull.Sci. Pharmacol., 38, 316-355, 1931, Chem.Abstr., 25:44, 26, (1931).

117. Skipin, A.I., Extraction of Castor Seed, Masloboino Zhirovoe Delo 13, No.3, 1-2, (1937); Chemie Industrie 40, 727, Chem.Abstr. 33: 22837 (1939)

118. Chernukhin, A., Oil Extraction with Dichloroethane, Masloboino Zhirovoe Delo, 13, No.3, 7-8, 1937, Chem. Abstr. 31:8967[9] (1937)

119. Chernukhin, A., D. Gilman, R.Sumenko, and V.Pchelintseva, Extraction of Castor Oil Cake with Dichloroethane at the Saratov Plant, Masloboino Zhirovoe Delo, 16, No.3, 9-10, 1940, Chem.Abstr. 35:923[5], 1941.

120. Goldovskii, A.M. A.A. Bozhenko, and N.F. Neiman, Masloboino Zhirovoe Delo, No. 1, 27-30, No.2, 28-32, No.3, 12-16, 1934.
A Physicochemical and Microscopic Investigation of the Processing of Ricinus Communis by Skipin's Method, Chem. Abstr., 29:8376, (1935)

121. Perkin, F.P., Solvent Extraction of Drying Oils, J.Amer.Oil Chemists' Soc., 27, 451-454, 1950. Chem. Abstr., 47:6672 (1953).

122. Eddy, C.F., Recovering Fats and Oils from Vegetable Materials, U.S. Patent No. 1,607,731. Chem.Abstr., 21:335 (1927).

123. Hassel, B., Solvents and their Proper Use in Oil and Fat Extraction, Seifensieder-Ztg., 56, 370-372, 1929. Chem.Abstr., 24:982, (1930.

124. Swisher, M.C. and G.W. Fiero, The Solvent Extraction of Castor Oil Seeds, J.Amer.Pharm. Assoc., 21, (6), 579-582, 1932.

125. Andre, E.A. Castor Oil, French Patent 677,360, Oct., 19, 1928, Chem. Abstr., 24:3124 (1930)

126. Andre, E.A., Castor Oil, French Patent No. 36,877, Oct., 30, 1928, Chem. Abstr., 25:1112 (1931)

127. Klein, C., Castor Oil, German Patent 580,452, July 11, 1933, Chem. Abstr., 27:4945 (1933)

128. Geyson, J., Extraction of Castor Oil, Belgium Patent No. 395,785, May 31, 1933. Chem. Abstr., 28:2559 (1936)

129. Otin, C. and G. Alexa, The Chemical-Technological Investigation of Castor Oil, An.Inst.Cercetari Agronom. Romanici, 6, 244-74, 1934. Chem. Abstr., 31:7683 (1937)

130. Lindenberg, A. Partition Coefficient Between Oil and Water of a Substance Completely Miscible with both Solvents. The Case of Acetone in the System Castor Oil-Water, Compt.rend.Soc.Biol., 116, 63-65, 1934. Chem. Abstr. 28:4964, 1934.

131. Lindenberg, A.,Partition Coefficients Between Oil and Water of Substances Entirely Miscible with either Methanol and Acetic Acid in the System Castor Oil-Water, Compt.rend. Soc.Biol., 118, 441-44, 1935. Chem. Abstr. 29:3217, (1935)

132. Lindenberg, A., Partition Coefficients Between Neutral Glycerides or their Fatty Acids and Water of a Substance Soluble in either Solvent, Compt.rend. Soc. Biol., 125, 138-140, 1937. Chem. Abstr. 31:6084, (1937)

133. Sly, G., Extraction of Castor Oil, Australian Patent No. 137,130, May 25, 1950. Chem.Abstr. 45:375 (1951)

134. Chayen, I.H., Recovery of Fats and Oils from Nuts and Seeds, German Patent No. 1,006,560, April 18, 1957. Chem.Abstr., 54:16878 (1960)

135. Colbeth, I.M., Solvent Extraction of Castor Oil from Castor Seeds, U.S. Patent No. 2,616,907, Nov. 4, 1952. Chem.Abstr., 47:6684 (1953)

136. Gathman, A., Castor Oil Solvent Extraction, U.S. Patent No. 3,377,368, 9, April 1968. Chem.Abstr. 69:3917.

137. Chatterjee, N.G., New Process for the Extraction of Castor Seed or Cake with Industrial Alcohol, Ind. & News Ed.J.Indian Chemical Society, 2,171-177,1939. Chem.Abstr. 34:2196 (1940)

138. Chatterjee, N.G. and R.S. Saxena, Laboratory Experiments on the Extraction of Castor Seed and Cake with Industrial Alcohol, J.Indian Chemical Soc., Ind. & News Ed., 3,23-28, 1940.
Chem. Abstr. 34:8315 (1940)

139. Ellis, S.R.M., Vapour Phase Extraction Processes, Brit.Chem.Eng., 16 (4-5), 358-361, 1971.

140. Panzner, F., S.R.M. Ellis, and T.R. Bott, The Extraction and Separation at Near Critical Conditions of Components in Some Natural Products, Int.Solvent Extraction Conf. 1977, Volume 2, pp.685-692. The Canadian Institute of Mining and Metallurgy.

141. Wilke, A., Extraction with Supercritical Gases - A Forward to Proceedings of a Symposium on Supercritical Extraction, Angewandte Chemie (International English Edition), Vol.17, No.10, pp.701-784, 1978.

142. Grimmett, C., The Use of Liquid Carbon Dioxide for Extracting Natural Products, Chemistry and Industry, 16 May 1981, pp.359-362.

143. Jolly, D.R.P., Wine Flavour Extraction with Liquid Carbon Dioxide, Process Biochemistry, Aug./Sept., 1981, pp.36-40.

144. Stahl, E., Schütz, E. and H.K. Mangold, Extraction of Seed Oils with Liquid and Supercritical Carbon Dioxide, J.Agric.Food Chem., 28, 1153-1157, 1980.

145. Peter, S. and G. Brunner, The Separation of Nonvolatile Substances by Means of Compressed Gases in Countercurrent Processes, Angew.Chem.Int.Ed. Engl., 17, 746-750, 1978.

146. Stahl, E., W. Schilz, E.Schütz, and E. Willing, A Quick Method for the Microanalytical Evaluation of the Dissolving Power of Supercritical Gases, Angew.Chem.Int.Ed. Engl., 17, 731-738, 1978.

147. Bernardini, E., Batch and Continuous Solvent Extraction, J.Amer.Oil Chemists' Soc., 53, (6), 275-278, 1976.

148. Wingard, M.R. and W.C. Shand, The Determination of the Rate of Extraction of Crude Lipids from Oil Seeds with Solvents, J.Amer.Oil Chemists' Soc., 26, 422-426, 1949.

149. Smith, C.T., A New Continuous Solvent Extractor for Oleaginous Substances, J.Amer.Oil Chemists' Soc., 28, (6), 274-277, 1951.

150. Keane, J.D. and C.T. Smith, Solution Hold-Up as a Factor in Oilseed Extractor Design, J.Amer.Oil Chemists' Soc., 35, (5), 199-203, 1958.

151. Wolf Hamm, "Extraction, Liquid-Solid," in Kirk-Othmer Encylopedia of Chemical Technology, Vol.9, pp.721-739, 3rd ed. Wiley & Sons Inc., New York. 1980.

152. Prabhudesai, R.K., "Leaching" in The Chemical Engineers' Handbook, Perry, R.H. and C.H. Chilton (eds.), Section 17, pp.3-8, 5th ed., McGraw-Hill Inc. 1973.

153. Treybal, R.E., Mass Transfer Operations, Chapter 13, pp.628-676, 2nd ed., 1968, McGraw Hill-Kogakusha (Int.Student Ed.)

154. Foust, A.S., L.A. Wenzel, C.W.Clump et al, Principles of Unit Operations, 2nd ed., John Wiley & Sons Inc., 1980.

155. Schweitzer, P.A. Handbook of Separation Techniques for Chemical Engineers, McGraw Hill Book Co., 1979.

156. Panzani, R. and L.L. Layton, Allergy to the Dust of Ricinus Communis (Castor Bean):Clinical Studies upon Human Beings and Passively Sensitized Monkeys, Intern.Arch.Allergy Appl. Immunology, 22,350-356, 1963.

157. Liener, I.E., "Removal of Naturally-Occurring Toxicants through Enzymatic Processing in Food Proteins: Improvement through Chemical and Enzymatic Modification," Feeney, R.E. and J.R. Whitaker (eds.), Adv.Chem.Ser.No. 160, (1977), Chapter 9, pp.283-300, Am. Chem.Soc., Wash. D.C.

158. Rayner, E.T., S.P. Koltun, and F.G. Dollear. Solvent Extraction of Aflatoxins from Contaminated Agricultural Products. JAOCS, 54(3), 242A-244A, 1977.

159. Corwin, A.H., Toxic Constituents of the Castor Bean, J.Med.Pharm.Chem., 4 (3), 483-496, 1961.

160. Clarke, E.G.C., Detection of Ricin, J.Pharm. & Pharmacol., 5, 458-459, 1953.

161. Koja, N., T. Shibta, and K. Mochida, Enzyme-Linked Immunoassay of Ricin, Toxicon, 18, 611-618, 1980.

162. Ghosh, M., B.K. Bachhawat, and A. Surolia, A Rapid and Sensitive Assay for Detection of Nanogram Quantities of Castor Bean (Ricinus Communis) Lectins, Biochem.J., 183, 185-188, 1979.

163. Millerd, A., Biochemistry of Legume seed Proteins, Ann.Rev. Plant Physiol., 26, 53-72, 1975.

164. Seal, R., "Industrial Soya Protein Technology," in Applied Protein Chemistry, Grant, R.A. (ed.), Chapter 4, pp. 87-111, Applied Science Publishers Ltd., London, 1980.

165. Youle, R.J. and A.H.C. Huang, Albumin Storage Protein and Allergen in Cottonseeds, J.Agric.Food Chem., 27, (3), 500-503, 1979.

166. Avers, C.J., Basic Cell Biology, D.Van Nostrand Co.,1978.

167. Armstrong, F.B. and T.P. Bennett, Biochemistry, Oxford University Press, New York, 1979.

168. Mange, A.P. and E.J. Mange, Genetics:Human Aspects, Saunders College/Holt, Rinehart and Watson, 1980.

169. Wagner, R.P., B.H. Judd, B.G. Sanders, and R.H. Richardson, Introduction to Modern Genetics, John Wiley & Sons, New York, 1980.

170. Watson, J.D. Molecular Biology of the Gene, 3rd ed. Menlo Park, California: W.A. Benjamin, Inc. 1976.

171. Watson, J.D. and F.H.C. Crick, Molecular Structure of Nucleic Acids, A Structure for Deoxyribose Nucleic Acid, Nature, 171, 737-738, 25th April 1953.

172. Nirenberg, M.W., The Genetic Code. II. Scientific American, 208 (3), 80-95, 1963.

173. Crick, F.H.C., The Genetic Code, Scientific American, 207, (4), 66-77, 1962.

174. Tietz, N. (ed.), Fundamentals of Clinical Chemistry, 2nd ed., Saunders, Philadelphia:London, 1976.

175. Hyde, R.M. and R.A. Patnode, Immunology, Reston Publishing Company, A Prentice-Hall Company, Reston, Virginian, USA 1978.

176. Scott, M.L., M.J. Thornley, R.R.A. Coombs, and A.R. Bradwell, Measurement of Human Serum lgE and lgA by Reverse Passive Antiglobulin Hemagglutination, Int. Arch. Allergy Appl. Immunol., 64, 222-229, 1981.

177. Bach, J.F. (ed.), Immunology - J.Wiley & Sons Inc., New York 1978.

178. Carmichael, E.B., The Influence of Chemical and Other Agents upon the Toxicity and Antigenic Power of Ricin. III. The Production of Immunity by means of Ricin and Detoxified Ricin. J.Pharmacol. Exptl. Therapeutics, 35, (3), 223-239, 1929.

179. Voller, A., Bidwell, D.E. and Bartlett, A., The Enzyme Linked Immunosorbent Assay (ElISA). A Guide with Abstracts of Microplate Applications. Dynatech Europe, Borough House, Rue du Pre, Guernsey,G.B. 1979.

180. Saltvedt, E., Ricinus and Abrus Agglutinin, Biological Properties and Application in Immunoglobulin Subfractionation, J. Oslo City Hosp., 27, 53-68, 1977.

181. Funatsu, M., "Structure and Toxic Function of Ricin," in Proteins Structure and Function, Funatsu, M., et al (eds.), pp.103-139. Wiley, 1972.

182. Lis, H. and N. Sharon, The Biochemistry of Plant Lectins (Phytohemagglutinins), Ann.Rev.Biochem., 42, 541-547, 1973.

183. Olsnes, S., "Abrin and Ricin" in Perspectives on Toxinology, Bernheimer, A.W. (ed.), Chapter 6, pp.121-147, Wiley, New York, 1977.

184. Osborne, T.B., L.B. Mendel, and I.F. Harris, A Study of the Proteins of thé Castor Bean, with Special Reference to the Isolation of Ricin, Am.J.Physiol., 14, 259-286, 1905.

185. Kabat, E.A., M. Heidelberger, and A.E. Bezer, A Study of the Purification and Properties of Ricin, J.Biol.Chem., 168, 629-639, 1947.

186. Ishiguro, M., T. Takahashi, G. Funatsu, K. Hayashi, and M. Funatsu, Biochemical Studies on Ricin. I. Purification of Ricin, J. Biochemistry (Tokyo), 55,(6), 587-592, 1964.

187. Kraus, K.A., Studies on Ricin, Ph.D. Dissertation. Johns Hopkins University, Baltimore, USA, 1941.

188. Kunitz, M. and M.R. McDonald, Isolation of Crystalline Ricin, J.Gen.Physiol., 32, 25-31, 1948.

189. Nicolson, G.L. and J. Blaustein, The Interaction of Ricinus Communis Agglutinin with Normal and Tumor Cell Surfaces, Biochim.Biophys.Actu., 266, 543-549, 1972.

190. Tomita, M., Kurokawa, T., K. Onozaki, N. Ichiki, T.Osawa, and T. Ukita, Purification of Galactose-Binding Phytoagglutinins and Phytotoxins by Affinity Column Chromatography using Sepharose, Experientia, 28, 84-85, 1972.

191. Gürtler, L.G. and H.J. Hortsmann, Subunits of Toxins and Agglutininins of Ricinus Communis, Biochim. Biophys.Acta, 295, 582-594, 1973.

192. Jaffe, W.G., "Hemagglutinins," in Toxic Constituents of Plant Foodstuffs, Liener, I.E. (Ed.), Chapter 3, pp. 69-102,1st ed. Academic Press Inc. 1969.

193. Liener, I.E. Phytohemagglutinins (Phytotoxins), Ann.Rev.Plant Physiol., 27, 291-319, 1976.

194. Nicolson, G.L., The Interaction of Lectins with Animal Cell Surfaces. Int.Rev.Cytol., 39,90-190, 1974.

195. Brown, J.C. and R.C. Hunt, Lectins, Int. Rev. Cytol., 52, 277-349,1978.

196. Lis, H. and N. Sharon, "Lectins : Their Chemistry and Application to Immunology," in Sela, M.(ed.) : The Antigens, Volume 4, Chapter 7, pp.429-529. Academic Press, 1977.

197. Olsnes, S. and A. Pihl, "Abrin, Ricin and their Associated Agglutinins" in Receptors and Recognition, Series B: The Specificity and Action of Animal, Bacterial, and Plant Toxins, Cuatrecasas, P. (ed.), Chapter 4, pp.130-173, Chapman and Hall, London, 1977.

198. Nicholson, G.L., J. Blaustein, and M. Etzler. The Characterization of Two Plant Lectins from Ricinus Communis and Their Quantitative Interaction with a Murine Lumphoma. Biochemistry, 13, 196-204, 1974.

199. Olsnes, S., Refsnes, K., Christensen, T.B. and A. Pihl, Studies on the Structure and Properties of the Lectins from Abrus Precatorius and Ricinus Communis, Biochim. Biophys. Acta, 405, 1-10, 1975.

200. Funatsu, M., G. Funatsu, M. Ishiguro, and S. Nanno, Chemical Structure and Toxicity of Ricin D, Japan J.Med. Sci. Biol., 23, 264-267, 1970.

201. Kimura, M. and G. Funatsu., Amino Acid Sequences of Two Cyanogen Bromide Fragments CBII and CBIII, and the Complete Sequence of Ala Chain of Ricin D. Agric. Biol. Chem., 45(1), 277-284, 1981.

202. Lin, T. and S. Li, Purification and Physico-Chemical Properties of Ricin and Agglutinin from Ricinus Communis, Eur. J.Biochem., 105 (3), 453-459, 1980.

203. Saltvedt, E., Structure and Toxicity of Pure Ricinus Agglutinin, Biochim.Biophys.Acta, 451, 536-548, 1976.

204. Funatsu, M., G. Funatsu, M. Ishiguro, S. Nanno, and K. Hara, Structure and Toxic Function of Ricin. II. Subunit Structure of Ricin D, Proc.Japan Acad., 47 , 718-723, 1971.

205. Ishiguro, M., Funatsu, G., and Funatsu, M., Biochemical Studies on Ricin. IV. Amino Acid Analysis, Carboxyl and Amino Terminal Amino Acids of Ricin D, Agr.Biol. Chem., 35, (5), 729-733, 1971.

206. Ishiguro, M., M. Tomi, G. Funatsu, and M. Funatsu, Isolation and Chemical Properties of a Ricin Variant from Castor Beans, Toxicon, 14, 157-165, 1976.

207. Li, S.L., C.H. Wei, J.Y. Lin, and T.C. Tung, Amino Terminal sequence of the Anti-Tumor Lectin Ricin A- and B-Chains., Biochem.Biophys.Res.Commun., 65, 1191-1195, 1975.

208. Olsnes, S., E. Satvedt, and A. Pihl, Isolation and Comparison of Galactose-Binding Lectins from Abrus Precatorius and Ricinus Communis, J.Biol.Chem., 249, 803-810, 1973.

209. Fuhrman, F.A., Tetrodotoxin, Scientific American, 217, (8), 60-71, 1967.

210. Moriyama, H. Jap. J. Exptl. Med., 12, 395-409, 1934. Cited by Kraus, K. Studies on Ricin, Ph.D. Thesis, 1941 (Ref. 187).

211. Balint, G.A. Experimentally Induced Contributions to the Therapy of Ricin Intoxication. Tokushima J. Exp. Med., 25, 91-98, 1978.

212. Lin, J.Y., K. Liu, C.G.Chen,
Effect of Ricin on Biosynthesis of Protein, RNA and DNA in Experimental Tumor Cells, Cancer Res., 31, 921-924, 1971.

213. Onozaki, K., M. Tometa, Y. Sakurai

The Mechanism of the Cytotoxicity of Ricinus Communis Phytoagglutinin towards Rat Tumor Cells, Biochem. Biophys.Res. Commun., 48, 783-788, 1972.

214. Olsnes, S., Refsnes, K., and A. Pihl, Mechanism of Action of the Toxic Lectins Abrin and Ricin, Nature, 249, 627-631, 1974.

215. Refsnes, K., S. Olsnes, and A. Pihl, On the Toxic Lectins Abrin and Ricin. Studies on their binding to and Entry into Ehrlich Ascites Cells, J.Biol. Chem., 249, 3557-3562, 1974.

216. Nicolson, G.L., M. Laccrbiere, and T.R. Hunter, Mechanism of Cell Entry and Toxicity of an Affinity-Purified Lectin from Ricinus Communis and its Differential Effects on Normal and Virus-Transformed Fibroblasts, Cancer Res., 35, 144-155,1975.

217. Montanaro, L., S. Sperti, and F. Stirpe. Inhibition by Ricin of Protein Synthesis in vitro. Ribosomes as the Target of the Toxin. Biochem. J., 136, 677-683, 1973.

218. Olsnes, S., Binding, Entry and Action of Abrin, Ricin, and Modeccin, Life Sciences Research Report, Transport of Macromolecules in Cellular Systems, Silverstein, S.C. (ed.), pp.103-116, Berlin: Dahlem Konferenzen, 1978.

219. Spies, J.R. and E.J. Coulson, The Chemistry of Allergens. VIII. Isolation and Properties of an Active Proteic-Polysaccharidic Fraction, CB-1A, from Castor Beans, J.Am.Chem.Soc., 65, 1720-1725, 1943.

220. Dasgupta, B.R. and H. Sugiyama, "Biochemistry and Pharmacology of Botulinum and Tetanus Neurotoxins" in Perspectives in Toxinology, Bernheimer, A.W. (ed.), p.93, Wiley and Sons Inc., New York, 1977.

221. Taira, E., N. Yoshizuka, G. Funatsu, and M. Funatsu, Biochemical Studies on Ricin, Part XXIII. Effects of Physical and Chemical Treatments on the Biological Activity of Ricin D. Agric.Biol.Chem., 42, (10), 1927-1932, 1978.

222. Liener, I.E. and M.L. Kakade, "Protease Inhibitors" in Toxic Constituents of Plant Foodstuffs, Liener, I.E. (ed.), Chapter 2, pp.7-72, 2nd ed., Academic Press, New York, 1980.

223. Nagel, O., Ricinus Cake, J.Soc.Chem.Ind., Lond., 21, 30-31, 1902.

224. Carmichael, E.B., The Influence of Chemical and Other Agents upon the Toxicity and Antigenic Power of Ricin. II. The Detoxification of Ricin by means of Various Agencies, J.Pharmacol. Exptl. Therapeutics, 35, (3), 193-221, 1929.

225. Tangl, H. The Feeding Value of the Extracted Castor-Oil Meal. Kiserletügyi Kozlemenyek, 41, 69-72, 1938. Chem. Abstr. 33: 7422 (1939).

226. Rudolph, W., Extracting the Poison from Castor Oil Residues, German Patent No. 698,200, Oct.3, 1940. Chem. Abstr., 35:6690, (1941).

227. Borchers, R., Castor Bean Oil Meal. I. Destruction of the Toxic Factor, Poultry Science, 28, 568-570, 1949, Cited by R.E. Young in "Further Studies on the Detoxification and Utilization of Castor Pomace" Master of Science Thesis, Oklahoma Agricultural and Mechanical College, 1951. Also see Chem.Abstr. 44:1208, (1950).

228. Kodras, R. The Detoxification and Utilization of Castor Pomace. Master of Science Thesis, Oklahoma Agricultural and Mechanical College, 1949.

229. Kodras, R., C.K. Whitehair, and R. MacVicar, Studies on the Detoxification of Castor Seed Pomace, J.Amer.Oil Chemists' Soc., 26, 641-644, 1949.

230. Young, R.E., Further Studies on the Detoxification and Utilization of Castor Pomace. A Master of Science Thesis, Oklahoma Agricultural and Mechanical College,1951.

231. Petrosyan, E. and V. Ponomarev, Use of Castor Cake for Pig Feeding, Schweinezucht, 4, 33, 1934.
Chem. Abstr. 31:7554 (1937).

232. Jenkins, F.P. Allergenic and Toxic Components of Castor Bean Meal : Review of the Literature and Studies of the Inactivation of these Components. J. Sci. Fd. Agric., 14, 773-780, 1963.

233. Massart, A., Feed for Farm Stock, Belgium Patent No. 438,744, 20 April 1940.
Chem. Abstr., 36:2950 (1942).

234. Thörls, F., Residues from Castor Oil Extraction, German Patent 671,716, Feb. 13, 1939.
Chem. Abstr., 33:6469, (1939).

235. Dreyer, D., and O. Hansen. Compt. rend, Paris, 1907, CXIV, 234. Cited by E.B. Carmichael (Ref. No. 224). Also see Chem. Abstr. 2: 147 (1908).

236. Balint, G.A., Experiments on the Detoxification of Ricin. Toxicology, 1, 175-178, 1973.

237. Harvey, N. and A.L. Loomis. J. Bact., XVII, 373, 1929. Cited by Nakahera, W. and R. Kobayashi, Jap. J. Exptl. Med., 12, 131-135, 1934 (Ref. No. 238).

238. Nakahara, W. and R. Kobayashi, Effect of Short Exposure to Supersonic Waves on Vaccine Virus and Some Bacteria, Jap.J. Explt.Med., 12, 131-135, 1934.

239. Pappenheimer, A.M., Jr., Diphtheria Toxin, Ann.Rev. Biochem., 46, 69-94, 1977.

240. Funatsu, G., S. Miyauchi, N. Yoshizuka, and M.Funatsu, Biochemical Studies on Ricin. XIV. Hybridization between the Heterologous Chains of Nature and Iodinated or Maleyl Ricin D., Agric. Biol. Chem., 41, (7), 1217-1223, 1977.

241. Carmichael, E.B., Detoxification of and the Immunity Production to Ricin by Sodium Ricinoleate, Proc. Soc. Exptl. Biol. Med., 24, 5-7, 1926.

242. Grabar, P. and K. Koutseff, Distinction between Ricinus Toxin, or Ricin,and a Ricinus Allergen, Compt.rend.Soc.biol., 117, 700-701, 1934, Chem.Abstr. 29:3023 (1935).

243 Ambekar, V.R. and K.K. Dole, Detoxification of Castor Cake, Indian J.Dairy Sci., 10, 107-122, 1957.

244. Clementi, A. and DeGaetani, Modifying the Toxicity of Acidified Zootoxins and Phytotoxins by the Addition of Bile, Boll.Soc. Ital. Biol. Sper., 3, 34-35, 1928. Chem. Abstr. 22:2613 (1928)

245. Delga, J., Resistance of Ricin to Various Chemical Agents, Compt.rend.Soc.Biol., 148, 302-304, 1954 Chem.Abstr. 48:12189 (1954)

246. Boquet, P., Attenuation of Ricin by Hydrogen-Peroxide in the Presence of Traces of Copper, Compt.rend.Soc.Biol., 132, 418-419, 1939. Chem.Abstr. 34:3362 (1940)

247. Boquet, P., Role of Copper in Infinitesimal Quantities in the Attenuation by Hydrogen Peroxide of Venoms of Vipera aspis and of Naga tripudians and of a Plant Toxin, Ricin, Ann.Inst. Pasteur, 66, 379-396, 1941.

248. Corwin, A.H. and staff, Preparation and Dispersion of Ricin, Entry No.34 in "Health Aspects of Castor Bean Dust, Review and Bibliography", Apen, E.M., Jr. et al, USDHEW, PHS, Cincinnati, Ohio, 1967, (Ref. No. 42).

249. Moriyama, H. Studies on Ricin. II. Jap. J. Exp. Med., 12, 437-453, 1934.

250. Avery, R.C. and F.B. Moreland, The Reversal of Iodine Detoxification of Ricin, J. Tennesse Acad. Sci., 12, 163-168, 137, Chem.Abstr. 32:9296 (1938)

251. Clark, C.L. and E.G.C. Clarke, The Action of Iodine on the Toxic and Agglutinating Factors of Ricin, Pharm.J., 158, 70-71, 1947, Chem.Abstr. 41:3262 (1947)

252. Lin, J.Y., Cheng, Y.C., Liu, K., et al, Carbohydrate in Abrin, Tocicon, 9, 353, 1971.

253. Darzins, E., Edible Castor Cake, U.S. Patent No. 2,920,963, Jan 12, 1960, Chem. Abstr. 54:10352 (1960)

254. Spies, J.R., Allergens, J.Agric. F.D.Chem., 22, 30-36, 1974.

255. Farrow, R.I., "Enzymes:Health and Safety Considerations" in Enzymes and Food Process, Birch, G.G. et al (eds.), Paper No.12, pp.239-260, Applied Science Publishers, London 1981.

256. Berrens, L., "The Chemistry of Atopic Allergens," in Monographs in Allergy, Vol. 7, Part IV. Allergens in Seeds, pp.74-103, 1971.

257. Lehrer, S.B., J. Taylor, and J.E. Salvaggio, Castor Bean Allergens: Evidence for Distinct Heat-Labile and Stable Entities, Int.Arch.Allergy Appl. Immunol., 65, 69-75, 1981.

258. Scott, M.L., M.J. Thornley and R.R.A. Coombs, Comparison of Red-Cell Linked Anti-IgE and I-Labelled Anti-IgE in a Solid-Phase system for the Measurement of IgE Specific for Castor Bean Allergen, Int.Arch. Allergy Appl. Immunol., 64, 230-235, 1981.

259. Grabar, P. and A. Koutseff, On the Preparation of Ricin Allergen and its Separation from Ricin, Compt. rend. Soc. Biol., 117, 702-704, 1934. Chem. Abstr. 29:3023 (1935)

260. Spies, J.R., E.J. Coulson, H. Bernton, and H. Stevens, The Chemistry of Allergens. II. Isolation and Properties of an Active Protein Component of Cottonseed, J.Am. Chem.Soc., 62, 1420-1423, 1940.

261. Clarke, E.G.C., The Preparation of Castor Seed Allergen, Int.Arch.Allergy Appl. Immuno., 7, 54-55, 1955.

262. Layton, L.L., B.T. Dante, L.K. Moss, N.H. Dye, and F. De Eds., Electrophoretic Fractionation of Soluble Antigenic Proteins from the Seed of Ricinus Communis (Castor Bean), J.Amer.Oil Chemists' Soc., 38, 405-410, 1961.

263. Spies, J.R., E.J. Coulson, D.C. Chambers, H.S. Bernton, and H. Stevens, The Chemistry of Allergens. IX. Isolation and Properties of an Active Carbohydrate-Free Protein from Castor Beans, J.Am.Chem.Soc., 66, 748-753, 1944.

264. Spies, J.R., E.J. Coulson, and H. Stevens, The Chemistry of Allergens. X. Comparison of Chemical and Immunological Properties of CB.1A Preparations from Domestic Castor Beans and Brazilian Castor Bean Pomace., J.Am.Chem.Soc., 66, 1798-1799, 1944.

265. Layton, L.L., L.K. Moss, and F.De Eds., The Complex Nature of Castor Sensitivity, J.Amer. Oil Chemists' Soc., 38, 76-80, 1961.

266. Alilaire, E., Studies on Ricin:Hypersensitivity to Ricin, Ann.Inst. Pasteur, 28, 605-607, 1914, Entry No. 1 in "Health Aspects of Castor Bean Dust,", E.M. Apen, Jr., et al (Ref. No. 42).

267. Schern, K., Experimental Studies of the Practical Utility of Anaphylexis, Arch.Tierheilk, 36:suppl. 590-610, Entry No. 109 in "Health Aspects of Castor Bean Dust,", E.M. Apen, Jr., et al. (Ref. No. 42).

268. Ratner, B. and H.L. Gruehl, Respiratory Anaphylaxis (Asthma) and Ricin Poisoning Induced with Castor Bean Dust, Am. J. Hyg., 10, 236,244, 1929.

269. Knight, B. Ricin - A Potent Homicidal Poison. Brit. Med. J., pp.350-351, 3rd Feb. 1979.

270. Bernton, H.S., On Occupational Sensitization to the Castor Bean, Am.J.Med.Sci., 196-202, 1923, cited by B. Ratner, Dust Hypersensitiveness with Special reference to Castor Bean, J.Allergy, 2, 1-5, 1930.

271. Snell, W.H., Hypersensitivity to the Castor Bean, Science, 59, 300, 1924, cited by B. Ratner, J. Allergy, 2, 1-5, 1930 (see Ref. 270)

272. Arnold, H.L., Poisoning from Castor Beans, Science, 59, 577, 1924, cited by B.Ratner, J.Allergy, 2, 1-5, 1930, (see Ref. 270)

273. Berto, R., and D. Bassi, Ricinus Asthma. 11 Policlinico, 58, 417-426, 1951. (A Complete English translation is included in "Health Aspects of Castor Bean Dust," E.M. Apen, Jr., et al., pp. 63-79, (see Ref. 42)

274. Follweiler, F.L., and D.E. Haley, Toxicity of the Castor Bean, J.Amer.Med. Assoc., 84, 1418, 1925, Cited by B. Ratner, J. Allergy, 2, 1-5, 1930, (see Ref. 270).

275. Corwin, A.H., and Staff, Preparation and Properties of Ricin, pp.29-34 in "Health Aspects of Castor Bean Dust," Apen, E.M., Jr., et al (see Ref. 42)

276. Lucchese, G., Bronchial Asthma and Allergy to Castor Bean Dust, Settimana Medica, 37, 165-170, 1949. (A complete English translation is included in "Health Aspects of Castor Bean Dust," E.M. Apen, Jr., et al, pp. 80-89, (see Ref. No. 42)

277. Rejsek, K., Allergic Manifestations During Processing of Castor Beans, Caspo. Lek. Cesk., 88, 609-613, 1949. (A complete English translation is included in "Health Aspects of Castor Bean Dust,", E.M. Apen, Jr., et al, pp. 115-123, (see Ref. No. 42)

278. Cooper, W.C., V.B. Perone, L.D. Scheel, and R.G. Keenan, Occupational Hazards from Castor Bean Pomace:Tests for Toxicity, Am.Ind.Hyg.Assoc. J., 25, 431-438, 1964.

279. Panzani, R., Respiratory Castor Bean Dust Allergy in the South of France with Special Reference to Marseilles, Intern.Arch. Allergy Appl. Immunol., 11, 224-236, 1957.

280. Topping, M.D., F.H. Tyrer, and R.K. Lowing, Castor Bean Allergy in the Upholstery Department of a Furniture Factory, Br.J.Ind.Med., 38, 293-296, 1981.

281. Figley, K.D. and R.H. Ebrod, Endemic Asthma due to Castor Bean Dust, J. Am. Med. Assoc., 90, 79-82, 1928, Entry No. 48 in "Health Aspects of Castor Bean Dust," E.M. Apen, Jr., et al (see Ref. No. 42)

282. Mendes, E., "Asthma Provoked by Castor-Bean Dust," in Occupational Asthma, Frazier, C.A. (ed.), Chapter 17, pp.272-282, Van Nostrand Reinhold Co., 1980.

283. Mendes, E. and A.B. Ulhoa-Cintra, Etiology of Epidemic Asthma in Bauru, Rev.Paul. Med., 43, 29-44, 1953. (English translation included in E.M. Apen, et al: Health Aspects of Castor-Bean Dust, pp. 90-106, (See Ref. 42)

284. Miskolczy, V., Mass Allergy Connected with the Manufacture of Castor Oil, Nepeges, 31, 253-256, 1950, (English translation included in E.M.Apen, Jr., et al, "Health Aspects of Castor Bean Dust, pp.106-114, (See Ref. 42)

285. Evans, J.M. and J.A.M. Keogh, Side Effects of Drugs: Adverse Reactions to Intravenous Anaesthetic Induction Agents, Brit.Med.J., 17 Sept., 1977, pp.735-736.

286. Mehta, S., Anaphylactic Reactions to Althesin, Anaesthesia, 28, 182-183, 1973.

287. Glen, J.B., G.E. Davies, D.S. Thomson,

An Animal Model for the Investigation of Adverse Responses to Intravenous Anaesthetic Agents and their Solvents, Br. J.Anaesth., 51, 819-827, 1979.

288. Watkins, J., A.M. Ward, and N.A. Appleyard, Adverse Reactions to Intravenous Anaesthetic Induction Agents, Brit. Med. J., 22 Oct. 1977, pp.1084-1085.

289. Hacker, M., M. Koeferl, C.B. Hong, and M.A. Fagan, Cremophor and Emulphor Induced Alterations of Serum Lipids and Lipoprotein Electrophoretic Pattern of Dogs, Res. Commun.Chem.Path.Pharmacol., 31, (1), 119-128, 1981.

290. Watkins, J., A. Padfield, and J.D. Alderson, Underlying Immunopathology as a Cause of Adverse Responses to Two Intravenous Anaesthetic Agents, Brit.Med.J., 6 May 1978, 1180-1181.

291. Spies, J.R., E.J. Coulson, H.S. Bernton, P.A. Wells, and H. Stevens, The Chemistry of Allergens, Inactivation of the Castor-Bean Allergens and Ricin by Heating with Aqueous Calcium Hydroxide, Agr. and Food Chem., 10, 140-145, 1962.

292. Layton, L.L., E. Yamanaka, S.Lee, and T.W. Green, Multiple Allergies to the Pollen and Seed Antigens of Ricinus Communis (Castor Bean), J. Allergy, 33, 232-235, 1962.

293. Layton, L.L., S. Lee, and F. De Eds., Passive Cutaneous Anaphylexis in the Detection of Seed Antigens of Ricinus Communis (Castor Bean), J.Amer.Oil Chemists' Soc., 38, 597-600, 1961.

294. Mottola, A.C., L. Eldridge, and G.O. Kohler, A Comparison of Passive Cutaneous Anaphylaxis Guinea Pig Responses using an Intravenous or Intradermal Route for Antigen Challenge, J.Amer.Oil Chemists' Soc., 41, 458-460, 1970.

295. Mottola, A.C., B. Mackay, H.G. Walker, and G.O. Kohler, Castor Meal Antigen Deactivation - Pilot-Plant Lime Process, J.Amer.Oil Chemists' Soc., 49 (12), 662-664, 1972.

296. Mottola, A.C., B. Mackay, V. Herring, and G. Kohler, Castor Meal Antigen Deactivation - Pilot-Plant Ammonia Process, J.Amer.Oil Chemists' Soc., 49, (1), 101-105, 1972.

297. Coulson, E.J., J.R. Spies, and H. Stevens, The Allergen Content of Castor Beans and Castor Pomace, J.Amer.Oil Chemists' Soc., 37, 657-661, 1960.

298. Kabat, E.A. and M.M. Mayer, Experimental Immunochemistry, 2nd ed., Charles C. Thomas, 1964.

299. Gutman, A.A., "Allergens and Other Factors Important in Atopic Disease," in Allergic Diseases: Diagnosis and Management, Patterson, R. (ed.), Chapter 5, pp.100-147, 2nd ed., J.B. Lippincott Co., Philadelphia, 1980.

300. Daussant, J., R.L. Ory, and L.L. Layton, Characterization of Proteins and Allergens in Germinating Castor Seeds by Immunochemical Techniques, J.Agric. Food Chem., 24 (1), 103-107, 1976.

301. Coombs, R.R.A., A. Hunter, W.E. Jonas, H. Bennich, S.G.O. Johansson, and R. Panzani, Detection of IgE (IgND) Specific Antibody (Probably Reagin) to Castor Bean Allergen by Red-Cell-Linked Antigen-Antiglobulin Reaction, Lancet, May 25, 1968, pp.1115-1118.

302. Prof. R.R.A. Coombs, University of Cambridge, Department of Pathology, Division of Immunology, Private Communication (23rd April 1981)

303. Roitt, I.M., Essential Immunology, 3rd ed., Blackwell Scientific Publications, 1978.

304. Perlman, F., "Allergens," in Toxic Constituents of Plant Foodstuffs, Liener, I.E. (ed.), Chapter 10, pp.295-328, 2nd ed. Academic Press, New York, 1980.

305. Mottola, A.C., G.O. Kohler, and R.T. Prescott, Allergen Deactivation in Castor Pomace with Steam, Feedstuffs, Vol.39, No. 43, p.20, 1967.

306. Corwin, A.H. and Staff, Preparation and Dispersion of Ricin, pp.39-40, in E.M. Apen, Jr., et al., "Health Aspects of Castor Bean Dust," (see Ref. 42)

307. Spies, J.R., E.J. Coulson, H.S. Bernton, H. Stevens, and A.A. Strauss, The Chemistry of Allergens. XIV. Effect of Heat and pH on the Precipitin Reaction and Reagin Neutralizing Capacity of the Castor Bean Allergen CB-1C, Ann.Allergy, 18, 393-400, 1960.

308. Layton, L.L. and F.C. Green, Deallergenizing Castor Beans by Treating with One Molar Ammonium Hydroxide and with at least one Part of Water per Part of Castor Bean Material, US Patent No. 3,294,776: Dec.27, 1966, Chem. Abstr. 66:45632 (1967).

309. Corwin, A.H., and Staff, Preparation and Dispersion of Ricin, Entry No. 32, pp.34-39, in E.M.Apen,Jr.,et al, Health Aspects of Castor Bean Dust, (see Ref. 42)

310. Mottola, A.C., A.P. Hendrickson, D.E. O'Connell, R.Patter, and G.O. Kohler, Pilot Plant Deactivation of Castor Meal Antigen-Lime Process, Agric. and Food Chemistry, 15 (5), 725-729, 1968.

311. Corwin, A.H. and Staff, Preparation and Dispersion of Ricin, Entry No. 34, pp.40-42, in E.M. Apen, Jr. et al, Health Aspects of Castor Bean Dust, (see Ref. 42)

312. Jones, D.B., Proteins of the Castor Bean - Their Preparation, Properties, and Utilization, J.Amer.Oil Chemists' Soc., 24, 247-251, 1947.

313. Raymond, W.D., Castor Beans as Food and Fodder, Tropical Science, 3, 19-24, 1961.

314. Murase, K., S. Kusakawa, C. Yamaguchi, et al., J.Agr.Chem.Soc. Japan, 40, 61-66, 1966 (Japanese). Cited by Fuller, G., H.G. Walker, Jr., A.C. Mottola, D.D. Kuzmicky, G.O. Kohler, and P.Vohra, Potential for Detoxified Castor Meal, J.Amer.Oil Chemists' Soc., 48 (10), 616-618, 1971.

315. Bris, E.J. and J.W. Algeo, Castor Bean By Products for Cattle Rations, Feedstuffs, Vol.42, No. 20, 26-28, May 16, 1970.

316. American Oil Chemists' Society, Official and Tentative Methods, Revised 1973.

317. Demint, R.J., Cucullu, A.F., and C.L. Hoffpauir, Determination of Moisture, Oil and Free Fatty Acids in Castor Beans, J.Amer.Oil Chemists' Soc., 30, (6), 225-227, 1953.

318. Pitt, M.J., Chemical Engineering Department, University of Aston in Birmingham, Unpublished Results.

319. Young, P. Miles Research Laboratories Ltd., P.O.Box 37, Stoke Court, Stoke Poges, Slough SL2 4LY, England; Private Communication.

320. Alders, L., Liquid-Liquid Extraction:Theory and Laboratory Practice, Elsevier Van Nostrand, 1955.

321. Biray, S., The Design of a High-Pressure Solvent Extraction Process Using Liquid Ammonia as Solvent, Ph.D. Thesis, The Chemical Engineering Department, The University of Aston in Birmingham, England, April 1979.

322. Youle, R.J. and A.H.C. Huang, Protein Bodies from the Endosperm of Castor Bean:Subfractionation, Protein Components, Lectins, and Changes During Germination, Plant Physiol., 58, 703-709, 1976.

323. Merma, G.C. and P.B. McNulty, Mechanisms of Mechanical Oil Expression from Rapeseed and Cashew, Solids Separation Processes, Institution of Chemical Engineers Symposium Series No. 59, 1980. pp. 1:2/1-1:2/11.

324. Merma, G.C., Mechanisms of Mechanical Oil Expression from Rapeseed and Casher, Ph.D. Thesis, Agricultural Engineering Department, National University of Ireland, Dublin 1979.

325. Knowles, P.F., "Sunflower:Morphology and Anatomy," in Carter, J.F. (ed.), Sunflower:Science and Technology, Agronomy 19, The American Society of Agronomy, 1978.

326. Yatsu, L., Cell-Wall Architecture of Peanut (Arachis Hypogaea, L.) Cotyledon Parenchyma Cells and Resistance to Crushing, J.Amer.Oil Chemists' Soc., 59, (2), 148A-150A, 1981.

327. Carter, J.F. (ed.), Sunflower:Science and Technology, p.415, The American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, Wisconsin, 1978.

328. Defromont, C., Dehulling of Sunflower Seeds, pp.353-361, Proceedings of the 5th Int. Sunflower Conference, 1972. Cited by Dorrell in J.F. Carter (ed.), Sunflower:Science and Technology, The American Society of Agronomy, Madison, Wisconsin, 1978.

329. Tachibana, T., Jap.J. Microbiol.Pathol., 35, 175-183, 1942 (Japanese)
Cited in G.A. Balint, Ricin:The Toxic Protein of Castor Oil Seeds, Toxicology, 2, 77-102, 1974.

330. Petersen, B.R., "The Impact of the Enzymic Hydrolysis Process on Recovery and Use of Proteins," in: Birch, G.G., N. Blakebrough, and K.J. Parker (eds.) Enzymes and Food Processing, Paper 8, pp.149-176, Applied Science Publishers, London, 1981.

331. Hudson, B.J.F., "Detoxifying Enzymes," in: Birch, G.G., N. Blakebrough, and K.J. Parker (eds.), Enzymes and Food Processing, Paper 13, pp.261-274, Applied Science Publishers, London, 1981.

332. Ballou, W.R., "Carbon Dioxide," in Kirk-Othmer Encyclopedia of Chemical Technology, Vol.4, pp.725-742, 3rd ed., Wiley, 1978.

333. Mustakas, G.C., "Recovery of Oil from Soybeans," in: Erickson, D.R., E.H. Pryde, O.L. Brekke, T.L. Mounts, and R.A. Falb (eds.): Handbook of Soy Oil Processing and Utilization, Chapter 4, pp.49-66, The American Soybean Association (St. Louis, Missouri) and the American Oil Chemists' Society (Champaign, Illinois), 1980.

334. United Nations Industrial Development Organization (UNIDO), Castor Oil Production and Processing, United Nations, New York, 1974.

335. Heilman, J.E. Safety in Solvent Extraction Plants - USA. J. Amer. Oil Chemists' Soc., 53, 293-294, 1976.

336. Her Majesty's Inspector of Factories. Private Communication.

337. Tavasolian, B. and S. Mottaghian. Isolation and Purification of Lectin from Iranian Ricinus Communis Seeds. Iranian J. Pub. Health, 8(3), 145-154, 1979.

338. Eggers, R. Large-scale Industrial Plant for Extraction with Supercritical Gases. Angew. Chem. Int. Ed. Engl., 17, 751-754, 1978.

339. Ridlehuber, J.M. and H.K. Gardner, Jn. Production of Good-Grade Cottonseed Protein by the Liquid Cyclone Process. J. Amer. Oil Chemists' Soc., 51( ), 153-157, 1974.

340. Knoepfler, N.B., J.J. Spadaro, E.J. McCourtney, and H.L.E. Vix. Filtration-Extraction of Flaxseed as Affected by Preparation Variables. J. Amer. Oil Chemists' Soc., 33, 272-276, 1956.

341. Hinkson, J.W., C.A. Elliger, and G. Fuller. The Effect of Ammoniation upon Ricinine in Castor Meal. J. Amer. Oil Chemists' Soc., 49(3), 196-199, 1972.

342. Siddiqi, A.I. and S.O. Freedman. Identification of Chlorogenic Acid in Castor Bean and Oranges. Can. J. Biochem. Physiol., 41,947- , 1963.

343. Freedman, S.O., R. Shulman, J. Knipey, and A.H. Sehon. Antigenic Properties of Chlorogenic Acid. J. Allergy, 35(2), 97-107, 1964.

344. Tros'ko, U.I., et al. Enzymatic Hydrolysis of Low Quality Industrial Fats. Maslo-Zhir. Prom-st., 7, 27-29, 1977. Chem. Abstr. 87: 86656 (1977).

345. Goldblat, L.A. Mycotoxins - Past, Present and Future. J. Amer. Oil Chemists' Soc., 54(4), 302A-309A, 1977.

GLOSSARY

This is a compilation for the definitions of some of the medical and related terms used in the thesis, and has been made by reference to a number of works.

Adjuvant:	A preparation that increases the immune response to an antigen when adminstered simultaneously with it.
Agglutination:	gathering of mobile bacilli into an immobile group.
Agglutinin:	A substance capable of causing agglutination.
Albumins:	A class of simple proteins, soluble in water and dilute salt solutions, and coagulable by heating.
Allergy:	Exaggerated or unusual sensitivity to a substance that is ordinarily harmless to most individuals of the same species.
Amino acids:	organic acids that contain both the acidic carboxyl group and the basic amino group, and are the basic units of which proteins are formed.

Anaphylaxis: IgE - mediated hypersensitivity provoked by injection of an antigen (preparing injection) followed by a later injection of the same intigen (provoking injection) and expressed in its acutest and clearest forms by anaphylactic shock.

Antibodies: Proteins that are elaborated by plasma cells in response to the influence of an antigen and which react specifically with that antigen.

Antigen: A substance that induces the production of an antibody which is able to react specifically with it.

Antiserum: serum containing antibodies

Asthma: (Bronchial Asthma): A chronic disease characterized by difficulty in breathing accompanied by wheezing and over-inflation of the lungs.

Atopy:	A form of immediate hypersensitivity which tends to occur only in those members of a species with a hereditary predisposition.
Bioassay:	The estimation of the activity or amount of a biologically active substance by measuring its effect on a living organism.
Codon:	Nucleotide triplet containing the information necessary to specify a particular amino acid to be used in assembly of a polypeptide chain.
Coenzyme:	A low molecular weight organic compounds that binds to an enzyme and permits its reaction with the substrate.
Cofactor:	A metallic ion necessary for enzyme activity (e.g. Mg, Cu, Fe, Zn).
Conjunctivitus:	Inflammation of the conjunctiva, the membrane that lines the eyelids.

Cyanogens:	substances that produce hydrocyanic acid on hydrolysis
Cytoplasm:	The protoplasm of the cell exclusive of the nucleus.
Deoxyribonucleic acid (DNA):	A macromolecule consisting of a double helix of paired nucleotides, the sequence of which constitutes the genetic code.
Dyspnea:	A general term describing difficulty in breathing
Electrophoresis:	Migration of a molecule (e.g. a protein) or a particle (e.g. a cell) in an electric field.
Erythrocytes:	Red blood corpuscles.
Freund's Adjuvant (complete):	Water-oil emulsion that contains killed mycobacteria in the oil phase.
Freund's Adjuvant (incomplete):	Water-oil emulsion without mycobacteria.

Gene:	Smallest, independently functional unit of genetic material.
Genetic Code:	mRNA codons and their corresponding meaning in protein synthesis.
Globulins:	A class of proteins which are generally insoluble in water, soluble in dilute salt solutions, coagulated by heat and precipitated from solution by half-saturation with $(NH_4)_2SO_4$
Glucosidases:	Enzymes which catalyse the conversion of glucosides to glucose and aglutones.
Glucosides:	A group of glycosides found in plants which, on hydrolysis, yield sugar (which is usually glucose).
Glycosides:	Compounds which if hydrolysed give a sugar and non-sugar (aglutone) residue, e.g. glucosides give glucose, galactosides give galactose.

Hemagglutination: agglutination of red blood cells

Histamine: A vasoactive amine which causes the contraction of smooth muscles. It is released when the cell bound reagin reacts with the antigen and causes the classical wheal and flare response of immediate hypersensitivity.

Homologous: Belonging to the same species

Immunoglobulin: A general term that describes all serum proteins (globulins) that are antibodies, presently divided into five classes: lgG, lgA, lgM, lgD, and lgE. Immunoglobulins are composed of two symmetric heavy and light chains.

Intradermal: Within a muscle

Intraparenteral:	Adminstered by injection, as opposed to oral administration.
Intraperitoneal:	In the heart cavity.
Intravenous:	Within a vein.
In vitro:	Outside a living organism (literally: in glass)
In vivo:	Within the living body.
Lectin:	Cell-agglutinating proteins that have the ability of binding to carbohydrates.
Lymphocytes:	Cells associated with all aspects of specific immunity.
Messenger RNA (mRNA):	RNA that serves as a template for protein sythesis.
Nucleic Acids:	Important constituents of a nucleus; made up of polymers of nucleotide monomers, and have significant functions in genetics. The two main nucleic acids are DNA and RNA.

Nucleotides: The subunits into which nucleic acids are split by the action of the enzyme nuclease; composed of a nitrogenous base (a purine or a pyrimidine), a pentose (sugar:ribose or deoxyribose) and phosphate.

Plasma: The fluid portion of blood after removal of formed elements, the cells and platelets.

Polypeptide: A polymer of amino acid monomers.

Polysome: A cluster of ribosomes held together temporarily by mRNA.

Reagin (Reaginic Antibody): Antibody of specialized immunoglobulin type (IgE in Man) which fixes to tissue cells of the same species so that, on reaction with the antigen, histamine and similar substances are released. Responsible for immediate hypersensitivity.

Rhinitis	Inflammation of the nasal mucous membrane.
Ribonucleic Acid (RNA):	Nucleic acid containing the pentose ribose; present in both cytoplasm and nucleus; intimately engaged in synthesis of proteins.
Ribosomal RNA (rRNA):	The nucleic acid component of ribosomes.
Ribosomes:	Small cellular particles (200Å in diameter) made up of rRNA and protein. Ribosomes are the site of protein synthesis.
Serology:	The study of antigen-antibody reactions in vitro.
Serum:	The fluid portion of the blood after removal of cells.
Subcutaneous:	Under the skin
Transfer RNA (tRNA):	Any of at least twenty structurally similar species of RNA, all of which have a MW 25,000. Each species of tRNA molecule is able to combine covalently with a

specific amino acid and to hydrogen bond with at least one mRNA nucleotide triplet. Also called adaptor RNA.

Appendix No. 1

Decortication Results

Bean size 8.0 mm — 150 psig

	weight (g)	
Charge	300.63	%
Intact	291.03	96.81
Cracked	8.46	2.81
Shattered	0.99	0.33
Charge	300.48	%
Intact	247.16	82.39
Cracked	13.87	4.62
Shattered	38.97	12.99
Charge	300.48	%
Intact	258.52	86.06
Cracked	9.66	3.22
Shattered	32.14	10.70
Charge	298.61	%
Intact	255.99	85.84
Cracked	19.53	6.55
Shattered	22.00	7.38

Bean Size 8.0 mm — 175 psig

	weight (g)	
Charge	300.74	%
Intact	284.85	94.72
Cracked	6.70	2.23
Shattered	8.61	2.86
Charge	300.24	%
Intact	283.27	94.35
Cracked	8.11	2.70
Shattered	7.93	2.64
Charge	300.80	%
Intact	282.69	93.98
Cracked	8.92	2.97
Shattered	8.40	2.79
Charge	299.56	%
Intact	215.22	71.85
Cracked	26.49	8.84
Shattered	57.32	19.15
Charge	299.10	%
Intact	205.10	69.07
Cracked	19.37	6.48
Shattered	71.80	24.01

Bean size 8.0 mm		200 psig
	Weight (g)	
Charge	299.15	%
Intact	168.95	56.48
Cracked	50.38	16.84
Shattered	80.13	26.79
Charge	299.54	%
Intact	194.56	65.04
Cracked	23.48	7.85
Shattered	80.46	26.11
Charge	299.43	%
Intact	222.53	74.32
Cracked	20.19	6.74
Shattered	55.26	18.46
Charge	298.46	%
Intact	277.95	76.37
Cracked	20.36	6.82
Shattered	49.44	16.56
Charge	299.21	%
Intact	246.33	82.33
Cracked	20.03	6.69
Shattered	31.63	10.57

Bean size 8.0 mm 225 psig

	Weight (g)	
Charge	300.74	%
Intact	186.10	61.94
Cracked	17.10	5.69
Shattered	97.23	32.36
Charge	300.67	%
Intact	141.99	47.22
Cracked	22.71	7.55
Shattered	135.03	44.91
Charge	300.06	%
Intact	157.91	52.63
Cracked	18.36	6.12
Shattered	123.61	41.19

Bean size 8.0 mm

250 psig

	Weight (g)	
Charge	300.39	%
Intact	169.86	56.55
Cracked	24.28	8.08
Shattered	106.25	35.37
Charge	300.49	%
Intact	155.98	51.91
Cracked	27.16	9.04
Shattered	116.90	38.90
Charge	300.66	%
Intact	164.75	54.80
Cracked	24.35	8.10
Shattered	110.60	36.79

Bean size 8.0 mm		275 psig
	Weight (g)	
Charge	300.73	%
Intact	113.82	37.85
Cracked	20.56	6.84
Shattered	164.81	54.80
Charge	301.35	%
Intact	114.15	37.88
Cracked	25.75	8.54
Shattered	160.53	53.21
Charge	300.94	%
Intact	105.38	34.89
Cracked	26.73	8.85
Shattered	168.81	55.89

Bean Size 9.5 mm		25 psig
	weight (g)	
Charge	285.06	%
Intact	277.50	98.67
Cracked	3.73	1.33
Charge	291.83	%
Intact	278.35	95.38
Cracked	12.10	4.15
Charge	290.26	%
Intact	277.76	95.69
Cracked	12.40	4.27
Charge	290.40	%
Intact	280.20	96.49
Cracked	10.10	3.48
Charge	290.61	%
Intact	283.57	97.58
Cracked	6.80	2.34

Bean Size 9.5 mm		50 psig
	weight (g)	
Charge	283.7	%
Intact	280.5	98.87
Cracked	-	
Shattered	3.0	1.06
Charge	290.29	%
Intact	282.94	97.47
Cracked	7.40	2.55
Shattered	0.57	
Charge	289.23	%
Intact	276.26	95.51
Cracked	11.43	3.95
Shattered	1.50	0.52
Charge	289.15	%
Intact	283.01	97.88
Cracked	6.04	2.09
Shattered	-	

Bean Size 9.5 mm		75 psig
	weight (g)	
Charge	285.17	%
Intact	268.83	95.38
Cracked	4.97	1.76
Shattered	8.05	2.86
Charge	296.1	%
Intact	283.4	95.71
Cracked	5.1	1.72
Shattered	7.4	2.50
Charge	290.48	%
Intact	283.43	97.59
Cracked	2.38	0.82
Shattered	4.26	1.47
Charge	290.65	%
Intact	232.92	80.14
Cracked	18.09	6.22
Shattered	39.02	13.42
Charge	290.46	%
Intact	283.96	96.76
Cracked	5.29	1.82
Shattered	0.72	0.25

Bean Size 9.5 mm

100 psig

	weight (g)	
Charge	297.5	%
Intact	204.1	68.61
Cracked	7.4	2.49
Shattered	84.7	28.47
Charge	290.3	%
Intact	265.2	91.35
Cracked	11.5	3.96
Shattered	13.6	4.68
Charge	287.59	%
Intact	199.34	69.39
Cracked	19.59	6.82
Shattered	68.36	23.79
Charge	291.62	%
Intact	284.38	97.52
Cracked	3.82	1.31
Shattered	3.11	1.07
Charge	290.53	%
Intact	286.28	98.53
Cracked	2.61	0.90
Shattered	1.28	0.45
Charge	285.54	%
Intact	212.15	73.13
Cracked	19.13	6.77
	51.11	18.10

Bean Size 9.5 mm		125 psig
	weight (g)	
Charge	291.2	%
Intact	104.0	35.71
Cracked	41.3	14.18
Shattered	144.2	49.52
Charge	290.48	%
Intact	66.68	22.95
Cracked	31.48	10.48
Shattered	191.61	65.96
Charge	290.39	%
Intact	58.40	20.11
Cracked	21.99	7.57
Shattered	208.74	71.87
Charge	290.81	%
Intact	29.54	10.16
Cracked	24.87	8.55
Shattered	235.19	80.87
Charge	285.53	%
Intact	67.79	24.09
Cracked	30.14	10.71
Shattered	183.49	65.20

Bean Size 9.5 mm		150 psig
	weight (g)	
Charge	295.1	%
Intact	24.8	8.4
Cracked	79.6	27.0
Shattered	190.3	64.5
Charge	292.6	%
Intact	23.2	7.9
Cracked	68.9	23.6
Shattered	199.7	68.2
Charge	277.4	%
Intact	48.6	17.52
Cracked	71.8	25.88
Shattered	156.5	56.42
Charge	288.93	%
Intact	260.50	90.16
Cracked	11.34	3.92
Shattered	17.00	5.88
Charge	287.75	%
Intact	247.95	86.17
Cracked	11.02	3.83
Shattered	28.73	9.98
Charge	290.68	%
Intact	267.66	92.08
Cracked	13.43	4.62
Shattered	9.19	3.16

Bean Size 9.5 mm		150 psig
	weight (g)	
Charge	285.74	%
Intact	50.26	17.79
Cracked	100.96	35.74
Shattered	131.23	46.6

Bean Size 9.5 mm		175 psig
	weight (g)	
Charge	291.69	%
Intact	65.46	22.44
Cracked	36.90	12.65
Shattered	188.08	64.48
Charge	290.70	%
Intact	46.20	15.89
Cracked	53.70	18.47
Shattered	189.58	65.21
Charge	291.03	%
Intact	177.38	60.95
Cracked	21.01	7.22
Shattered	92.25	31.70
Charge	292.27	%
Intact	204.46	69.95
Cracked	16.26	5.56
Shattered	71.30	24.39
Charge	294.95	%
Intact	212.58	72.07
Cracked	20.44	6.93
Shattered	61.59	20.88
Charge	291.23	%
Intact	207.07	71.10
Cracked	20.46	7.03
Shattered	63.37	21.76

Bean Size 9.5 mm — 175 psig (Cont.)

	weight (g)	
Charge	290.04	%
Intact	226.37	78.05
Cracked	18.09	6.24
Shattered	45.48	15.68
Charge	285.32	%
Intact	10.54	3.78
Cracked	70.92	25.42
Shattered	197.51	70.80
Charge	285.50	%
Intact	15.07	5.33
Cracked	62.05	21.95
Shattered	205.57	72.22
Charge	286.86	%
Intact	21.27	7.41
Cracked	152.99	53.33
Shattered	112.60	39.25

Bean Size 9.5 mm		200 psig
	weight (g)	
Charge	291.41	%
Intact	146.24	50.18
Cracked	59.86	20.54
Shattered	84.36	28.95
Charge	290.41	%
Intact	31.94	11.00
Cracked	93.31	32.13
Shattered	163.15	56.18
Charge	290.59	%
Intact	57.03	19.63
Cracked	76.76	26.41
Shattered	156.75	53.94
Charge	291.21	%
Intact	174.45	59.90
Cracked	40.04	13.75
Shattered	76.07	26.12
Charge	290.20	%
Intact	38.82	13.38
Cracked	100.20	34.53
Shattered	149.05	51.36
Charge	292.08	%
Intact	168.00	57.52
Cracked	38.27	13.10
Shattered	85.81	29.38

Bean Size 9.5 mm — 200 psig (Cont.)

	weight (g)	
Charge	292.61	%
Intact	171.47	58.60
Cracked	44.13	15.08
Shattered	76.90	26.28
Charge	290.36	%
Intact	105.95	36.49
Cracked	66.39	22.86
Shattered	116.87	40.25
Charge	291.85	%
Intact	164.83	56.48
Cracked	41.46	14.21
Shattered	85.03	29.13
Charge	292.47	%
Intact	55.70	19.04
Cracked	93.72	32.04
Shattered	141.23	48.29

Bean Size 11.2 mm		75 psig
	weight (g)	
Charge	285.56	%
Intact	263.56	92.30
Cracked Shattered	{22.00	7.70
Charge	286.81	%
Intact	247.28	86.22
Cracked	14.39	5.01
Shattered	25.14	8.77

Bean Size 11.2 mm		100 psig
	weight (g)	
Charge	281.87	%
Intact	242.05	85.87
Cracked	30.70	10.89
Shattered	9.02	3.20
Charge	268.67	%
Intact	239.39	89.10
Cracked	27.30	10.16
Shattered	1.70	0.63
Charge	268.35	%
Intact	227.20	84.66
Cracked	32.70	12.19
Shattered	8.20	3.06
Charge	283.63	%
Intact	270.05	95.21
Cracked	6.92	2.44
Shattered	6.01	2.12
Charge	281.75	%
Intact	250.05	88.75
Cracked	27.40	9.72
Shattered	4.10	1.46
Charge	276.6	%
Intact	85.9	30.72
Cracked	33.8	12.09
Shattered	158.9	56.83

Bean Size 11.2 mm

100 psig (cont.)

	weight (g)	
Charge	285.5	%
Intact	194.9	68.27
Cracked	19.2	6.73
Shattered	71.2	24.94
Charge	287.48	%
Intact	146.55	50.98
Cracked	18.28	6.36
Shattered	122.65	42.66
Charge	287.50	%
Intact	136.06	47.33
Cracked	24.78	8.62
Shattered	126.66	44.06

Bean Size 11.2 mm — 125 psig

	Weight (g)	
Charge	286.46	%
Intact	56.60	19.76
Cracked	67.70	23.63
Shattered	162.08	56.58
Charge	286.56	%
Intact	102.63	35.81
Cracked	46.23	16.13
Shattered	130.43	45.51
Charge	281.0	%
Intact	53.5	19.04
Cracked	68.3	24.31
Shattered	157.1	55.91
Charge	286.59	%
Intact	66.48	23.20
Cracked	108.90	38.00
Shattered	111.21	38.80
Charge	286.81	%
Intact	110.20	38.42
Cracked	107.53	37.49
Shattered	63.60	22.17
Charge	285.75	%
Intact	49.24	17.23
Cracked	83.79	29.32
Shattered	152.21	53.27

Bean Size 11.2 mm		150 psig
	weight (g)	
Charge	283.9	%
Intact	23.1	8.1
Cracked	85.6	30.2
Shattered	174.0	61.3
Charge	286.4	%
Intact	22.9	8.0
Cracked	88.8	31.0
Shattered	173.3	60.5
Charge	286.13	%
Intact	62.93	21.99
Cracked	196.35	68.62
Shattered	26.41	9.23
Charge	285.83	%
Intact	82.65	28.92
Cracked	115.65	40.46
Shattered	87.53	30.62
Charge	267.24	%
Intact	103.50	38.73
Cracked	97.27	36.39
Shattered	66.47	24.87

Bean Size 11.2 mm		175 psig
	weight (g)	
Charge	286.60	%
Intact	53.20	18.56
Cracked	193.70	67.58
Shattered	39.00	13.61
Charge	285.33	%
Intact	21.00	7.36
Cracked	188.83	66.18
Shattered	75.03	25.30
Charge	285.06	%
Intact	46.59	16.34
Cracked	76.27	26.76
Shattered	160.60	56.34
Charge	285.26	%
Intact	11.02	3.86
Cracked	157.64	55.26
Shattered	112.86	39.56

Appendix No. 2

Table 1 The Solubility Data for the System Cyclohexane-Castor Oil-Water at 25°C (wt.%)

Castor Oil	Water	Cyclohexane	Castor Oil	Water	Cyclohexane
71.90	0.50	27.60	-	99.10	0.90
59.50	1.40	39.10	0.80	-	99.20
24.30	1.50	74.20	1.50	0.90	97.60

Table 2 The Solubility Data for the System Ethanol-Castor Oil-Water at 25°C

Castor Oil	Water	Ethanol	Castor Oil	Water	Ethanol
99.60	0.40	-	15.83	12.92	71.25
79.69	2.82	17.49	13.58	6.76	79.76
76.69	0.29	23.02	9.69	16.06	74.25
71.50	2.10	26.40	2.13	23.14	74.73
66.58	1.57	31.85	0.90	24.88	74.22
46.18	7.33	46.49	0.40	79.40	20.20
36.75	6.51	56.74	0.20	90.10	9.70
20.37	10.32	69.31	0.10	38.60	61.30

Table 3 The Solubility Data for the System Acetone-Castor Oil-Water at 25°C

Castor Oil	Water	Acetone	Castor Oil	Water	Acetone
85.59	2.38	12.03	0.16	91.45	8.38
80.37	2.36	17.27	0.74	79.74	19.53
74.98	2.76	22.26	0.35	64.39	35.27
68.18	3.82	28.00	0.19	60.42	39.39

Appendix No. 3

Chemicals for Transmission Electron Microscopy

The material for this appendix is from Glauert (*). The

* Glauert, A.M. Practical Methods in Electron Microscopy. Volume 3, Part 1. Fixation, Dehydration, and Embedding of Biological Specimens. North-Holland Publishing Co, 1974.

Appendix No. 4

Castor Oil Specifications

Table 1

1.1 General Properties

Flash point, Tag closed cup, ^{o}C	230
Flash point, Cleveland open cup, ^{o}C	285
Autoignition Temperature, ^{o}C	449
Fire Point, ^{o}C	322
Boiling point	Decomposes
Polarimeter, 200 mm	+7.5 to +9.0
Coefficient of expansion per ^{o}C	0.00066
Pour point, ^{o}C	-33
Surface tension at 20^{o}C, dyn/cm	39.9

1.2 ASTM Standards

Viscosity (Gardner-Holdt) at 25^{o}C	
specific gravity, 20/20^{o}C	0.957-0.963
Acid value	0.4-4.0
Saponification value	176-184
Unsaponifiable matter, max.%	0.7
Iodine value (Wijs method)	82-88
Appearance	clear
Colour (Gardner), max.	Not darker than 3
Refractive index, 25^{o}C	1.477-1.478
Solubility in alcohol at 20^{o}C	No turbidity
Acetyl value	145-154

1.3 Characteristics of U.S. No. 1 and No. 3 Castor Oil

Property	No. 1	No. 3
Sp. gr., 15.5/15.5°C	0.961-0.963	0.957-0.963
Viscosity at 25°C (Gardner Holdt)		
Colour (Gardner)	3 (max.)	7 (max.)
Acid value	3 (max.)	10 (max.)
Iodine value (Wijs)	82-88	80-88
Saponification value	179-185	177-182
Unsaponifiable Matter, %	0.5 (max.)	1.0 (max.)

Table 2 : British Pharmacopia Specifications for Castor Oil

Descripiton:	A nearly colourless or faintly yellow, viscid oil
Solubility:	Soluble in 2.5 parts of alcohol (95 per cent); miscible with dehydrated alcohol, and with glacial acetic acid.
Identification:	Miscible with half its volume of light petroleum (boiling range 40° to 60°C), and only partially soluble in two volumes.
Acetyl Value:	Not less than 140
Acid Value:	Not more than 2.0
Iodine Value:	82 to 90 (Iodine monochlorite method)
Optical rotation:	Not less than + 3.5°
Refractive Index:	1.477 to 1.481
Saponification value:	177 to 187
Weight per ml.:	0.953 to 0.964 g

Table 3 : U.S. Pharmacopia (1970) Specification for Castor Oil

Description:	Pale yellowish or almost colourless, transparent, viscid liquid. Has a faint mild odour.
Solubility:	Soluble in alcohol; miscible with dehydrated alcohol, with glacial acetic acid, with chloroform, and with ether.
Specific gravity:	Not less than 0.957, not higher than 0.961.
Free Fatty Acids:	10 g sample requires no more than 3.5 ml of 0.1 N NaOH.
Hydroxyl value:	160-168
Iodine value:	83-88
Saponification value:	176-182