INTERACTIONS OF STEROID HORMONES WITH CENTRALLY ACTING DRUGS

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A B S T R A C T

A study has been made of the effects of steroid hormones on the pharmacological activity of certain centrally-acting drugs.

The Introduction contains a brief description of the known hormonal effects of steroids together with the historical background to the introduction of the synthetic steroids into clinical medicine. Some emphasis is placed on the ways in which steroids may affect central nervous sensitivity to drugs; their possible intra-cellular mechanisms of action and pathways of metabolic degradation are also discussed.

The experimental part of this project has involved the pretreatment of mice or rats for a number of days with a synthetic glucocorticoid, mineralocorticoid, oestrogen or progestin. Subsequently, these animals have received some centrally-acting drugs, for example, an analgesic, sympathomimetic stimulant , anti-convulsant or barbiturate, and their responses compared with those in animals of un-altered hormonal state. A number of techniques and pieces of apparatus have been devised, important amongst which are a method for the assay of plasma barbiturate levels and devices for measuring anti-nociceptive activity and hypnotic activity.

The most marked and sustained effects were produced by pretreatment of mice with oestrogens or progestins. The effects of certain barbiturates and related agents were markedly attenuated by progestin pretreatment, whereas, in

direct contrast, their effects were markedly enhanced by oestrogens. A study of plasma barbiturate levels suggested that there were effects in the rates of metabolism of these drugs, and similar changes in the metabolism of morphine may also have been produced. Nevertheless, a number of other observations, for example, certain minimal changes in brain biogenic amine levels and the response of mice to centrally-acting sympathomimetics suggest that there occurred also changes in the inherent sensitivity of the central nervous systems of these animals. The mice were more sensitive to the effects of the sex steroids than they were to pretreatment with adrenocorticoids, whereas experience with rats was the reverse.

Finally, the Discussion summarises the possible interactions of steroids and centrally-acting drugs in experimental animals, and concludes with a study of the possible clinical implications of combining long-term steroid treatment with the concomitant use of other drugs such as hypnotics, analgesics, anti-convulsants and stimulants. The simultaneous use of centrally-acting drugs and long-term steroids, including those taken for contraceptive purposes, should be attended by caution.

C O N T E N T S

I N T R O D U C T I O N

1. NATURE AND PHYSIOLOGICAL ROLE OF THE ENDOCRINE SYSTEM.

The mammalian endocrine system comprises a group of ductless glands whose secretions are termed hormones, and which empty directly into the blood. Steroid hormones are all derivatives of cylopentaphenanthrene, are related to cholesterol and are produced by the adrenal cortex and the gonads. They appear to have a dual role in the body, namely: (i) morphogenesis, involving the growth, differentiation and maturation of the organism; (ii) homeostasis, maintaining a steady but dynamic equilibrium of the components of the animal. The majority of physiological functions is under the control of both hormonal and neural control and in order to achieve morphogenesis and homeostasis there is a functional integration of these two systems. The key to the integrative process lies in: (a) the so-called central transmitters, or more correctly, neuro-humors: noradrenaline, dopamine, 5-hydroxytryptamine (other candidates are tyramine, substance P, cerebellar factor, histamine, etc.) which are produced by neurons in the brain, together with (b) the trophic hormones or neuro-secretions of the adenohypophyseal cells. The former are short-range, brief-acting agents whilst the latter are long-range long-acting compounds. Since they both exert regulatory function, the inclusive term of neuro-hormones has been accepted. These neuro-hormones constitute the link between the central nervous system and the endocrine glands, carrying neural messages in a chemical form.

That this integration is profound and deep rooted and

not superficial is borne out by the occurrence of psychiatric maladjustments in sexual and other endocrine disorders. The converse also occurs with changes in gonadal and sexual behaviour being associated with neurologic disorders.

2. ADRENOCORTICAL HORMONES

(a) History and nature

Addison first related the adrenal glands to human disease over a century ago but a detailed knowledge of the function of these glands has advanced only during the last 35 years. Mason et al (1936), Reichstein (1936), and Wintersteiner and Pfiffner (1936) isolated various steroids from the adrenal cortex.

The three steroid-producing organs (adrenal, ovary and testis), have a common embryological origin in the splanchnic mesoderm. The adrenal gland elaborates and secretes not only the hormones peculiar to this tissue but also hormones ordinarily associated with the testis and ovaries. In recent years many of the biochemical pathways attributed to a particular organ have been shown to exist in all three (Axelrod, 1961). Thus progesterone is an intermediary in the production of the corticosteroids (desoxycorticosterone, aldosterone, corticosterone, 21-desoxycortisol and hydrocortisone; See fig. 1) from cholesterol. Which particular metabolic pathway takes preference in each gland may be determined by the trophic hormones of the adenohypophysis

The principal steroids secreted by the adrenal cortex are corticosterone and hydrocortisone (from the cells of the

zona fasciculata) and aldosterone (from the cells of the zona glomerulosa). The former group is glucocorticoid, exerting a profound effect upon carbohydrate metabolism whilst the latter is mineralocorticoid, regulating sodium and potassium balance (a property shared by desoxycorticosterone).

The discovery by Hench, Kendall, Slocumb and Polley (1949, 1950) that the glucorticoid, cortisone, has the capacity to reverse the inflammatory reactions of rheumatoid arthritis stimulated great research activity for steroids with greater anti-inflammatory activity. Chemically modified adrenocortical steroids may be divided into six distinct classes (Boland, 1962) :-

(i) Prednisolone and prednisone
(ii) 6-methyl analogues
(iii) 16-methyl analogues, e.g. dexamethasone
(iv) 16-hydroxy analogues, e.g. triamcinanolone
(v) 6-fluoro analogues
(vi) Miscellaneous, e.g. fludrocortisone

) See fig. 2.

The first indisputable evidence that the funtions of the natural corticoids could be altered selectively was supplied by Fried and Sabo (1953, 1954). They observed that addition of halogen atoms at the 9th carbon position of hydrocortisone caused enhancement of several of its biological activities. Thus, 9-alpha fluorohydrocortisone (fludrocortisone) is ten to fifteen times more powerful than the parent hormone in promoting glycogen deposition and suppressing anti-inflammatory responses in the rat and it is fifty times more potent in retaining salt.

(Borman & Singer, 1954).

Herzog and co-workers (1955) then introduced a double bond at C positions 1 and 2, thereby producing their detla-1 analogues, prednisone and prednisolone. Dehydrogenation at this position was found to enhance anti-inflammatory potency and glycogen deposition without causing a corresponding increase in electrolyte activity. Arth et al (1958) developed a family of analogues bearing a methyl group at C-16 (16-methylation like 16-hydroxylation markedly reduces the salt-retaining properties of 9 alpha fluorinated compounds). Biological tests have demonstrated that this analogue is one of the most potent anti-inflammatory steroids yet devised. Its activity as determined by thymus involution, is 140 - 200 times greater than hydrocortisone (and by granuloma inhibition, 200 times greater, and by glycogen deposition, 20 times greater than hydrocortisone). Sodium retention in animals, however, is negligible even at high dose levels. (Boland, 1962).

(b) Action and effects

Both natural and synthetic glucocorticoids increase blood sugar levels and antagonize the effects of insulin but they also promote the deposition of glycogen in the liver. This is due to an increased rate of gluconeogenesis and inhibition of peripheral utilization of glucose. Consequently there is a drain on protein reserves leading to loss of nitrogen in the urine. Prolonged exposure to high glucocorticoid activity (e.g. adrenal hyperactivity or steroid therapy) leads to symptoms of Cushing's syndrome in man. Under normal conditions glucocorticoids increase

the resistance to stress, increase the efficiency of muscular contraction and reduce inflammatory reactions.

Aldosterone is the most important natural mineralocorticoid (desoxycorticosterone and fludrocortisone are also potent mineralocorticoids); yet despite its high potency on salt and water metabolism it has significant glucorticoid activity. Mineralocorticoids promote tubular reabsorption of sodium and excretion of potassium in the kidney. After adrenalectomy, the loss of sodium and chloride ions (together with water) leads to haemoconcentration, a fall in blood pressure and bradycardia. In the adrenalectomized rat intake of sodium offsets the renal loss and prolongs the survival time.

(c) Control of secretions

The adrenocortical steroids (with the exception of aldosterone) are secreted in response to adrenocorticotrophic hormone (ACTH) release from the adenohypophysis or administered exogenously. Sayers and Sayers (1948) postulated the cortical hormone titre theory which states that a decrease in blood titre of adrenocortical steroid accelerates production of ACTH by the pituitary and high levels of cortisol depresses this process. There seems little doubt that the production of pituitary corticotrophin is controlled by a hormone from the median eminence of the hypothalamus (Harris, 1951). This has been termed the corticotrophin-releasing factor (C.R.F.), which is polypeptide similar in chemical structure to vasopression.

In summary the available evidence suggests that regulation of ACTH release involves both a hormonal and

neural mechanism whilst subsequent levels of ACTH may be regulated by the blood titre of adrenocorticosteroids.

3. FEMALE SEX HORMONES

(a) History and nature

Zondek (1928) showed that large amounts of oestrogenic hormone are excreted in the urine of pregnant women. Butenandt (1929) and Doisy (1929 & 1930) separately extracted and purified the active crystalline substance. The corpus luteum of pregnancy is a temporary endocrine gland producing a hormone which takes up the direction of pregnancy begun by the oestrogens. The progestational hormone was discovered and named in 1929 by Corner and Allen as progestin.

Reports of the influence of a gonadotrophic hormone from the anterior pituitary on the ovary were first published independently in 1927 both by Ascheim and Zondek, and Smith and Engle. However, Ascheim and Zondek believed that there were two compounds which they called Prolan A and Prolan B. Prolan A stimulated the maturation of the ovarian follicle and ovary, and the production of oestrogen. Prolan B stimulated the luteinisation of the follicles, namely the formation of the corpora lutea and the secretion of progestin. These anterior pituitary hormones are now known respectively as follicle stimulating hormone (FSH) and the luteinising hormone (LH).

The control of FSH and LH was formerly thought to involve the direct negative feedback system between the gonads and the anterior pituitary. (Moore & Price 1932).

Subsequent studies have shifted the emphasis from direct negative feedback to an indirect one via the hypothalamus.

The term oestrogen refers to a class of substances possessing oestrogenic activity. They may be divided into three categories:-

(i) Naturally occurring oestrogens;

(ii) Semi-synthetic oestrogens;

(iii) Synthetic oestrogens.

(i) The follicular hormone is not an entity in itself but a collection of oestrogenic compounds which differ in oestrogenic activity. It is convenient to relate their structure to perhydro-cyclopenta-phenanthrene since the phenanthrene nucleus of most steroids is almost completely saturated. The chemical characteristics of the oestrogens are the unsaturation of the A ring, a phenolic hydroxyl group at position 3, and a methyl group at position B (See Fig. 3). The crystalline material "theelin" named by Doisy was officially called oestrone by the Committee on the Standardisation of Sex Hormones of the League of Nations (1935). Oestradiol - 17B, the most potent natural oestrogen was isolated from the ovary by MacCorquodale in 1935. Reversible interconversion of oestrone and oestradiol occurs in the body.

(ii) Semi-synthetic oestrogens: The manufacture of the ethinyl and its 3-methylether derivatives of oestradiol provided oestrogens which are stable in the gastro-intestinal tract and provided a useful step forward towards the problem of oral contraception.

(iii) Synthetic oestrogens: These are non-steroidal derivatives of diphenylethylene (stilbene). Dodds and co-workers (1938) showed that substitution with alkyl groups in positions R_1 and R_2 in the basic structure of stilboestrol provide the most active compounds. (See Fig. 3).

In 1934, after Corner and Allen had demonstrated the presence of a hormone in the corpus luteum, four groups of chemists isolated a crystalline material progesterone. Subsequently derivatives of progesterone were identified in the body, namely, pregnanolone and pregnanediol.

The progestational hormones used in oral contraceptive formulations fall into several chemical categories but it is interesting to note that no simple non-steroid substance has been found which possesses significant progestational activity. (Review - Goldzieher, 1964).

The chemical categories are :-

(1) 19-nortestosterone derivatives, e.g. norethynodrel and norethisterone acetate;
(2) 3-desoxy 19-nortestosterone derivatives, e.g. lynestrenol;
(3) 17-acetoxyprogesterone derivatives, e.g. megestrol acetate;
(4) retro-progesterone derivatives.

Category (2) contains compounds lacking the ketonic oxygen which was until recently considered essential for biologic activity (See Fig. 4).

There is good evidence that the first ring of the 19-nor steroids may be transformed in vivo to an aromatic

ring to give ethinyl oestradiol which may be the active moiety in the ovulation inhibitory effect of 19-nor steroids (Langecker, 1961; Paulson et al, 1962).

(b) Actions and effects

In rodents oestrogens are responsible for the development of:-

(i) the accessary organs of generation;

(ii) cycle of oestrous;

(iii) continuation of pregnancy in its early stages.

More particularly, in humans oestrogens are responsible for the maturation of primary female sex organs and the development of secondary sex characteristics. During the menstrual cycle they are responsible for the maturation of the ovum, and later essential for the maintenance of pregnancy, growth of mammary tissue, inhibition of lactation until parturition, relaxation of the pelvic girdle, and sensitisation of the uterus to the action of oxytocin during labour. During the post partum period they maintain lactation, cause involution of the uterus and prepare for a fresh menstrual cycle.

With the advent of complex analytical techniques, workers have shown that specific protein-like receptors for oestrogenic substances of different chemical compositions exist in the hypothalamus, endometrium and breast. Jenson et al (1968) believe that the receptor substances, probably histones, prepared from such varied sources as mice, rats, rabbit and calves, act as transport vehicles transferring oestradiol through the cytoplasm to the

nucleus. Baulieu (1967) showed that an isolated fraction from an endometrial homogenate (from the sow) bound diethyl-stilboestrol better than the dimethyl analogue - the latter also having less oestrogenic activity. Kellie (Dodds Review, 1969), also showed that oestradiol metabolites do not compete with free oestradiol for receptors. Eisenfeld and Axelrod (1967) using tritiated ^{3}H-17B-oestradiol provided evidence for the occurrence of specific oestrogen binding sites in the hypothalamus. It appears that there exists a biological system which can recognise activity in compounds differing entirely in chemical constitution.

In rodents, progestins are responsible for:-

(i) Inhibition of ovulation and oestrous changes in accessory organs;

(ii) The sensitisation of the uterus for implantation of the fertilized ova;

(iii) Maintenance of pregnancy.

More particularly, in humans progestins cause hypertrophy and increase the vascularity and secretory power of the endometrium. Progestins are also essential for the maintenance of pregnancy; nidation and the inhibition of maturation of further follicles. It relaxes uterine muscle and causes growth of mammary tissue. It also maintains lactation during the post-partum period.

Progesterone is frequently hyperthermic, in contrast to the known hypothermic effect of oestrogen (Israel et al, 1950). This is the basis of basal body temperature measurement in the detection of ovulation, a rise in

temperature indicating luteal function.

Progesterone exerts a catabolic effect on certain tissues, which is reflected by a rise in nitrogen excretion, (Landau et al, 1955). Dietary protein enhances the catabolic influence of administered progesterone (Landau et al, 1961). They attributed this in man to increased utilization of amino acids in liver due to an increase in the metabolic pool of these substances.

It has been shown recently that progesterone has a chloruretic and natriuretic effect in man (Landau et al, 1958), through a competitive antagonism of aldosterone at peripheral sites. Progesterone also decreases both experimental hypertension in dogs as well as elevated blood pressure in man, (Armstrong, 1959). An intravenous infusion of 500mg of progesterone in albumin induces somnolence, and Merryman et al (1954) have postulated that increasing the dose might actually result in clinical anaesthesia. This has been confirmed in rodents (Selye, 1941).

(c) Control of secretions

The classic theory of Moore and Price advanced a direct negative feedback mechanism between the gonads and anterior pituitary hormone LH and FSH. Harris (1961) outlined a theory which shifted the emphasis from the direct feedback to that of an indirect one mediated via the hypothalamus. Work with various hypothalamic lesions and with direct hypothalamic implants of sex hormones (Bogdanove, 1963; Kanematsu & Sawyer, 1963 & 1965), has

pointed to the hypothalamus as the primary target organ for the ovarian feedback mechanisms.

There is a growing body of evidence that higher brain structures participate in the regulation of anterior lobe hormones which control the release of female sex hormones. According to MacLean (1958) the limbic system is concerned with two essential functions: self preservation and preservation of the species. The former is related to the frontotemporal region, while the circuit formed by the septum, the cingulate gyrus and the hippocampus, might be concerned with sexual and reproductive activities. Electrical stimulation of the anterior cingulate region produces sexual excitement in monkeys (MacLean et al, 1958). Nerve fibres from these regions end in the mamillary nuclei of the hypothalamus and supra optic region and these regions are closely connected with the secretory mechanisms of neurohormones. The final pathway from the hypothalamus to the pituitary was thought by Harris (1951) to consist of two parts: one neural, ending in the median eminence; and the other vascular, via the hypophyseal portal vessels. Recently a third system, comprised of neurons and ending in the secretory cells of the anterior pituitary, has been shown to exist (Metuzals, 1959). According to various workers, including McCann and Ramirez (1964), there exists in the hypothalamus a factor or factors facilitating ovulation. Possible adrenergic mechanisms of control are discussed later, page 28.

The neural, neuroendocrine and hormonal interactions which govern the release of FSH and LH are very complex. As well as the presence of a feedback mechanism existing

between the ovarian steroids and the anterior pituitary, the hypothalamus has an integrative function, bearing hormone-sensitive receptors and receiving neural messages from higher brain centres. These messages are relayed by either direct neural or neuro-endocrine pathways to the anterior pituitary. Such varied stimuli as environmental factors (temperature, light, food, social conditions), hormonal milieu, neurophysiological or neurosurgical intervention, can also complicate and influence these mechanisms. (Bajusz, 1966). Nevertheless it may be assumed that the sequence of events leading to ovulation involves the production of oestrogen by the growing follicle under FSH influence; the oestrogen via the hypothalamus, stimulates the release of adenohypophyseal LH which is responsible for both the pre-ovulatory swelling of the follicle and its subsequent rupture. Progesterone produced by the corpus luteum in turn induces the hypothalamus to terminate the secretion of LH.

(d) Suppression of ovulation

The search for oral contraceptives has been in progress since the time of Hippocrates who suggested such varied remedies as crocus, laurel, nettle seeds, peony root and other plant and mineral sources.

Recent research has produced ovulation-inhibiting compounds which are currently used for this purpose. Progesterone is known to inhibit ovulation in mammals and Pincus in 1956 suggested this might also be true in women. The idea is untenable since progesterone has little activity when administered orally. However, with the advent of

potent stable 19-nortestosterone derivatives in the early 1950s, the possibility of using these steroids as ovulation-inhibitors was explored by Rock, Pincus and co-workers (1957) and successfully mass-tested in extensive studies in Puerto Rico. Oestrogens also prevent ovulation but the incidence of unpleasant side-effects limits their clinical use.

Whether the steroids have an effect on the hypothalamus-hypophyseal system or a direct effect on the reproductive glands is a subject of controversy. Administration of gonadotrophins to rabbits treated with ovulation suppressing steroids results in ovulation (Edgren and Carter, 1962) and also in rats (Husain & Pincus, 1968). There is evidence that oestrogens exert their action at the ovarian or follicular level, while progestins exert their action centrally. Since oestrogen/progestin oral contraceptives do not suppress the stimulatory effects of luteinizing hormone release factor (LRF) on LH, it is probable that they act mainly on the hypothalamus or higher brain centres and not the pituitary, according to Schally et al (1968). Nelson and Patanelli (1960) maintain that ovulation inhibiting compounds may not depend on gonadotropin suppression as on the upset LH-FSH balance since some of these compounds do not suppress urinary gonadotropin levels, (Brown & Matthew, 1962).

Changes in the reproductive female tract may be influenced by amounts of oestrogen and/or progestin which do not prevent ovulation but do prevent conception nidation. Pincus (1958) pointed out that the histology of the endometrium of subjects on oral contraceptives is not synchronous with that of the normal cycle. Even if

ovulation and fertilization were to take place the blastocyst would not find the endometrium favourable to implantation. Also, under progestational influence, the cervical mucus becomes viscous, crystallizes upon drying and the "Spinnbarkeit" (thread formation) is considerably lowered. In vitro tests on sperm penetrability is poor and there is considerable obstruction to the passage of sperm through the cervix (Zanartu, 1964).

4. EFFECTS OF STEROID HORMONES ON THE CENTRAL NERVOUS SYSTEM

When describing the clinical manifestations of adrenocortical insufficiency, Addison in 1855 noted that several of his cases exhibited neurological and psychological symptoms including depression, anxiety and mind-wandering. In 1899, Klippel associated Addison's disease with epileptic convulsions and post mortem examination revealed cellular changes in the cerebellum. Since then the administration of both sex steroids and adrenal steroids has been associated with changes in excitability of the central nervous system, behaviour, and brain morphology.

(a) Excitability and EEG

Hans Selye (1941) demonstrated that intraperitoneal injection of either progesterone or deoxycorticosterone acetate (DOCA) produces anaesthesia in young male rats. Injection of leptazol antagonises this anaesthesia although the steroids protected the animals from the lethal effects of leptazol.

Subsequently in 1942 Selye produced anaesthesia in

partially hepatectomized female rats with 75 different synthetic and natural steroids. Of these, the most active fell into three classes of hormonal activity, e.g. progesterone (luteoid), pregnanedione (small folliculoid), and DOCA (corticoid).

Late in 1964 Atkinson et al showed that 67 pregnane derivatives possessed hypnotic activity whilst other steroids they used proved to be convulsant.

The anaesthetic properties of steroids do not depend on specific hormonal activity, nor in fact any hormonal effect at all, but Figdor (1957) pointed out that certain potent derivatives of progesterone and DOCA which he used to produce anaesthesia had been shown by previous workers to be metabolites of progesterone and DOCA in rat liver, e.g. progesterone → pregnanedione and 3α pregnenalone DOCA - allepregnane → 3β - 21 - diol - 20 - one. The potent clinically used anaesthetic hydroxydione was developed from these studies.

Gowers (1885) was the first to suspect a relationship between incidence of epileptic seizures in susceptible women and menstruation. The role of sex steroids in epilepsy has received considerable attention over the past 20 years. Logethetis and co-workers (1959) provided both clinical and experimental evidence that oestrogens and the occurrence of oestrogenic predominance near the menstrual period is coincidental with aggravation of both seizures and EEG abnormalities. That oestrogens may alter all membrane permeability or interfere in some way with acetylcholine metabolism was discussed.

As long ago as 1899, Klippel reported an Addisonian case characterised by attacks of delirium and epileptic convulsions followed by coma. This was later confirmed when adrenalectomy in experimental animals resulted in convulsions. (For historical discussion see Woodbury, 1958; Quarton et al, 1955).

Stephen and Noad (1951) reported a case of status epilepticus in a 15 year old girl receiving cortisone therapy. Previous to this she had suffered no convulsive seizures, epileptic variants nor head injuries.

Brain excitability is usually measured by:-

(1) Threshold for electrically-induced seizures;

(2) Threshold of leptazol-induced seizures;

(3) Susceptibility to audiogenic seizures.

Woodbury (1954) showed that changes in brain excitability (electroshock threshold; EST) produced by changes in adrenal hormone status could be explained by changes in electrolyte balance in the brain. Adrenocortical insufficiency increases brain excitability (decreased EST) which may be reversed by 0.9% NaCl administration. Hypophysectomy increases excitability in rats for about 10 days after which excitability then decreases. The adrenocortical steroids which markedly affect electrolyte metabolism, (e.g. DOCA), decrease excitability, whereas those which predominantly affect carbohydrate metabolism (e.g. cortisone, hydrocortisone) increase excitability. Those with intermediate effects (e.g. corticosterone) have intermediate effects on brain excitability.

Although the evidence is conflicting, the wealth of

information supports the conclusion that, like EST, leptazol seizure thresholds are decreased by DOCA and increased by cortisone, (Woodbury, 1958).

Again with audiogenic seizures in mice and rats, the effects of adrenocortical steroids are not clear cut. DOCA usually decreases susceptibility whereas reports with cortisone are variable.

Woolley and Timiras (1962) investigated excitability in female rats by two methods, EST and minimal electro-shock (they timed shortening of tonic flexor and/or lengthening of tonic extensor muscle contractures). They found that mature female rats show greater convulsability than rats ovariectomized before maturity. Administration of oestradiol to intact male and ovariectomized immature and mature female rats results in a lowering of EST. As this phenomenon is observed in hypophysectomized rats the effect is not mediated through the pituitary. Progesterone rapidly and significantly raises EST in female rats. Thus sex steroids are capable of altering thresholds and convulsive patterns in the brain apart from their sexual function.

The resting EEG of Addisonian patients exhibits definite abnormalities characterized by high voltage, slow bursts (5 to 8/sec which is slower than the normal rhythm) and a predilection for exaggerated EEG response to hyper-ventillation. The EEG of these patients is improved by adequate replacement therapy. (Reviews of Woodbury, 1958; Quarton et al, 1955).

Heofner and Glaser (1958) made extensive inquiries into the effects of ACTH and cortisone. Both cause the

appearance of slow wave activity (4 to 7 sec). Glaser and Merritt (1952) had shown that both ACTH and cortisone lead to increased abnormalities of EEG in epileptic patients. It is interesting to note that no correlation exists between EEG alteration and serum electrolyte changes (Woodbury,1958).

Kawakami andSawyer (1959) postulated two target zones in the brain for sex hormone activity, based upon observations of EEG arousal thresholds in two opposing cerebral systems in rabbits.

(1) EEG arousal threshold - brain stem and reticular formation;

(2) EEG after reaction threshold - rhinencephalon and hypothalamus.

The former is closely related to sexual behaviour and the latter correlated with the release of ovulatory hormones.

Arai et al (1967) demonstrated a dose-dependent inhibitory influence of intravenous progesterone on the cortical activity of immobilized female rats. Administration of oestradiol tended to antagonise this effect. They postulated that the intravenous administration of progesterone might facilitate sexual behaviour by suppressing a cortical inhibitory centre.

Komisaruk et al (1967) showed that progesterone induces a sleep-like EEG which lasts 20 - 60 min in rats. It exerts differential effects on neurons present in the cerebral cortex, hypothalamus and thalamus through a non-specific suppression of arousal.

(b) Biochemistry, intermediary metabolism and enzyme systems

Steroids may affect seizure thresholds and EEG patterns by affecting the neurochemical processes in the brain. Many reports indicate the importance of the blood-brain barrier, synaptic transmission, carbonic anhydrase activity, brain electrolyte and water concentration, brain, brain ACh and cholinisterase levels, noradrenaline, gamma aminobutyric acid and monoamine oxidase levels.

Electrolytes:

The summarised results of many workers on brain Na^+ and K^+ levels and turnover in adrenalectomized animals reveal an increased concentration of intracellular Na^+, a decreased ratio of extra-cellular to intracellular Na^+ concentration, and a decreased turnover of Na^+. Total brain K^+ concentration is unchanged, but the ratio of intracellular to extracellular K^+concentration is decreased, whilst the turnover of K^+ is questionably increased.

DOCA decreases brain intracellular Na^+ and questionably increases turnover of Na+; the ratio of intracellular to extracellular K^+ is increased. The effects of cortisone (and ACTH) on brain Na^+ and K^+ are minimal during chronic pretreatment with this steroid and also in Cushing Syndrome when there are raised levels of endogenous corticosteroids. However acute administration of cortisone does increase intracellular Na^+ (in contrast with DOCA above) (Review of Woodbury, 1958).

Physiological Barriers:

Steroids may produce their effects by modifying the

permeability of the barriers separating various fluid compartments in and about the brain. Four barriers are usually distinguished :-

(a) Blood-brain barrier;

(b) Blood-spinal fluid barrier;

(c) Spinal fluid-brain barrier;

(d) Inter-intracellular barrier.

Steroids may render these barriers more or less sensitive to such factors as the solubility of any compound, its molecular or ionic size, the electrical charge, lipid solubility, the protein-binding characteristics or the toxicity of the compound.

Work on isolated membranes indicates that cortisone decreases permeability while DOCA increases permeability. However, occasionally patients on ACTH and cortisone have elevated protein levels in their cerebrospinal fluid.

Thus altered permeability could greatly influence the concentration of electrolytes, could allow protein and other molecules not normally present to affect enzyme systems and could permit toxic substances to influence brain metabolic processes.

Brain oxygen and oxidative metabolism:

The preferential metabolism of glucose in the brain is relatively independent of hormonal control (Quarton & Clark, 1955). Experimentally produced adrenal insufficiency (by adrenalectomy) produces a decrease in brain oxygen consumption which may be explained by the decreased cerebral blood flow (Woodbury,1958).Administration of

pregnenalone, cortisone or adrenocortical extract (ACE) reversed these effects whilst DOCA produced little or no effect. However, in clinical hypocorticism (Addison's disease) no such effects are seen; the importance of the original observation remains to be determined.

There is no decisive clinical evidence that administration of ACTH, cortisone or DOCA influences cerebral blood flow or brain metabolism. In vitro work suggests that DOCA and progesterone decrease respiration in the presence of glucose and pyruvate, but not succinate (Gordan and Elliot, 1947). Hayano et al (1950) maintain that this depression is not as a result of an interference with the cytochrome C - cytochrome oxidase system. Nevertheless, Gordan and Elliot (1947) maintain that this inhibition is at the level of the dehydrogenases, and consequently can increase hypnosis. Since adrenaline increases aerobic glycolysis in posterior hypothalamic tissue (an opposite effect to cortisone), they may have opposite controlling influences on neural pathways in this location. Steroids acting as anaesthetics may be uncoupling agents where respiratory chain phosphorylations are uncoupled from oxidations - a theory first postulated by Brody and Bain (1951), to explain barbiturate anaesthesia. However, observed increases in high energy intermediates (ATP, etc.) may be the result of rather than the cause of the anaesthesia (McIlwain, 1962).

Acetylcholine and cholinesterase:

Hydroxydione, a potent anaesthetic developed from steroid work, induces an acute increase of brain ACh levels in the rat. (Giarman and Pepeu, 1962). The levels follow

anaesthesia remarkably well and within one minute of revival from anaesthesia the levels are back to normal; 5HT levels do not rise after hydroxydione whereas they do with other anaesthetics. Barbiturates also increase ACh levels which parallel the pharmacological course of anaesthesia (Review P'an & Laubach, 1964). Torda and Wolff (1952) showed that ACh synthesis is decreased by hypophysectomy, unaffected by adrenalectomy, yet increased by ACTH and cortisone. In vitro, cholinesterase was not affected by low concentrations of either ACTH or cortisone.

Oestrogens also cholinesterase activity according to Christianson (1956) and Logothetis et al (1958) postulate that the EEG changes and pro-convulsant activity due to oestrogen treatment may be due to modified ACh metabolism at the site of the epileptic lesion.

Monoamines and monoamine oxidase:

The fact that the median eminence and other parts of the brain stem of various mammals contain a dense network of catecholamine containing nerve-endings (Carlsson et al, 1962; Dählstrom & Fuxe, 1964), which terminate in or near the hypophyseal portal system, suggests that the anterior pituitary may be under nervous control. Leonardelli (1966) demonstrated a particular richness of MAO (and also acetylcholinesterase) in the paraventricular nuclei and the infundibulum and postulated an adrenergic mechanism in the control of the guinea pig anterior pituitary. Also cyclic changes in the rat hypothalamus have been reported by Kuwabara et al (1967) who found that MAO and biogenic amine activity reached a maximum at pro-oestrous.

Lichtensteiger (1968) demonstrated cyclic variations in the catecholamine content of the hypothalamic nerve cells during the oestrous cycle of the rat. More particularly they postulated a possible role for the tubero-infundibular catecholamine-containing neurons in the control of female gonadotrophins. The catecholamines (thought to be primarily dopamine) were greatest during dioestrous, falling at pro-oestrous and lowest during oestrous.

Grant and Pryse-Davies (1968) noticed a significant increase in the incidence of depression in women receiving strongly progestogenic compounds which was coincident with an increased MAO activity in plasma and the endometrium. They suggested that brain MAO activity might be altered and account for the observed changes in mood. This work, together with evidence of oestradiol binding sites in the hypothalamus (Eisenfeld & Axelrod, 1968), involves adrenergic mechanisms in the control of oestrous and menstrual cycles and the observed mood changes associated with the latter.

(c) Mammalian behaviour

Psychological maladjustments have been accepted as an integral part of Addison's disease for some time. Engel and Marjolin (1942) were the first workers to investigate the incidence of mental disorders in their Addisonian patients of whom 3 out of 25 were plainly psychotic. The derangements, however, differed from congenital madness in that the symptoms were entirely relieved by replacement steroid therapy and a high carbohydrate diet.

The decrease in spontaneous activity seen in both domestic and wild rats upon adrenalectomy (Griffith, 1949)

has been variously ascribed to decreased central excitability (seen with DOCA administration), upset carbohydrate metabolism (cortisone replacement corrects this and normalises activity), or decreased creatinine phosphate in the hypothalamus (Goldman & Abood, 1953). In mice also the "eosinophil rhythm" which parallels the peaks of their spontaneous locomotor activity is disrupted by adrenalectomy (Halberg et al, 1953).

The literature contains a mass of information involving corticosteroids with psychological maladjustments. The reviews of D. Woodbury (1958) and Quarton et al (1955) discuss in detail these associations. Mental abnormalities are associated with Cushing's disease, adrenogenital syndrome, and administration of cortisone or ACTH. Trethowen and Cobb (1952) in describing 25 Cushinoid patients stated that at least 21 of them had psychiatric disorders. Most of them had degrees of depression associated with either retardation or one or more other symptoms such as irritability, anxiety, un-cooperative behaviour, etc. Hench and co-workers (1950) reported euphoria in 6% of individuals given large doses of cortisone or ACTH in the treatment of arthritis, while other workers report higher percentages (up to 60%). Rome and Braceland (1952) graded psychological reactions to ACTH and corticosteroids in order of severity into 4 groups: from feelings of euphoria and elevation at one end of the scale to psychoses and paramia at the opposite end.

Observation of behavioural effects in animals compared with clinical descriptions of psychic and behavioural changes due to hypercorticism, is sparse. Liddell and

others (1935) noticed a dramatic improvement in conditioned neuroses in sheep upon administration of ACE, while Mirsky et al (1953) demonstrated that ACTH reversed a marked, conditioned, fear-reaction linked to reward bar-pressing activity in monkeys.

Premenstrual psychic disturbances and emotional instability, together with more prolonged mental states during certain periods of life such as puberty, pregnancy and menopause, have been associated with varying endocrine conditions. Both oestrogens and progestins have been variously blamed for these conditions. Reviews include Rees (1953); Stieglitz and Kimble (1949); and Bajusz (1966).

The presence of sex steroid hormones in most animals is essential for the animals to exhibit their characteristic behaviour patterns, sexual behaviour being an integral part of the animal's "personality". Berthold's work (1847) on gonadectomized cockerels was reviewed by Steinach (1912). The absence of sexual behaviour in the castrated cockerels reappears unaltered after grafting of testicular tissue. The intravenous administration of progesterone induces sexual receptivity in the oestrogen-primed rat within a matter of minutes, according to Lisk (1960).

Recently the occurrence of mood changes associated with anti-ovulatory steroids in women has received widespread attention. Significant variations in the incidence of depression and loss of libido occur with strongly progestogenic compounds (Grant & Pryse Davies, 1968). Also Daly et al (1967) reported the occurrence of psychoses in two women receiving sequential type of oral contraceptives.

These Authors' work implicated the progestin, since the oestrogen was well tolerated.

(d) Morphology

It has been said that specific pathological changes occurring in the CNS due to adrenal insufficiency are unlikely (Gordon, 1915). Yet the development of brain connective tissue and of the variety of glial cells in many animal species can be suppressed by ACTH and cortisone (Woodbury, 1958). Overdosage of cortisone in adult rats produces functional and structural changes in rat brain (Selye, 1950) whilst Malamud and Saver (1954) observed no pathological changes in the hypothalamus and thalamus of patients who had ACTH or cortisone for long periods. Neuropathological changes in the diencephalic region due to ACTH or cortisone appear to be variable. Chronic pretreatment with DOCA to animals fed on Na^+ rich diet will cause severe and permanent brain lesions, usually by a combination of hyalinosis of the brain blood vessels, thrombus formation and finally necrotic liquefaction and oedema of the parenchymatous brain cells, (Selye et al, 1948). Also DOCA overdosage has been reported to induce encephalopathy in man (Costa et al, 1946). Most interesting is the observation that glial cell proliferation may occur.

Trethowen and Cobb (1952) reviewed the pathological effects of Cushing's disease in which they described atrophy of certain thalamic and hypothalamic nuclei and some hydrocephalus. See also Reviews of Bajusz (1966) and Woodbury (1958).

Foetal gonads are known to begin to secrete hormone

early in intrauterine life and may be important in the development of that neural organization which determines future recognizable sexual characteristics. Both tumour and rickets in some way modify brain development so that the normal inhibitory influence of the hypothalamus on pituitary function before maturity is removed leading to sexual precosity. Also destruction of the posterior hypothalamus leads to delayed puberty, sexual infantilism and obesity, the characteristic signs of "Frohlich's Syndrome".

5. STEROIDS AS ENZYME-INDUCERS IN VARIOUS TISSUES AND THEIR INFLUENCE ON DRUG METABOLISM

In the uterus of ovariectomised rats oestradiol - 17B increases the activity of pyruvate-kinase, the rate-limiting step in glycolysis. Pyruvate-kinase induction is selective and not a consequence of a general increase in protein synthesis (De Asua et al, 1968).

The rate of synthesis of glutamic-alanine transaminase (GAT) in rat liver is measurably increased after prednisolone or hydrocortisone pretreatment. (Kim, 1967).

Administration of glucocorticoids increases the activities of the enzymes tryptophan pyrrolase, tryptophan - and tyrosine - a - ketoglutarate transaminase in the liver of rats (Knox, 1963).

Alanine aminotransferase is induced by corticosteroid in the hepatic and pancreatic tissue of rats (Keller et al, 1969). These workers report that binding of the steroids occurs and the free : bound ratio regulates the level of induction. The increased binding of corticosterone by the plasma of females explains the observed lower level of

induction that occurs in the female pancreas.

Thompson et al (1966) showed that tyrosine a keto-gluterate transaminase can be induced by various steroid hormones in tissue culture cells obtained from a rat hepatoma. Inhibiters of RNA synthesis - Actinomycin D and Mitomycin C - interfere with this enzyme induction which implicates the steroid activity at the DNA-directed RNA synthesis level. The arrangement of nucleotides in the molecules of RNA is determined by the arrangement of nucleotides in DNA - (template). Messenger RNA molecules carry information corresponding to the genetic code which results in amino acids being assembled together in a specific sequence according to the template to form proteins - (transcription).

Further to this Beato et al (1968) obtained increased RNA polymerase activity when rat liver nuclei were incubated in the presence of cortisol. Concommitantly they observed a 15-20% increase in the template activity of the chromatin (DNA). Barker and Warner (1967) observed a direct effect or oestradiol on chromosomal apparatus after prolonged 'in vitro' incubation of the hormone with isolated chromatin, resulting in increased template activity. Since the integrating of the nuclear membranes is necessary before increased RNA polymerase can occur, two processes influenced by the steroid appear to be involved. The first is the activation of the primer DNA and the second the rate of transcription of the active chromatin.

The exact locus of action of the steroids in enzyme induction appears to be at the level of the nuclear proteins.

Modification of the histone molecule by acetylation, methylation or phosphorylation or by a direct interaction between the histone and the hormone to form histone-steroid complexes, could lead to changes in template activity.

It has been shown that progesterone induces the synthesis of a specific protein in the chick oviduct. Because of the appearance of a new species of nuclear RNA (messenger RNA), progesterone is thought to exert its activity at the nuclear or transcriptional level of protein synthesis, (O'Malley & McGuire, 1969).

Since steroids induce enzymes and protein production in various organs, it is likely that the observed difference in response to drugs and environment during hormonal imbalance may be due to alterations in metabolism. Various workers have shown that liver microsomes contain enzyme systems dependent on reduced nicotinamide adenine dinucleotide phosphate (reduced coenzyme II or NADPH), which hydroxylate steroids such as oestradiol, testosterone, androsterone and progesterone. These enzyme systems resemble those which oxidise drugs and it has been suggested by Kuntzman et al (1964) that steroid hormones are naturally occurring substrates for drug-metabolising enzymes in liver microsomes. Recently steroids have been shown to compete with drugs for enzyme systems, and this led Tephly and Mannering (1968) to conclude that certain drugs and steroids are alternate substrates for a common microsomal mixed-function oxidase system. Pretreatment with barbiturates increases the metabolism of endogenous and exogenously administered steroids. Conversely, administration of steroids influences the metabolism of barbiturates (See Reviews of

Conney, 1967 and Kuntzman, 1969). The urinary excretion of pethidine and promazine and their metabolites is altered by pregnancy and administration of oral contraceptive steroids (Crawford & Rudofsky, 1966).

The microsomal haemoprotein cytochrome P_{450}, which is increased during steroid and barbiturate pretreatment, has been implicated as the terminal oxidase of the enzyme system which occurs in the liver and which hydroxylates a number of drugs and steroids. Recently Davies et al (1967) have found a good correlation between the amount of NADPH cytochrome P_{450} reductase and the rate of metabolism of ethylmorphine in the rat, rabbit, mouse and guinea pig, i.e. it is the rate limiting enzyme in the oxidation of the drug (See Review Kuntzman, 1969).

Kato et al (1968) have studied the effect of sex on the drug oxidizing activity of rat liver microsomes. There is a clear sex difference in the microsomal drug-oxidizing enzyme activity of rats but not other species (Quinn et al, 1958). Kato et al (1968) showed that the increased metabolism of hexobarbital, aminopyrine and aniline in male rats, compared with female rats, and in both males and females of other species (mice and rabbits), was due to an increase in the activity of NADPH - cytochrome c reductase (and other electron transport systems) and higher content of P_{450} haemoprotein.

Summary: Steroids are capable of inducing enzyme systems. There is a close correlation between enzyme systems which metabolize drugs and those which metabolize steroids. There is a sex difference in rats and their metabolism of drugs, which is linked with NADPH-linked electron transport and the terminal oxidase.

6. EFFECT OF STEROIDS ON THE RESPONSE OF ANIMALS TO CENTRALLY ACTING DRUGS

(a) Possible modes of interaction

Hormonal imbalance produced by surgical intervention or steroid therapy may alter the physiological environment of the animal so that centrally administered drugs may exhibit changes in potency and/or duration of action. Hormones may modify absorption, distribution,metabolism or excretion of the drug; the plasma binding of either drug or hormone may be changed, or the sensitivity of the CNS influenced by the hormonal status.

For example, adrenalectomy produces inadequate blood circulation which might impair absorption of drugs from the gastro-intestinal tract or from parenteral sites of injection. (Woodbury, 1958). Steroid drugs induce enzyme production in those liver microsomes capable of oxidising barbiturates and other drugs, and results in an increase in metabolism of the drugs and a reduction of their potency.
Oestrogens reduce while progestins elevate body temperature (Israel & Scheller, 1950), which results in different metabolic rates in the liver and other parts of the body. The rate of urinary excretion of non-metabolised drugs like

barbitone is most important but the effect of steroids at this site is thought to be unimportant. The distribution of drugs is known to be important with respect to their potency. The effects of barbiturates are proportional to plasma or brain levels. Thus, any agent which can modify these levels will profoundly affect the activity of the barbiturate. Fat depots which show a great affinity for certain barbiturates have been implicated in causing short sleeping times with the short acting barbiturates. It is well known that steroids modify carbohydrate metabolism and thus may interfere with fat depots. The degree of plasma binding will profoundly alter the activity of both steroids and centrally acting drugs. If brain excitability is altered by hormone status, then the sensitivity of the brain to central depressents or stimulants may also be changed. Thus, both adrenalectomy and the administration of DOCA decrease brain excitability and prolong pentobarbitone anaesthesia, (Robillard & D'Iorio, 1954), while cortisone increases brain excitability and shortens pentobarbitone anaesthesia. Progesterone decreases brain excitability and in larger doses produces anaesthesia (Selye, 1941), while oestradiol decreases EST.

(b) Historical evidence:

i. Barbiturates. Differences between the sexes in their response to drugs have been well documented. As far back as 1932, Nicholas and Baron, during a brief report of a new anaesthetic barbiturate, sodium amylobarbitone, reported a difference in response to the drug in male and female rats, the females requiring half the dose of anaesthetic compared with the males.

In 1937, Holck and co-workers made a study of the sex difference in rats' intolerance to certain barbiturates and also to nicotine. They showed the existence of male resistance to the short-acting barbiturates (e.g. hexobarbitone and pentobarbitone), but not to the long-acting barbiturates (e.g. barbitone). These studies were reproduced in the dog, cat, rabbit, guinea pig, mouse and frog - in these species no sex difference to the action of these barbiturates could be produced. They also showed that testosterone extracted from human urine counteracted the effect of castration in male rats and shortened the sleeping time in intact females. In 1947, Homberger and co-workers, whilst studying the factors affecting the susceptibility of rats to various barbiturates, showed that sex, age, weight and strain all influenced their reaction to barbiturates. In 1961, Kato and others showed sex differences in the metabolism of various drugs, including barbiturates. Robillard and co-workers (1954) made an extensive study of the effects of testicular, ovarian, adrenal and thyroid hormones on the liver detoxification of pentobarbitone. They believed that testosterone-induced shortening of pentobarbitone sleeping time is mediated through the release of cortisone from the adrenals. Quinn and co-workers (1954) showed that there are sex differences in response of rats to certain drugs, for instance, the hypnotic effect of hexobarbitone is of much shorter duration in male rats than in female rats. They believed this was due to the greater hexobarbitone metabolising ability of enzymes in the male rat. Testosterone administration to female rats increases the activity of the hexobarbitone metabolising enzymes and consequently shortens

the response to the barbiturate. Conversely, in male rats receiving oestradiol sleeping time is prolonged and the enzymes inhibited.

Most of this type of work was carried out in rats, although Westfall (1964) decided to experiment with steroid hormones and their interactions with barbiturates in mice. Using a single dose of the sex steroid 12 days prior to the administration of pentobarbitone, they showed that testosterone lengthened sleeping time in females, whilst stilboestrol significantly shortened sleeping time in males. In direct contrast, Gessner and co-workers (1966) showed that chronic pretreatment of male and female mice with sex hormones produced opposite results. Thus, ethinyl-oestradiol prolonged sleeping time in females and stilboestrol prolonged sleeping time in males. Also testosterone significantly reduced sleeping time in both sexes. Pretreatment with a single large dose of testosterone at varying times before the administration of barbiturate revealed a biphasic action of testosterone; sleeping time was prolonged if the hormone was given shortly before the hypnotic but shortened if a long time elapsed. In the former case, the testosterone may have had a direct hypnotic synergism with the hypnotic drug or a direct depressant action on the C.N.S.

There is little in the literature about the interaction of progestins with the barbiturates. The little work that has been done implies that progestins mimic oestrogens in their effects on barbiturate hypnosis (Robillard et al, 1954), despite physiologically that progestins act as anti-oestrogenic compounds.

Adrenalectomy significantly prolonged barbiturate anaesthesia in mice (Shibata & Komiya, 1953) and rats (Tureman et al, 1952 & Robillard et al, 1954). Also increase of glucocorticoids by stress (Bousquet et al, 1965 & Driever et al, 1965) in rats, or injection of cortisone acetate hydrocortisone acetate or ACTH in mice (Winter et al, 1952; Komiya & Shibata, 1953) or injection of ACTH and corticosterone in rats (Bousquet et al, 1965) shortened sleeping time to various short-acting barbiturates while the mineralocorticoid DOCA prolonged or had no effect upon sleeping time.

Remmer showed that adrenalectomy of either male or female rats decreased the activity of liver microsomal enzymes that oxidise hexobarbitone while Gillette (1963) and Kato et al (1965) found that adrenalectomy decreased hexobarbitone metabolism in vitro in male rats, but not females. Tureman and co-workers (1952), however, had previously shown that sex was unimportant in the effects of adrenalectomy upon pentobarbitone sleeping time.

ii. Analgesics: Paroli in 1957 undertook a series of experiments on the interactions of morphine with the sex hormones. He studied the influence of an 18-day course of injections of oestradiol (1 mg per male rat per day) on the mean lethal dose of morphine. The LD50 of morphine was reduced by 50% in treated rats. He also noticed atrophy of the gonads, increase in the chromophil cells of the hypophysis, and also hypertrophy and hyperplasia of the adrenal cortex. However, gonadectomy and chronic androgen administration had no effect on the resistance to morphine in rats.

Using hexoestrol and stilboestrol the analgesic effect of morphine, as determined by the tail-flick method of D'Amour and Smith (1941) was not augmented nor antagonised by either oestrogen, not were morphine intoxication and associated anatomical changes. However, a fall of bound morphine was observed, indicating that more of the free morphine was persisting in the blood stream. The result suggests that oestrogens influenced the fate of morphine, i.e. decrease metabolism and thus increase the levels of toxic concentrations of the drug in the rat. Paroli then determined the development of tolerance to the analgesic effect of a given dose of morphine (8 mg/kg). Tolerance occurred more quickly in the oestrogen treated rats than in the controls. This occurred together with a decrease in resistance of the treated animal to toxic doses of morphine.

Morphine is metabolised by N-demethylation in the liver. Since oestrogens are known to depress liver metabolism (Quinn et al, 1954) one would expect to find in the oestrogen-treated rats higher levels of circulating morphine as opposed to its metabolite nor-morphine. This would account for the greater toxicity of injected morphine in oestrogen-treated rats. The decrease in analgesic potency of morphine associated with oestrogen treatment might be explained by the work of Beckett and others (1956) who maintain that central demethylation of morphine provides the active moeity, nor-morphine, which is responsible for the analgesic action.

Crawford and Rudofsky (1967) have provided clinical evidence that altered hormonal status (for example, that associated with pregnancy, oestrogen therapy, and oral

contraception) will alter the metabolism of the synthetic narcotic analgesic pethidine. In each case, there was a definite increase in the amount of unmetabolised pethidine in the urine.

Kleiner et al (1965) have shown a similar picture in patients taking the contraceptive preparation 'Enovid' (which is norethynodrel together with mestranol). Administration of antifertility drugs produces a state of 'pseudo pregnancy' in women. Presumably arresting administration of oral contraceptives would have a similar effect to termination of pregnancy.

Since ACTH and cortisone relieve pain in joints swollen by rheumatoid arthritis, many workers believe these drugs to have analgesic properties (Review of Woodbury, 1958). This prompted Winter and Flataker (1951) to study the effects of DOCA, cortisone and ACTH on the response of animals to analgesic drugs. They measured the reaction time of the tail-flick response to thermal stimuli in normal rats under various treatments. Cortisone, DOCA and ACTH did not alter the normal reaction time, but pretreatment with ACTH and cortisone antagonised, while DOCA potentiated the prolonged reaction time after morphine and methadone. In 1963 Paroli demonstrated similar effects with hydrocortisone, and ACTH, where both the analgesic and respiratory depression due to morphine were antagonised. (These results were not reproducible with the other potent glucocorticoids dexamethasone and prednisolone).

Winter and Flataker (1951) also showed that cortisone exerted a synergistic effect with the narcotic antagonist nalorphine, enhanced the excitatory effect of morphine in

cats and moderately increased the LD50 of methadone in mice. The latter effect is probably due to antagonism of the respiratory depression due to methadone. They concluded that cortisone is stimulatory to the entire cerebrospinal axis. These results are in accord with those previously discussed in this Chapter.

iii. Stimulants: Very little evidence is cited in the literature regarding interaction between steroid hormones and amphetamine-like stimulants. Recently evidence of oestrogen/progestin effects on catecholamine and MAO levels in the brain (See page 28) has stimulated interest in the possible effects of these steroids on amphetamine's spectrum of activity. This is discussed later in Chapter III of the results.

iv. Anticonvulsants: With the advent of chronic administration of steroids acting as oral contraceptives (which may induce or inhibit hepatic microsomal drug metabolising enzymes) the possibility arose of drugs given concurrently becoming inadequate or toxic (Conney, 1967). During the course of the work for this thesis clinical (Espir et al, 1969) and pharmacological (Rümke & Noordhoek, 1969 & 1969) evidence of interaction between sex steroids and anticonvulsants was demonstrated. This is discussed further in Chapter IV of the results.

7. BASIS OF PROJECT

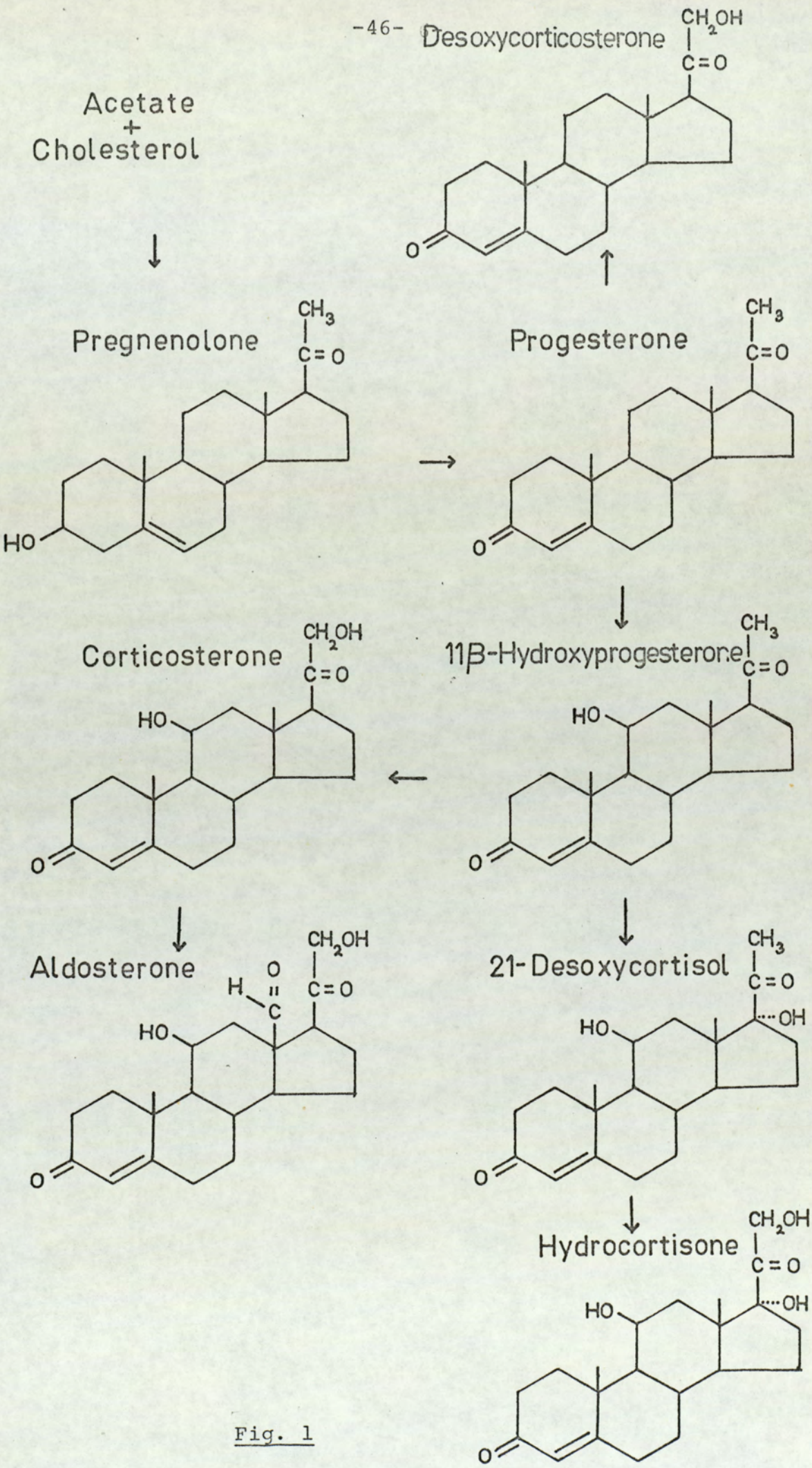
With the growing widespread clinical use of steroid hormones, either for replacement therapy in various hormonal deficiency states or as antifertility preparations in oral

contraception, there is an increasing percentage of the population receiving chronic steroid treatment together with intermittent or continuous medication with other drugs. The latter may be barbiturates, stimulants or anorexic agents, narcotic analgesics, or anticonvulsants.

The effects of steroids upon brain excitability and general body metabolism and the history of steroid drug interactions previously discussed, prompted this further investigation. It was decided to use the more potent, newly developed synthetic steroids to promote a hyper-hormonal state in mice and rats and then re-examine the spectrum of activity of hypnotic, stimulant, narcotic analgesic and anticonvulsant drugs in these animals.

In some cases the work has entailed confirmation of previous observations, using synthetic rather than natural steroids, (e.g. work with barbiturates and analgesics). In contrast the interaction of the synthetic steroid hormones with the stimulants and anticonvulsants is wholly new.

It is hoped that this work will help to clarify some of the factors to be considered during combined drug therapy.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Pregnenolone
Progesterone
Desoxycorticosterone
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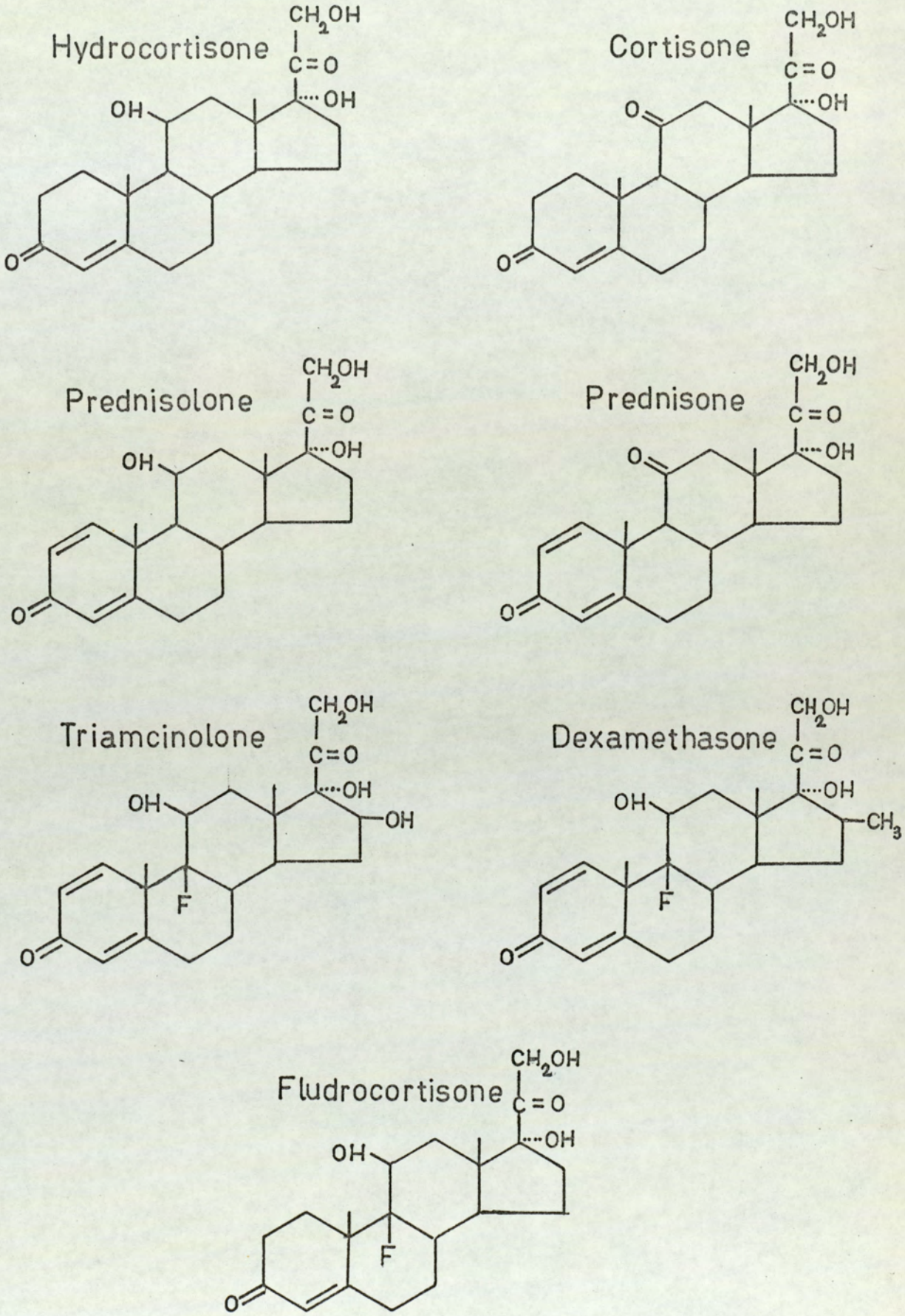
HO
O
CH3
CH2OH
C=O
OH

Fig. 1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Cortisone
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OH
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Prednisolone
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OH
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Prednisone
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C=O
OH
O
O
Triamcinolone
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OH
OH
OH
F
O
Dexamethasone
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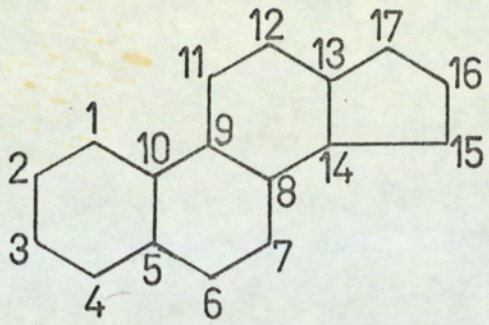
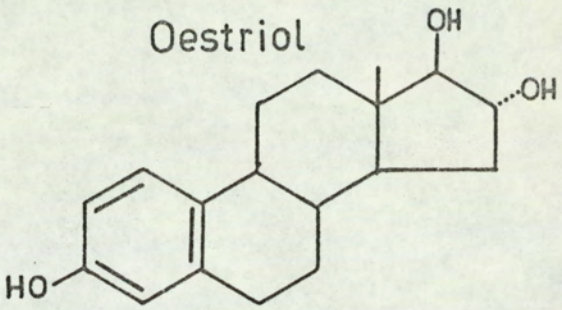
F
O

Fig. 2

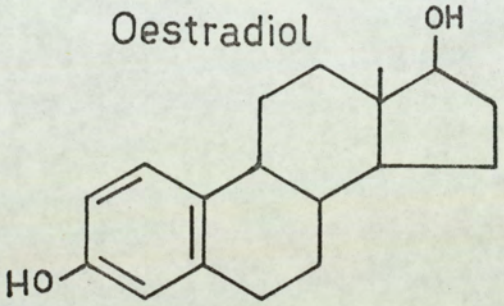


PERHYDROCYCLOPENTAPHENANTHRENE

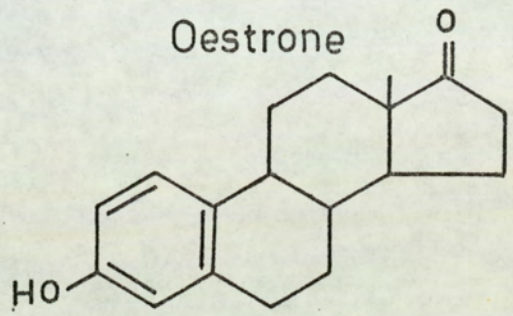
Oestriol



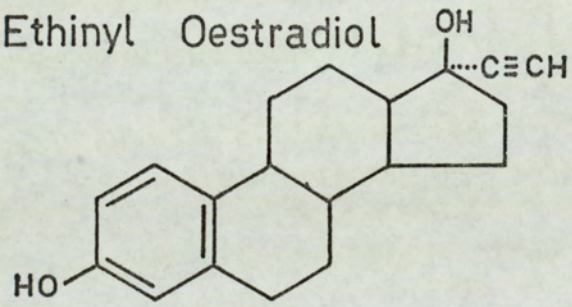
Oestradiol



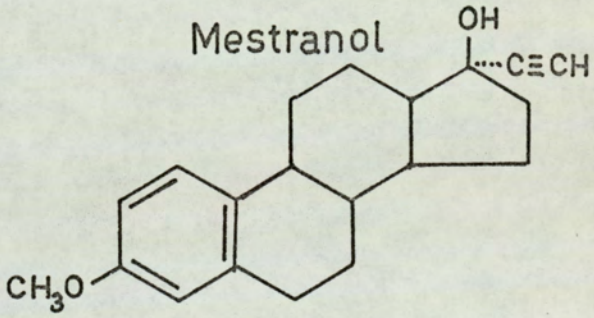
Oestrone



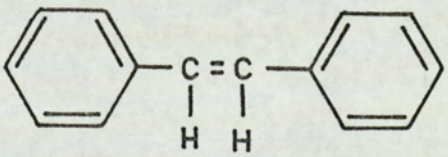
Ethinyl Oestradiol



Mestranol



Stilbene



Stilboestrol

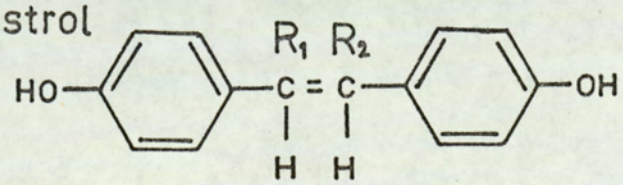
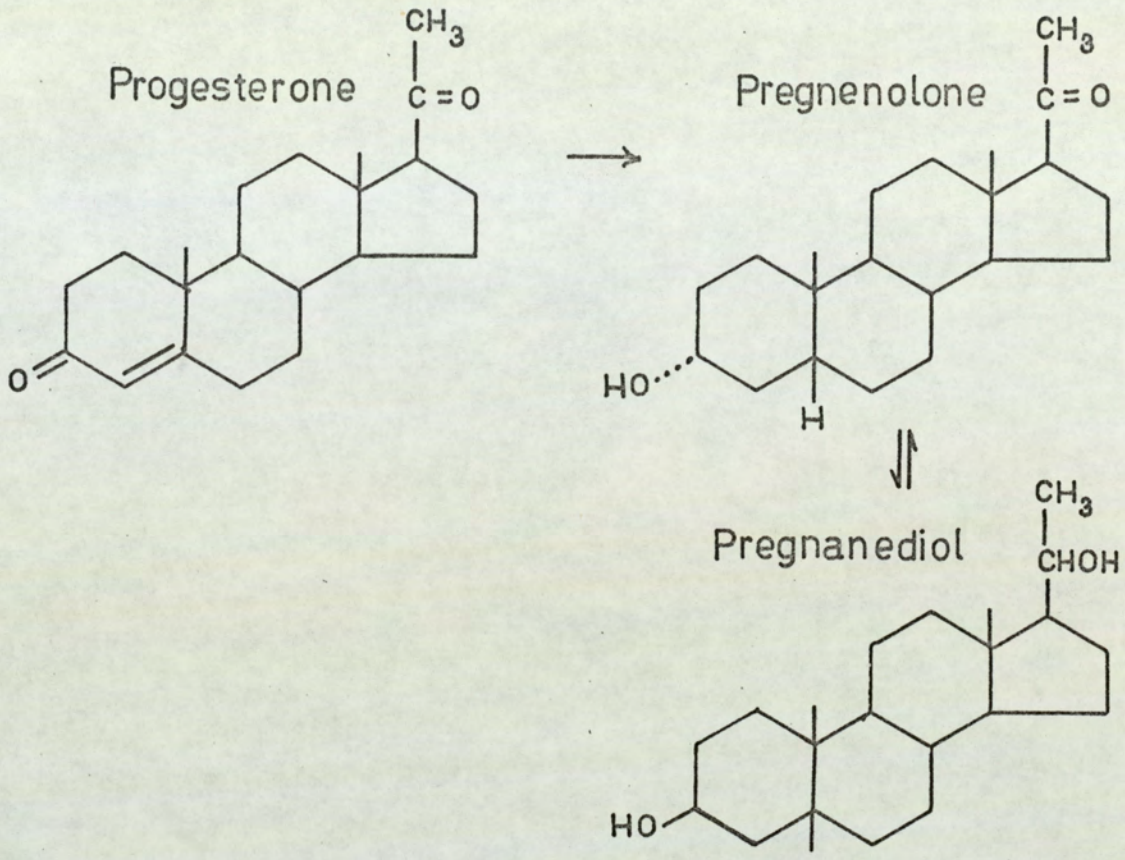


Fig. 3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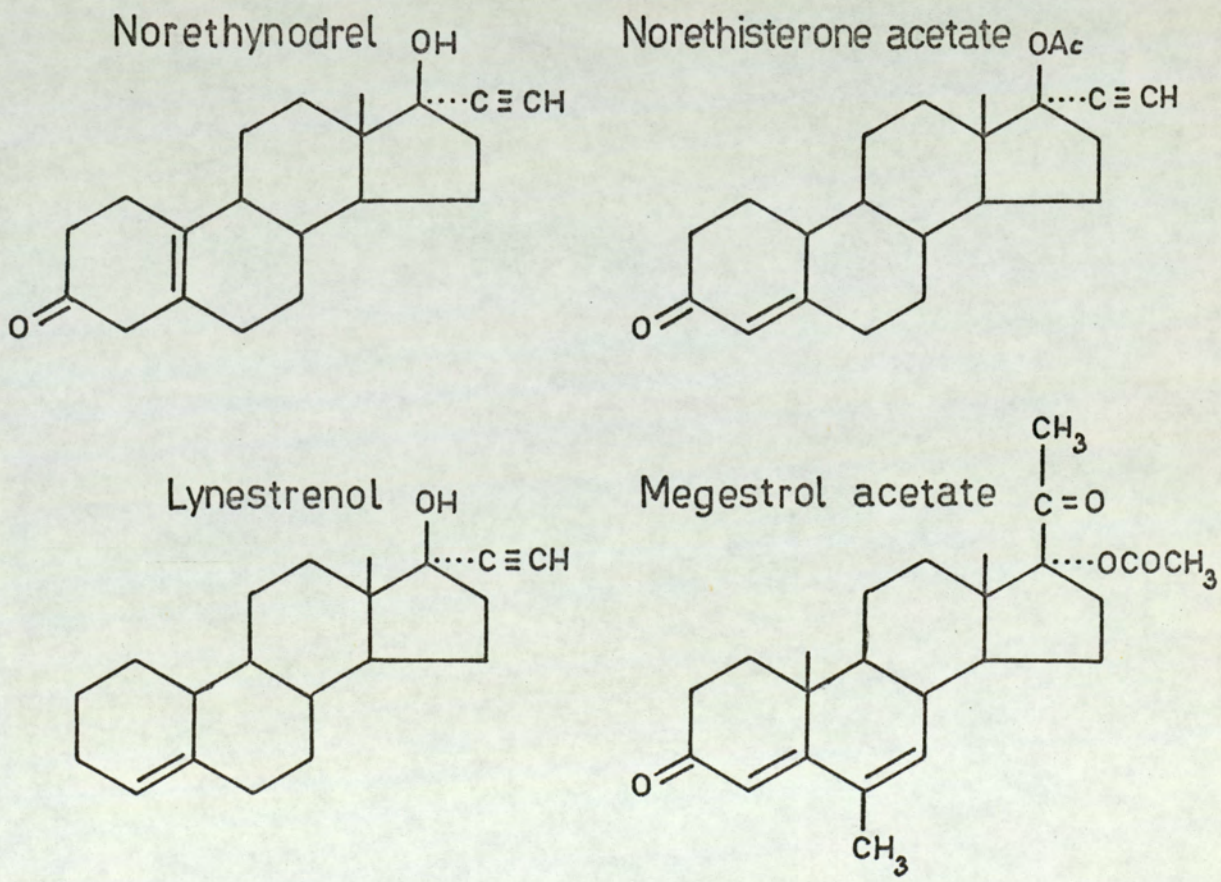
CH3
C=O
HO
H
Pregnanediol
CH3
CHOH
HO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Norethisterone acetate
OAc
C≡CH
O
Lynestrenol
OH
C≡CH
Megestrol acetate
CH3
C=O
OCOCH3
O
CH3

Fig. 4

METHODS

1. ANIMALS

Three week old Wistar female rats, and male and female TO mice were purchased and kept in animal house conditions for four to five weeks before experimental use. At this age the female mice were usually between 18 and 25g, male mice between 20 and 30g, and the female rats between 100 and 150g.

2. ANIMAL CARE AND LABORATORY CONDITIONS

The animals were fed on a conventional 41B cube diet and had access to a plentiful supply of tap water. The mice were kept in groups of either 25 or 50 and the female rats in groups of 8. The smaller groups of mice were kept in opaque plastic cages measuring 30cm x 26cm x 15 cm, whilst the larger groups of mice and the groups of rats were kept in large opaque plastic cages measuring 50cm x 30cm x 15 cm. The animal house was supplied with means to provide adequate ventilation and heating during the winter. The temperature usually remained between 20°C and 23°C. Experiments were performed in a temperature controlled room maintained between 20°C and 21°C.

3. STEROID PRETREATMENT

Animals were pretreated with daily injections of steroid hormones usually for four days but occasionally longer- this is stated in the text. The following synthetic steroid hormones were administered subcutaneously in oily solution in a dose volume of 5ml/kg for mice and

2.5ml/kg for rats: mestranol, (oestrogen); lynestrenol (progestin); dexamethasone, (glucocorticoid); fludrocortisone, (mainly mineralocorticoid); deoxycorticosterone, acetate - DOCA, (mineralocorticoid). The steroids were dissolved in a small volume of benzyl alcohol, 2-4% of total volume, and the volume made up to the mark with arachis oil. When proprietory preparations of the antifertility steroids were used, the tablets were ground up in a glass mortar and the powder suspended in 2% compound acacia powder and the resulting suspension administered to the mice orally in a dose volume of 5ml/kg.

4. ANIMAL OPERATIVE TECHNIQUES

(a) Adrenalectomy

Adrenalectomies were performed on female rats weighing between 100 and 150g. The rats were anaesthetised with a mixture of 3% Halothane, (reducing to 1.5% for maintenance), nitrous oxide 1 litre/min and oxygen 2.5 litre/min. They were shaved on their lower backs and two dorsal incisions made, one on either side of the spine, approximately 2 cm from the midline. Each incision measured 2 cm in both the skin and body wall. The adrenal glands were located and brought as near to the wound opening as possible. At this point a pair of splinter forceps was used to separate the adrenal gland from the kidney while a second pair of splinter forceps was used to nip off the gland. Sham operations comprised a similar operation except that the glands were not severed from their

connections. They were performed on an equal number of female rats to provide controls.

(b) Ovariectomy

Ovariectomies were performed on female rats weighing between 100 and 150g. The female rats were anaesthetised and incised in a similar manner to that outlined above, except that the two dorsal incisions were made a little lower in the back. The ovaries were located and drawn to the exterior using splinter forceps. The ovaries were separated from the fallopian tubes and surrounding fatty tissue with two pairs of splinter forceps. In this way haemorrhage was kept to a minimum. Sham operations (controls) involved a similar operation without removal of the ovaries.

In both operations the wound was closed using one silk stitch to close the body wall incision and a metal clip to close the skin incision. The animals were allowed minimum of two days for recovery before steroid pretreatment was begun.

5. PHARMACOLOGICAL TECHNIQUES

(a) Analgesia; 3 methods

i. Phenylquinone writhing (antagonism of).

Groups of 5 mice received an I.P. injection of 0.02% phenylquinone freshly prepared in 5% ethanol/water mixture using a dose volume of 0.25ml/20g body weight, (after the method of Siegmund, Cadmus and Go Lu, 1957), and maintained

at 37°C. The total number of writhes in the group of 5 mice in the following 20 min period was counted, (Hendershot & Forsaith, 1959). The reduction in this control value caused by pretreatment with an analgesic drug was used as a measure of analgesia. The potency and duration of action of morphine was studied in control and steroid pretreated mice. (Reduction by 50% was taken as the ED50 of the drug).

ii. Tail-clip response (antagonism of).

Groups of 5 mice received a painful stimulus to the tail by means of a lightly loaded spring clip applied about half way along the tail. The time taken for the mouse to make a conscious effort to remove the clip was measured with a stop-watch (after the method of Bianchi & Franchescini,1954). Morphine was used to increase the time before the mouse made any such effort. A points system was adopted: mice reacting between one and five seconds received 0 points, between six and sixteen seconds received 1 point, between seventeen and thirty seconds received 3 points and above thirty seconds received 5 points. In this way a cut-off time of thirty seconds was allowed. Definite analgesia was signified by a high score and poor analgesia by a low score. The potency and duration of action of morphine was studied in control and steroid pretreated mice. The maximum score possible would be 50 and a score of 25 was taken as the approximate ED50 of the analgesic drug.

iii. Tail flick method.

The method of D'Amour and Smith (1941) was modified so that the light produced by a pre-focused projector bulb

supplied by 6v D.C. current at 4.5A (= 27W) was directed on to the tail of a mouse restrained in a glass container (fitted with a close fitting lid through which the tail projected). This painful stimulus was enough to cause the mouse to flick its tail away in less than five seconds.(See Fig 5) Groups of five or ten mice were tested initially three times and their average reaction time measured. Upon receiving morphine the reaction time was shown to increase proportionally with the dose of analgesic. The dose of morphine to produce a 50% increase (or 100% when cut-off time 15 sec) in reaction time was measured (this was taken as the ED50); also the duration of action of the analgesic was determined. A plot of response time (sec) versus elapsed time after drug administration was made at various dose levels in both control and steroid pretreated mice. A cut-off time of ten seconds was used for most experiments. A study of a morphine antagonist was also made by this method. Doses of morphine which produced 'analgesia' ranging from 20 - 80% were obtained in preliminary experiments for each test.

(b) Hypnosis

Groups of ten or sixteen albino TO mice, both male and female, were used to determine the duration of sleep produced by various barbiturates. When the sleep-time was relatively long a semi-automatic apparatus was employed. This comprised a horizontal cylindrical glass container, pivoted freely by means of a glass rod attached along the length of its exterior surface and resting on two clamps. Attached at right angles to the rod was a writing lever which

wrote on a smoked kymograph drum rotating slowly. The barbiturate treated mouse, already asleep, was placed on its back inside the container and the lever made to write on the kymograph. When the mouse awoke it caused an oscillatory vertical movement of the cylindrical container which was recorded as a vertical stroke on the drum. (See fig. 6); concurrently a time marker recorded passage of time. Short sleeping times were measured with a stop-watch as the time between loss of righting reflex and the return of the righting reflex in both rats and mice. In the case of the non-metabolised barbiturate, barbitone sodium, when it was difficult to initiate true sleep in the mice, a slight modification was employed; the mice were assumed to be asleep if, when placed upon their backs, they failed to right themselves within 15 sec. They were tested for loss of righting reflex every 15 min.

(c) Measurement of Body Temperature

Temperature experiments were performed in a temperature controlled laboratory maintained at 20 - 22°C. The body core temperatures of mice were measured with an oesophageal probe as described by Brittain and Spencer (1964) and recorded on an electric thermometer (Light & Co., Brighton).

(d) i. Leptazol infusion and anticonvulsant activity in mice.

A 0.5% solution of pentylene-tetrazol (leptazol) was made up in normal saline. A 'Repette' syringe was filled with this solution and calibrated to deliver 0.05ml at every stroke.

Polythene tubing was used to connect the syringe to the broken-off shank of a No. 20 hypodermic needle which was used to cannulate the tail vein of the mouse. 0.05ml of the leptazol solution was delivered every 10 sec and the mouse observed for the following responses :

I. Initial twitch of the mouse's whole body;

II. Clonus, squeaks and resting phases - 'pseudo-convulsions';

III. Flexor/extensor tonic contraction usually ending in death.

(Method of Orloff et al, 1949)

ii. Maximal Electroshock and anticonvulsant activity in mice.

MES was produced by a shock of 70v, at 100 pulses per sec each of 0.2 msec duration, for 0.3 sec, delivered through silver ear electrodes. The current was supplied by an S.R.I. square wave stimulator, and activated by an A.E.I. timing switch. This method is based on that described by Cashin and Jackson (1962). The ED50 of various anti-convulsant drugs against MES, defined as the dose which protected 50% of the mice from a tonic extensor convulsion, was measured and analysed by the method of Litchfield and Wilcoxon (1949). Also the duration of action of the anticonvulsant drugs were determined in controls and steroid pretreated mice. Groups of ten mice were used at each dose level in the former experiment and percentage protection against dose curves were plotted on log/probit paper. Ref. Chartwell ref. 5575 log 3 cycle x probability. The duration of action experiments were

performed in groups of ten mice at various times after pretreatment with the anticonvulsant drug. In this way each mouse received only one maximal electroshock.

(e) Activity Counting

The activity of groups of five mice were compared using a Faraday system comprised of two activity cages fitted with metal grill bases and covered by perspex tops, drilled with holes to allow adequate ventillation. A spacing of at least one cage width was required between individual cages. Four aerial plates were positioned as described in manual supplied by Faraday Electronic Instruments Ltd. The cages were balanced against activity at the beginning of an experiment and the gain setting left at position seven. The mice were allowed at least half an hour in the activity cages before receiving the stimulant drug. A record of the activity produced by injection of stimulant drugs was compared in test and control animals for the duration of action of the stimulant drug. Pen recorders noted every hundred counts on a paper roll driven by a small electric motor. The experiments were repeated a number of times reversing the test and control groups in the two cages to obviate any residual imbalance in the activity recorder.

(f) Oxygen Consumption

An oxygen consumption chamber devised by Maclaghan and Sheahan (1951) and modified by S.R.I. Limited, was used to measure the metabolic rate of mice. Temperature inside the chamber was maintained at 20 - 21^{o}C by an outer water jacket,

whilst a soda lime compartment was employed to absorb the expired CO_2. A small fan inside the chamber was used to circulate the air. Each mouse was allowed a few minutes to acclimatise itself to the conditions before the apparatus was hermatically sealed. At this juncture 5ml of air was introduced into the chamber causing a rise in the internal pressure. This was recorded as a 100% displacement of a pen recorder attached to a low pressure transducer. By the law of partial pressures, the time taken for the animal to use up 5ml of oxygen was then equivalent to the time for the pressure to return to normal. Groups of five or ten test and control mice were compared.

(g) Blood Pressure in anaesthetised mice

The mouse tail vein was cannulated with a No. 18 needle (devoid of its stock) fitted with a polythene tube. The needle was previously dipped in a weak solution of heparin. Once in position the needle and tubing were tied to the mouse's tail in two places with cotton. When the tubing was completely filled with heparinised blood the tubing was sealed from the outside with a tightly fitting pin. The mouse was then anaesthetised with a mixture of oxygen and fluothane delivered from an apparatus devised for use with small animals by G.D. Parbrook (1967). In his method oxygen at 500ml/min was passed into the apparatus through tube A (see fig. 7). The majority of O_2 was passed directly to the animal by way of tap B. A small proportion was passed over the volatile anaesthetic in tube C by partly closing B, thus creating a "back pressure". However, it was discovered that even when

tube C was full and the manometer reading at a maximum the concentration of halothane was not great enough for either induction or maintenance of anaesthesia in mice. In fact in the present experiments the tap B was closed further so that bubbles of oxygen actually passed through the halothane. By adjustment of B and oxygen flow rate the number of bubbles passing through the halothane was kept constant. When induction of the venous cannulated mouse was complete the animal was laid on its back on a piece of expanded polystyrene which served as an operating board. The animals' limbs were tied to matchsticks mounted in the side of the polystyrene block by means of cotton thread. A midline incision was made in the neck region and the skin and neck muscle teased apart and secured with opthalmic eyelid spreaders to reveal the carotid and jugular vessels. The left carotid artery was located, dissected free of surrounding tissue and tied off distal to the heart. Blood flow from the heart was interrupted by means of a taut (not tied) cotton thread, and the vessel (magnified tenfold by a binocular microscope) was nicked with a fine pair of curved scissors. The artery was cannulated with a metal cannula manufactured from the bent shank of a No. 20 needle whose end had been filed square. The cannula and attached polythene tubing (containing heparinized saline) was tied into place securely. Upon releasing the taut cotton thread the animals' pulse was apparent immediately, and pressure recorded by a pressure transducer attached to a Devices electronic pen recorder. Records of blood pressure in the untreated anaesthetised mouse were recorded for as long as six hours by this method.

Drugs were administered through the venous cannula in dose volumes not greater than 0.05ml. The drugs were freshly prepared in normal saline.

(h) Acute toxicity - lethal dose 50% (LD50) uncrowded conditions

Groups of eight female TO mice received intraperitoneal injections of the stimulant drug under test at 5 dose levels in a dose volume of 10ml/kg. They were then placed in medium sized plastic cages measuring 36cm x 26cm x 15cm, allowing a floor area of about 78 cm^2 per mouse. The experiments were performed in the afternoon and the number of deaths recorded in the morning of the next day, about 20 hr later. LD50s of the drugs were calculated by the method of Litchfield and Wilcoxon (1949). Differences between the test and control animals are quoted at 5% significance levels.

- lethal dose 50% (LD50) crowded conditions

Groups of eight female TO mice received intraperitoneal injections of the stimulant drug at 5 dose levels, a dose volume of 10ml/kg. Immediately afterwards they were placed in the bottoms of standard two litre beakers lined with sawdust. The beakers were wrapped in brown paper to exclude the light through the side walls. This allowed a floor space of 10 cm^2 per mouse. Again the experiments were performed in the early afternoon andthe number of deaths recorded about 20 hr later. LD50s were calculated as above. The experiments were repeated in test and control animals and results pooled and compared.

6. ASSAY PROCEDURES

(a) Spectrophotometric assay of blood and plasma barbiturate levels.

SOLUTIONS:-

(i) Phosphate buffer pH 5.5

contains: a. Potassium dihydrogen orthophosphate; KH_2PO_4 - 136.09 (ANALAR B.D.H. LTD.)

500ml of 0.8M solution contained 54.44g of KH_2PO_4.

b. Disodium hydrogen orthophosphate; $Na_2HPO_4 12H_2O$ - 358.15 (ANALAR B.D.H. LTD.)

500ml of 0.5M solution contained 89.54g of $Na_2HPO_4 12H_2O$.

80ml of 0.5M $Na_2HPO_4 12H_2O$ was added directly to 500ml of 0.8M KH_2PO_4. The final few ml of the former solution were then added dropwise , the pH of the solution being monitored continuously by a pH meter, until pH 5.5 was reached.

(ii) Phosphate buffer pH 11.

To approximately 400ml of the remaining 0.5M $Na_2HPO_4 12H_2O$, a solution of 10N NaOH (Analar) was added dropwise to pH 11.

(iii) Petrol-ether, boiling range 100 - 120°C.

The solvent was purified by shaking with 1/5 volume of 1N NaOH. It was then dried over Na_2SO_4 and redistilled.

(iv) Petrol-ether, boiling range 40 - 60°C.

This solvent was purified as for (iii) above.

(v) Isoamyl alcohol, Analar, free of organic bases.

ULTRAVIOLET ABSORPTION:-

Pentobarbitone sodium exhibited an absorption peak at 240 mu in pH 11 buffer (See fig. 8). It was taken into pH 11 buffer for spectrophotometric measurement because it was noted by Brodie et al (1953) that in a strongly alkaline medium the ultraviolet absorption of the drug was decreased by 25% and the reagent blanks were less reproducible. The method described below was specific for pentobarbitone sodium, its metabolites remained unextracted.

(1) Assay of Pentobarbitone Sodium in Mouse Whole Blood

The initial assay of pentobarbitone sodium was based upon that used by Brodie et al (1953). In their method 2ml of urine or plasma was subjected to the assay procedure and 25 to 50 ug. of pentobarbitone sodium was recoverable with adequate precision ($95 \pm 3\%$). They found that normal plasma run through the same procedure gave a blank reading equivalent to 1 or 2 ug of pentobarbitone sodium per ml. In order to obtain results from individual mice it was thought necessary in the early experiments to use whole blood since the maximum volume of blood obtainable from each mouse was only 0.6 - 0.7ml. Also the volumes of reagents used were reduced.

Mice received 50 mg/kg of pentobarbitone sodium intravenously in saline, dose volume 5ml/kg, at various times before decapitation. The pentobarbitone sodium contained in mouse whole blood was assayed by the method described by Brodie et al (1953). 0.5ml of mouse whole blood was pipetted into a 15ml polypropylene centrifuge tube (I.E.C.) containing 0.5g of sodium chloride and 1ml of pH 5.5 buffer. 6ml of petrol ether (B.Pt. range 100 - 120°C) containing

1.5% isoamyl alcohol was then added. The whole was shaken for 20 min and separated by centrifugation at 15,000 rev/min for 10 min. 4ml of the organic phase was transferred to another centrifuge tube and extracted with 4ml of pH 11 buffer; after a further centrifugation at 7,500 revs for 10 min the organic phase was removed by aspiration. The absorbance of the clear buffered aqueous phase was measured in a SP 500 spectrophotometer using as zero reading the pH 11 buffer. 1 cm path length silica cells were used.

Solutions of pentobarbitone sodium, 100, 50 and 25 ug per ml, were prepared in 0.333N NaOH. 1 ml of each of these solutions was made up to 4 ml with pH 11 buffer to make standard solutions. Absorbance readings of each of these concentrations against pH 11 buffer were made on nine separate occasions, using freshly prepared standard solutions each time. (See Table 1). From the average absorbance readings a graph of absorbance against concentration of pentobarbitone sodium was drawn. (See fig.9). Using the graph, determinations of the blood barbiturate levels at various times after intravenous administration were calculated. A graph of blood concentration against time (See fig.10) indicates that the blood levels fell rapidly during the first 10 min and very much more slowly for the next 100 min. Using this method the waking blood levels of pentobarbitone sodium were studied in mice pretreated with sex steroids (See Chapter 2).

The results obtained using blood were not very satisfactory for the following reasons :-

(1) The results were not always reproducible;

(2) The blanks made by extracting whole blood were

sometimes too high;

(3) pH 11 buffer saturated with petrol-ether boiling range 100 - 120°C showed absorbance at 240 mu when compared with pH 11 buffer alone;

(4) Pentobarbitone sodium recovery was usually between 80 and 90% compared with 95 $\pm$ 3% claimed by Brodie et al (1953).

(2) Assay of Pentobarbitone Sodium in Mouse Plasma

It was decided to estimate the plasma concentrations of pentobarbitone sodium using the method described above. The method for obtaining 1 ml of mouse plasma was similar to that used by Noordhoek (1968). He also suggested using petrol-ether boiling range 40 - 60°C. Thus the final assay was essentially the same as that used for blood with the following modifications :-

(a) 1 ml of plasma, obtained from the pooled blood of four decapitated mice, was transferred to a centrifuge tube 4 mg of potassium oxalate deposited as a smear over the inside of the tube prevented the blood from clotting. The red cells were spun down at 3,000 revs/min for 12 min and 1 ml of plasma aspirated off.

(b) 0.5ml of pH 5.5 phosphate buffer was used instead of the original 1 ml.

(c) 8ml instead of 6ml of petrol-ether was used.

(d) Petrol-ether boiling range 20 - 40°C instead of 100 - 120°C was used.

(e) 6ml instead of 4ml petrol-ether was extracted by pH 11 buffer.

(f) A reagent blank exposed to the above extraction procedure was used as a zero reading instead of pH 11 buffer. In this way the blank readings were reduced and the percentage recovery rates were increased above 90%.

Solutions of pentobarbitone sodium 100 and 50 ug per ml were prepared as previously described. Absorbance readings of each of these concentrations against the reagent blank were made on ten separate occasions using freshly prepared standard solutions each time. These were very similar to those obtained previously (See Table 2). Again a graph of average absorbance against concentration of pentobarbitone sodium was drawn (See fig.11). From this graph it was possible to calculate plasma barbiturate levels at various times after intravenous administration of pentobarbitone sodium (See fig.12and Table 3).

During experiments in sex steroid pretreated mice, log plasma concentrations of pentobarbitone sodium against time was plotted and the results examined by regression analysis.

(b) Spectrophotofluorometric Assay of Noradrenaline dopamine and 5HT

Groups of eight female TO mice were killed by cervical dislocation, the brains were dissected out, weighed, and homogenized in 4ml of 0.4N perchloric acid at $0^{o}C$. The homogenate was centrifuged at 15,000 g for 8 min at $0^{o}C$ and the supernatant stored at $0^{o}C$. A second homogenization using a further 2 ml 0.4N perchloric acid was performed on the original sample and recentrifuged as before, the second supernatant being bulked with the initial supernatant.

The total clear supernatants from two groups of brains were combined, shaken and divided into two equal portions, one for dopamine and noradrenaline determination and the other for 5-hydroxytryptamine determination. Known amounts of the three amines were added to some extracts as a check on the recovery of these amines.

Noradrenaline and dopamine estimation

The aliquot of clear supernatant was titrated to pH 6.5 using 5N potassium carbonate at a pH meter. The precipitate of potassium perchlorate thus produced was removed by centrifugation at 15,000 g for 6 min at 0°C and the clear supernatant passed on to a Dowex 50 W.X.8 resin (100 mg dry weight) column which had been washed previously with: (1) 8ml 2N hydrochloric acid; (2) 10 ml distilled water; (3) 5ml 0.5M phosphate buffer pH 6.5; (4) 10 ml distilled water; (5) Two further 10 ml volumes of water. The dimensions of the washed resin column were 4 mm diameter and 12 - 15 mm in length.

The supernatant was passed through the resin at a flow rate not exceeding 1 ml in 2 min. After absorption of the amines, the columns were washed with 10 ml distilled water. Then, after passing 0. 5ml 0.4N hydrochloric acid on to the column to displace the water, the noradrenaline was eluted with 8 ml 0.4N hydrochloric acid at a flow rate not exceeding 1 ml every 2 min. The dopamine was then eluted with 8ml 2N hydrochloric acid at the same flow rate (having first displaced any 0.4N hydrochloric acid with 0.5ml 2N hydrochloric acid). This procedure was a modification of that used by Bertler, Carlsson and Rosengren (1958).

The noradrenaline was assayed by a trihydrocyindole method evolved from those of Euler and Floding (1955) and Bertler et al (1958). Phosphate buffer was used instead of acetate buffer, and zinc sulphate was omitted from the method. In the alkaline ascorbate, sodium borohydride was found to stabilize fluorescence, (Gerst, Odd, Steinsland & Walcott, 1966), although it was necessary to use a concentration of sodium borohydride ten times higher than that suggested by these workers. This stabilized the fluorescence of noradrenaline for at least 60 min. The fluorescence of noradrenaline was read at the activation and emission wavelengths 395/500 mu respectively in an Aminco Bowman spectrophotofluorometer.

Dopamine was assayed by the method of Carlsson and Waldeck (1958), with the modification of Carlsson and Lindqvist (1962. However, only 0.05ml iodine solution was used instead of 0.1 ml in the oxidation and maximum fluorescence developed without the use of ultraviolet irradiation. The fluorescent principle produced by this procedure was unstable in that it faded rapidly when subjected to the activation light in the fluorimeter, but if the tubes were immersed in a boiling water back for 5 min immediately after the oxidation and then allowed to cool to room temperature the dopamine fluorescence was stabilized at its maximum for at least 60 min. The fluorescence was then read at the activation and emission wavelengths 325/378 mu respectively.

5-hydroxytryptamine estimation

The 5HT aliquot was neutralized with 5N potassium

carbonate and centrifuged as for the catecholamine determination and the clear supernatant passed on to a column of Dowex 50 W.X.8 resin (100 mg dry weight) previously prepared in the sodium form with: (1) 8ml 1N sodium hydroxide; (2) 15ml distilled water; (3) 15ml 0.1N sodium hydroxide containing 0.2% w/v EDTA; (4) 10ml distilled water; (5) Two further 10ml volumes of distilled water. The clear supernatant was passed through this column at a flow rate not exceeding 1 ml every 2 min. The 5HT was then eluted from the column with 15ml 0.1N sodium hydroxide, (containing 0.2% w/v EDTA) into 1.5ml sodium acetate buffer, pH 4.6, and read directly in the spectrophotoflucrimeter at the activation and emission wavelengths 295/345 mu respectively. This method is similar to that used by Cox and Potkoniak (1967), but these authors eluted the catecholamines from this column with 1M potassium chloride before elution of 5HT. In our hands the presence of potassium chloride gave rise to higher blanks and significantly reduced the sensitivity of the 5HT assay.

The recoveries of the three amines by the methods outlined above were :-

Noradrenaline	— 73%
Dopamine	— 59%
5HT	— 69%

All values given are uncorrected for recovery.

7. ROUTES OF INJECTION, VEHICLES AND DRUGS USED

(i) Analgesics:

Morphine sulphate

Pentazocine hydrochloride

These drugs were dissolved in normal saline and administered subcutaneously.

(ii) Anticonvulsants:

Phenytoin sodium

Phenobarbitone sodium

Chlordiazepoxide hydrochloride (LIBRIUM - Roche)

Diazepam (VALIUM - Roche)

The first three anticonvulsants were dissolved in normal saline and administered intraperitoneally. Diazepam was obtained already dissolved as ampoules of Valium 10mg in 2.5ml. This was diluted out with distilled water immediately before intraperitoneal injection.

(iii) Enzyme inhibitors:

SKF 525A (PROADIFEN - SKF)

Nialamide

SKF 525A was dissolved in normal saline and administered intraperitoneally. Nialamide was dissolved in N/10 HCl and partially neutralised with N/10 NaOH to pH 5.5 and administered intraperitoneally.

(iv) Trophic hormones:

ACTH

Chorionic gonadotrophin

ACTH was dissolved in normal saline and administered 90 units per mouse, hourly, for three hours before pharmacological analgesic testing. Chorionic gonadotropin was administered 10 units per mouse, daily, for seven days before receiving the hypnotic drug. They were both injected subcutaneously.

(v) Hypnotics:

Barbitone sodium

Pentobarbitone sodium (NEMBUTAL - Abbott)

Hexobarbitone sodium (CYCLONAL - Abbott)

All three were dissolved in normal saline and administered intravenously.

(vi) Steroids:

Previously discussed (See page 51).

(vii) Stimulants:

dl-amphetamine sulphate

Dexamphetamine sulphate

Fencamfamin hydrochloride (Euvitol - A.& H)

They were all dissolved in normal saline and administered intraperitoneally.

ABSORBANCE at 240 mU		
25µg	50µg	100µg
0.265	0.475	0.910
0.225	0.468	0.920
0.225	0.500	0.960
0.218	0.468	0.935
0.242	0.475	0.970
0.242	0.475	0.970
0.237	0.445	0.945
0.200	0.434	0.945
0.200	0.434	0.945
MEAN 0.228	0.463	0.944

TABLE I

Absorbance of various concentrations of pentobarbitone sodium in pH 11 buffer.

ABSORBANCE at 240 mU	
50µg	100µg
0.452	0.910
0.452	0.918
0.472	0.920
0.470	0.940
0.450	0.880
0.458	0.910
0.480	0.920
0.470	0.920
0.437	0.900
0.448	0.905
MEAN 0.459	0.912

TABLE 2

Absorbance of various concentrations of pentobarbitone sodium in pH 11 buffer.

Time after pentobarbitone sodium, 50 mg/kg, I.V., in min	Absorbance at 240 mU after correction	Concentration of pento-barbitone sodium in 1 ml of plasma
0	0.896	96 µg
2½	0.430	47 "
5	0.354	39 "
10	0.286	31 "
20	0.256	28 "
40	0.203	22 "
60	0.176	19 "
90	0.157	17 "

TABLE 3

Concentration of pentobarbitone sodium in mouse plasma at various times after pentobarbitone sodium (50 mg/kg) injected intravenously.

Fig. 5

Apparatus devised for 'analgesia' testing of mice by the 'tail-flick' method of D'Amour and Smith (1941). The light produced by a pre-focused projector bulb directed on to the tail of a mouse restrained in a glass container.

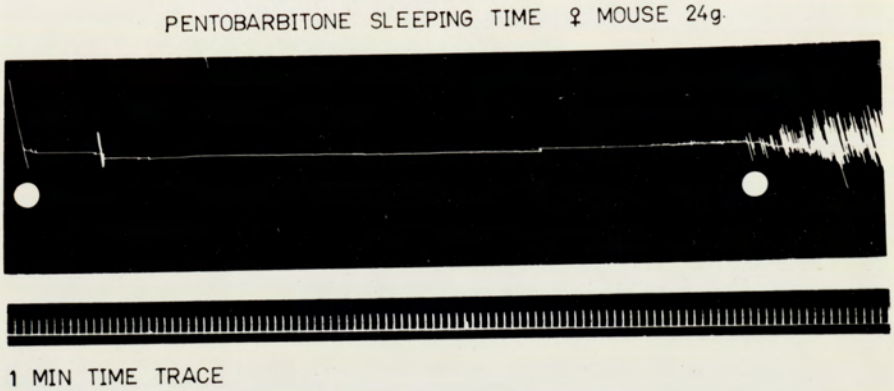


Fig. 6

Kymograph tracing produced by the apparatus devised to measure barbiturate sleeping-time in mice. The white circle on the right indicates the beginning of the oscillatory movement of mouse-container when the mouse first awoke.

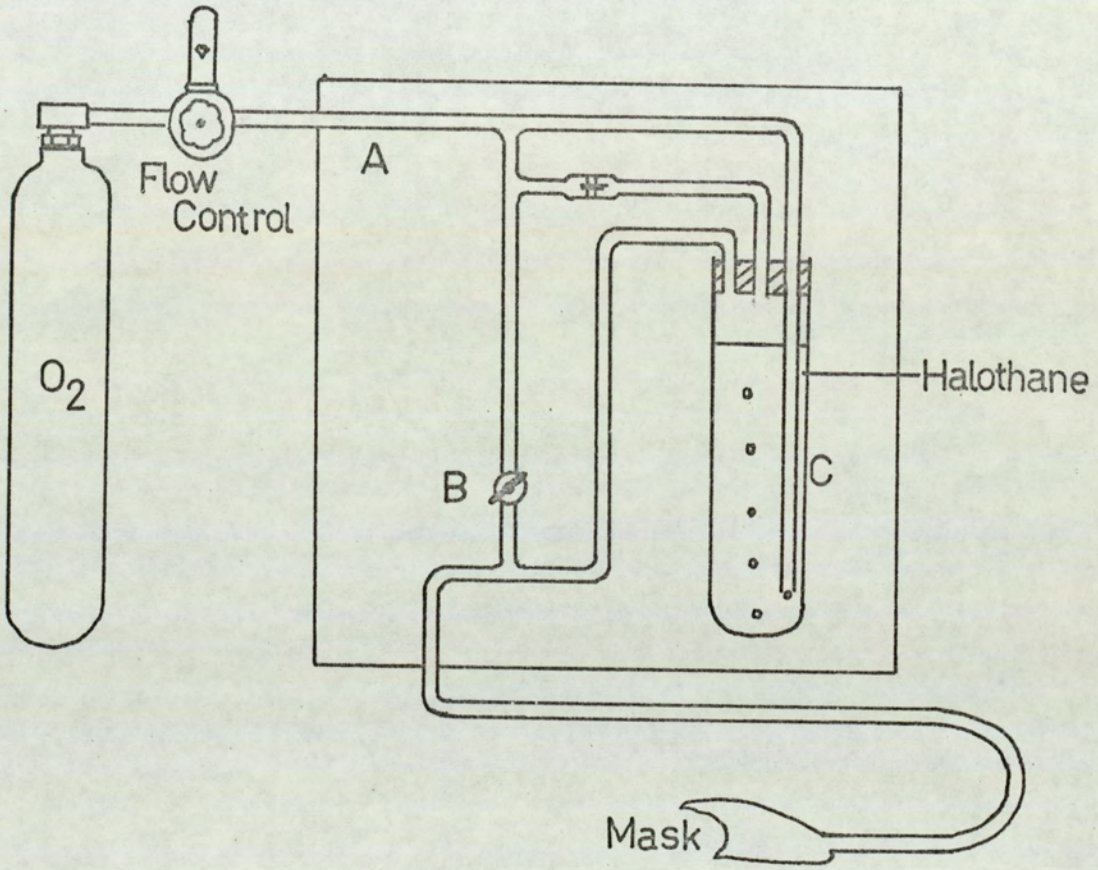


Fig. 7

Diagram of the apparatus devised by Parbrook (1967) as used by us for anaesthetising mice using a halothane/O_2 mixture.

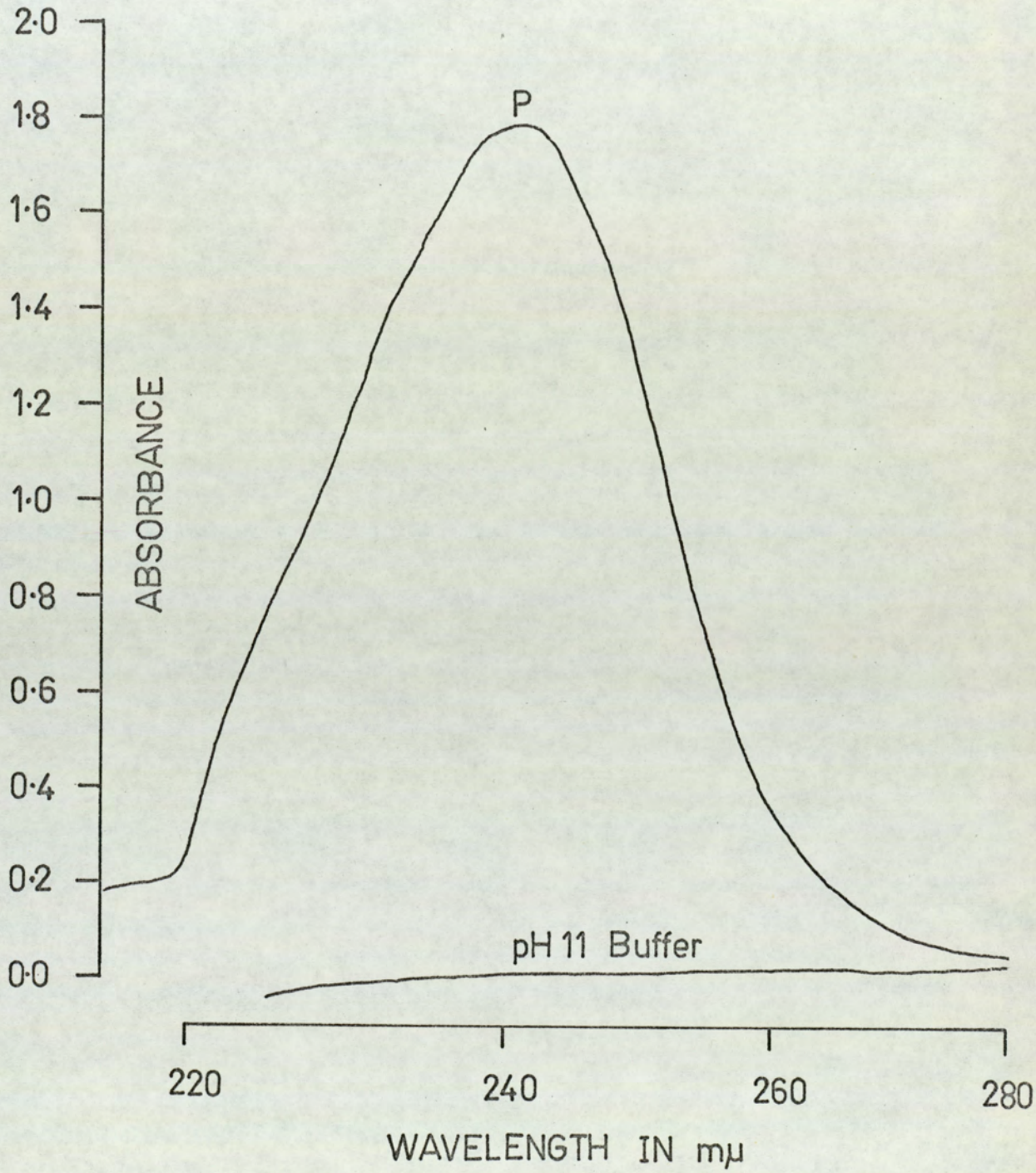


Fig. 8

U.V. absorption peak (240 mU) of pentobarbitone(P) sodium in pH 11 buffer.

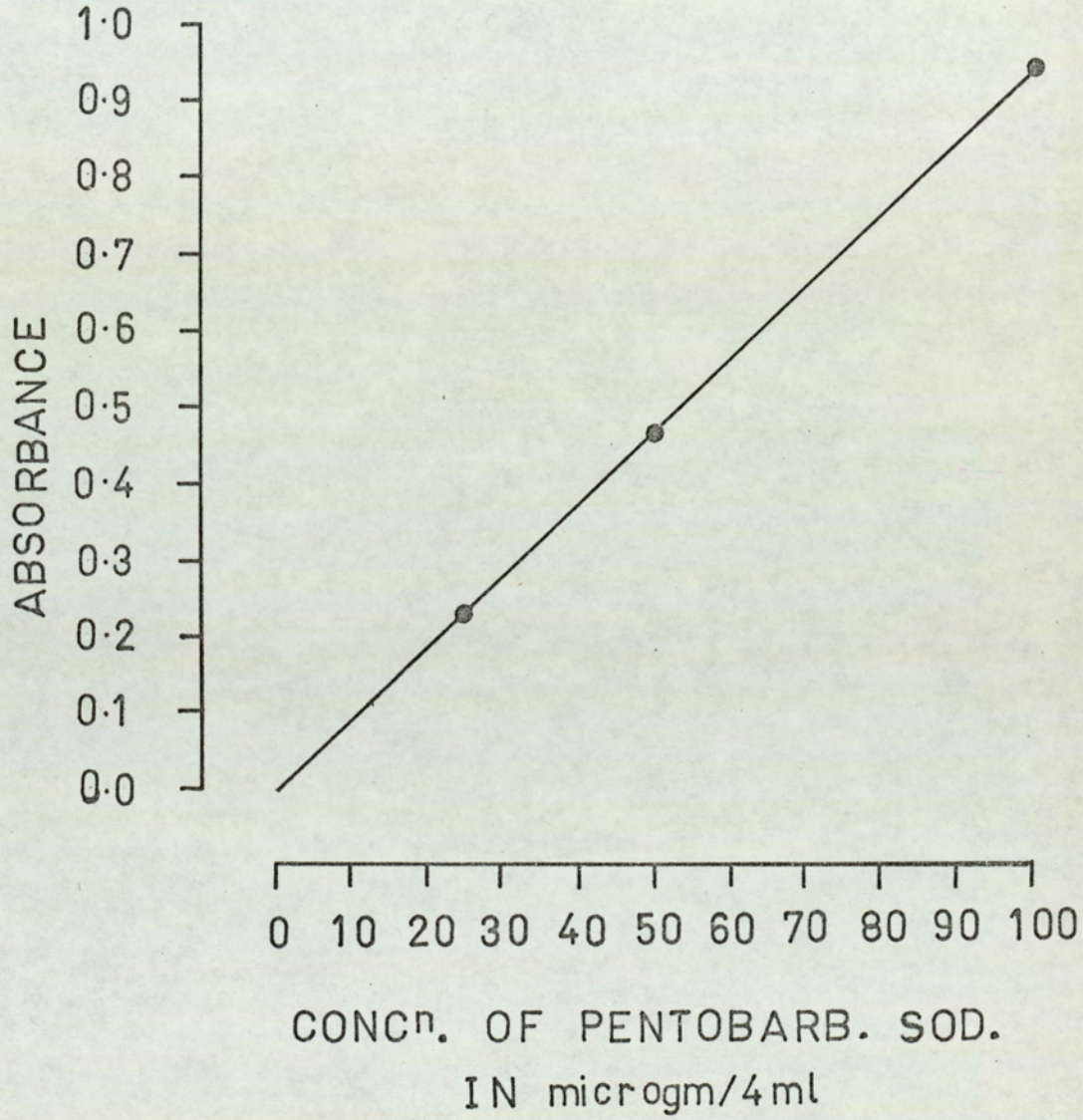


Fig. 9

I. Graph of absorbance (240 mU) vs. concentration of pentobarbitone sodium in pH 11 buffer.

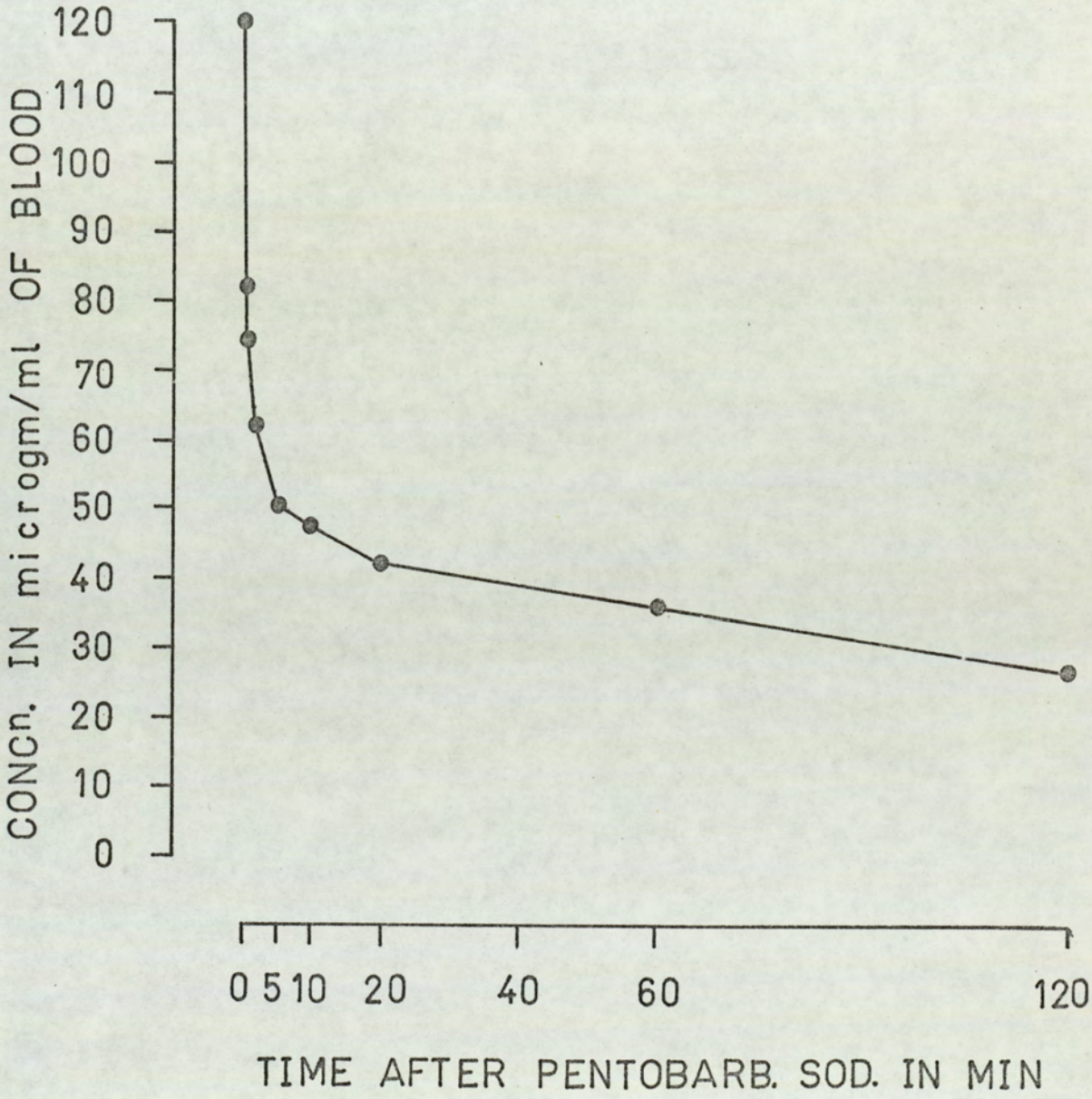


Fig. 10

Blood pentobarbitone levels in female mice at various times after intravenous injection of the drug (50 mg/kg).

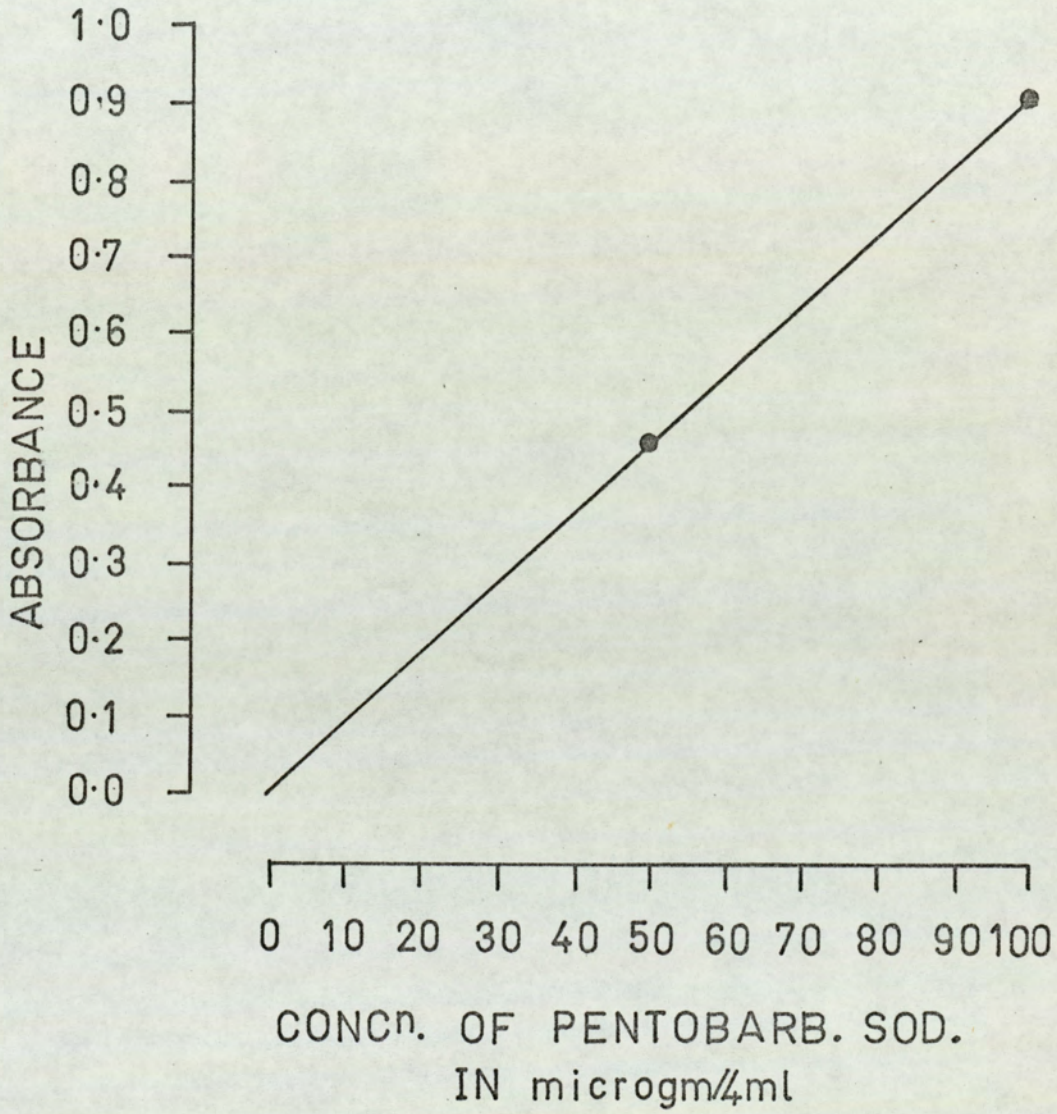


Fig. 11

II. Graph of absorbance (240 mU) vs concentration of pentobarbitone sodium in pH 11 buffer.

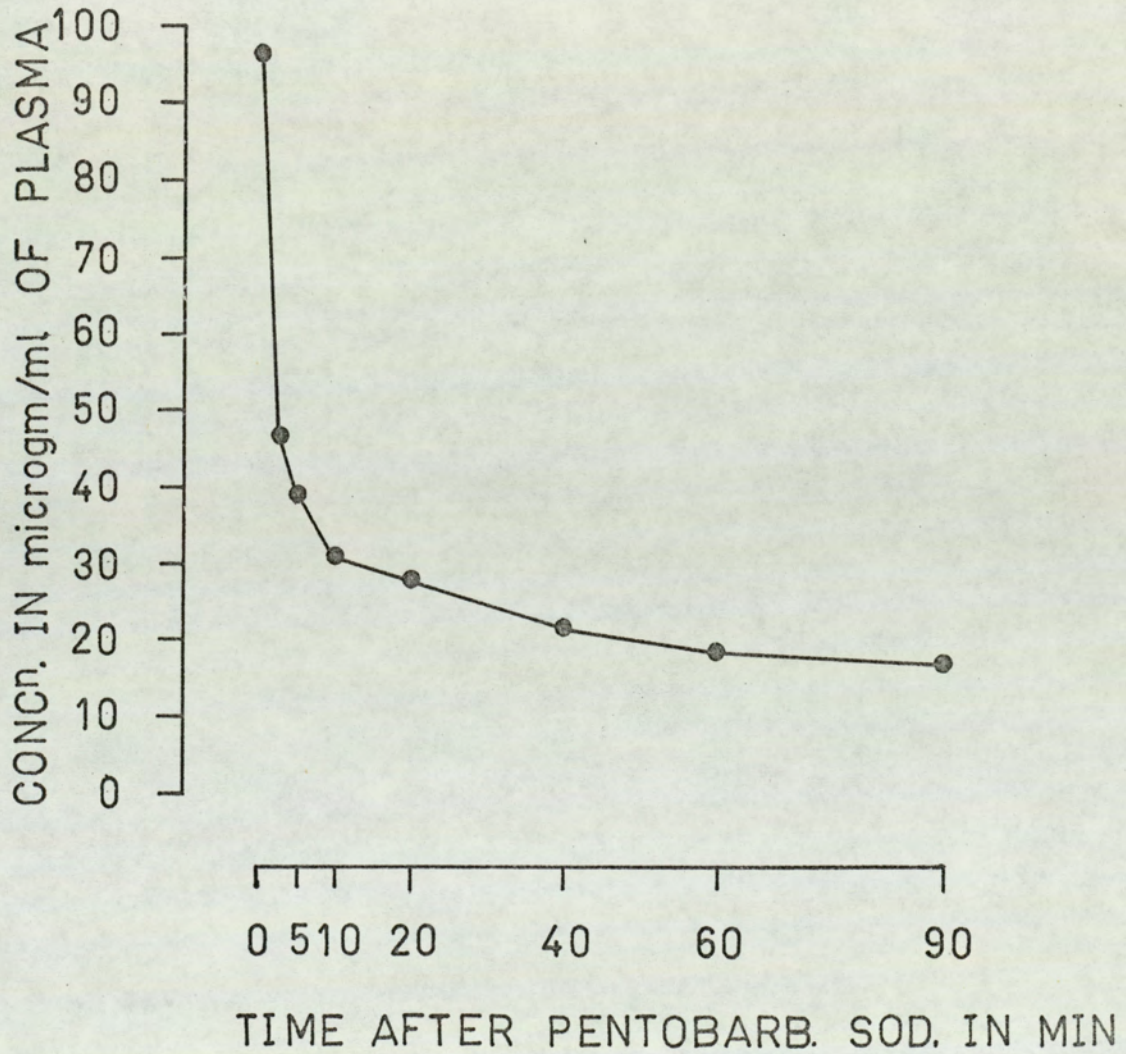


Fig. 12

Plasma pentobarbitone levels in female mice at various times after intravenous injection of the drug (50 mg/kg)

R E S U L T S

R E S U L T S

CHAPTER I - STEROID HORMONES AND ANALGESICS

Introduction :-

The aetiology of pain, comprising the anatomical sites of pain reception and the mechanism of analgesia has been the subject of extensive discussion over the years. This Chapter deals with the interactions of steroid hormones and analgesic drugs in steroid-pretreated mice. This by necessity demands a look into analgesic testing methods, which follows in the Discussion.

Analgesia is described as a 'state without pain' whilst an analgesic (or more correctly analgetic) is a substance which relieves pain without causing loss of other sensations. In man narcotic analgesics relieve pain of pathological origin but do not reliably raise the pain threshold. Thus, there is a lack of correlation between analgesia in man and prolongation of reaction time with analgesic agents in animals. It seems clear that the principal effect of any analgesic agent in relieving pain in man is upon the 'psychic reaction component' (Beecher, 1957). However, it is expedient to assume a perceptual nociceptive (as distinguished from pain) response to a 'painful' stimulus in analgesic testing methods upon animals.

It was decided to investigate in mice the effects of steroid pretreatment (both sex and adrenocortical types) upon the analgesics morphine and pentazocine. The former is

active in animals in the three analgesic tests used, namely phenylquinone writhing test (Siegmund et al, 1951), tail-clip test (Haffner, 1929; Bianchi & Franchescini, 1954), and tail-flick test (D'Amour & Smith, 1941), whereas pentazocine is active only as a morphine antagonist in animals. The analgesic effect of morphine is thought to be exerted at the thalamocortical projection system and associative areas of the brain (Sinitsen, 1961) since the classical sensory pathways as far as the thalamus remain unaffected by relatively high doses of morphine. However, small doses of morphine (0.5 - 3mg/kg) do not depress the reticular neuron system, but selectively block the connection of this system with the classical somatosensory pathways. Drug receptor interaction outlined by Van Rossum (1963) was extended by Beckett et al (1956) to morphine in the brain. They described the receptor as a charged anionic site 6.5 x 8.5. Å separated from a flat surface by a cavity. The aromatic nucleus attaches to the flat surface by Van der Waal's forces, the basic group to the anionic site, and the alkyl group fits into the cavity.

Paroli (1957) demonstrated that oestrogens influence the fate of morphine in rats, causing an increased toxicity and more rapid onset of tolerance (but he could show no difference in the morphine-prolonged reaction time in treated rats). He maintained that oestrogen pretreatment modified the metabolic fate of morphine. There is clinical evidence of a decrease in pethidine metabolism in women taking contraceptive preparations (Crawford & Rudofskey, 1967; Kleiner et al, 1965). Both natural and synthetic glucocorticoids (hydrocortisone) and ACTH

antagonise the prolonged reaction time after analgesic agents in rats (Winter & Flataker, 1951; Paroli, 1963) whilst DOCA has been shown to have the reverse effect (Winter & Flataker, 1951).

In the present study, the onset, duration and intensity of morphine 'analgesia' in mice pretreated with various synthetic steroids were examined in three analgesic tests. The control ED50 for morphine was found to be approximately 0.5mg/kg in the 'phenylquinone writhing'test and 7.5mg/kg in the 'tail-clip' and 'tail-flick' tests. The initial experiments were carried out in mice pretreated with dexamethasone in an oral suspension. It was later thought that an oily solution of this and other steroids would be more slowly absorbed and better tolerated when administered subcutaneously. Various doses of dexamethasone for different pretreatment times were examined but the lower dose of dexamethasone was found to be sufficient to induce adrenal weight changes after 4 days. This pretreatment time was found to be adequate for most of the steroids to produce their changes in this and further work.

SECTION I

I. Influence of steroid pretreatment on the reaction times of female TO mice to various nociceptive stimuli used in analgesic testing methods

It was noticed during the course of the work in this Chapter that pretreatment with steroid hormones altered the normal reaction times of mice in the three analgesic tests used. Dexamethasone (glucocorticoid) was the most fully investigated and the effects of this steroid on female TO mice in the 'phenylquinone writhing' test, 'tail-clip' test

and the 'tail-flick' test are considered in this section.

Groups of 15 female mice received dexamethasone (2mg/kg) orally in 1% sodium carboxymethyl cellulose (S.C.M.C.) daily for 2, 4 and 7 days. Controls groups received the vehicle (10 ml/kg) orally for the equivalent periods. On the third, fifth and eighth days, each group was divided into sub-groups of 5 mice each. Each mouse in the test and control sub-group then received an intraperitoneal injection of phenylquinone (0.02%) in 5% ethanol/water. The total number of writhes in each sub-group in the following 20 min period was counted and the results for the test and control mice compared (See Table 4).

All three steroid pretreatments produced a diminution in response (10 - 30%) when the mice were challenged with the noxious agent. However, there was a gradual reduction in the response of control animals during the course of the experiment. Dexamethasone is a well-known anti-inflammatory agent and may produce its antagonism by reducing the 'inflammatory response' to phenylquinone in the peritoneum. Control mice daily subjected to the stress of oral injection may have increased levels of corticosteroids which may explain the reduction with handling to the phenylquinone injection.

The experiment was repeated using a large dose of dexamethasone (10 mg/kg), subcutaneously, dissolved in arachis oil/5% benzyl alcohol. 3 and 5-day pretreatments were examined in groups of 15 female TO mice, control groups receiving the vehicle (5 ml/kg) subcutaneously, for the equivalent periods. Similar but more marked effects to those previously observed were obtained (See Table 5).

However, obvious local toxic reactions to these large doses of steroid were observed. After 3 days the mice developed lesions at the site of injection, their skin was parchment thin, their fur coarse and dishevelled; they appeared sedated and spontaneous activity was markedly reduced. By the fifth day there was a marked improvement in both activity and response to the noxious agent, phenylquinone. Thus it appeared that the animal developed tolerance to the steroid possibly by increased liver metabolism.

A further group of 10 mice having received a 4-day course of steroid pretreatment were allowed to recover for 3 days. Controls were similarly pretreated with vehicle and allowed to recover for 3 days. When these mice were challenged with phenylquinone it was observed that the test animals showed a marked increase in response to a level greater than control mice (40%; See Table 5). Dexamethasone produces a decrease in adrenal weight in the test animals. Upon withdrawal of the dexamethasone injections for 3 days some adrenal insufficiency would be expected which might account for the increased number of writhes in the phenylquinone test.

Groups of 10 female TO mice were pretreated with dexamethasone (2 mg/kg) subcutaneously daily for 4 days. Controls received the vehicle (5 ml/kg) during this period. On the fifth day the time for each mouse to react when a spring loaded clip was placed on the tail was measured with a stop-watch (See Methods, page 53). The average reaction time for groups of 10 test and control animals was calculated. The experiment was repeated in further groups of 10 animals (See Table 6). In this test pretreatment

with dexamethasone does not influence the average reaction time of the mice to the painful stimulus; the variation in reaction time ranged from 0.2 sec to 16.2 sec in a group of animals.

Groups of 10 female TO mice were pretreated with dexamethasone (2 mg/kg) subcutaneously, daily, for 4 days as above. Control mice received the vehicle only during this time. On the fifth day the reaction time of each mouse to a painful thermal stimulus applied to the tail was measured using a stop-watch (the 'tail-flick' test, Methods, page 53). This procedure was repeated at 15 min intervals. A cut-off time of 15 sec was employed since a longer stimulation produced tissue damage. The average reaction time for the first stimulation tended to be longer than subsequent ones, but not always so. The 'normal' reaction time was taken as the mean of the second and third average reaction times. In three experiments the 'normal' reaction time of control mice lay between 4.55 and 5.0sec whilst the 'normal' reaction time of dexamethasone pretreated animals lay between 5.05 and 5.45 sec, a significant increase (See Table 7). Thus as in the phenylquinone test dexamethasone confers some 'analgesic' action in mice subjected to these tests. The most likely explanation of this phenomenon is the anti-inflammatory action of the steroid rather than a central effect.

Thus these experiments demonstrate clearly that changes in potency of analgesic drugs believed to be brought about by a steroid may not be due entirely to a steroid/drug interaction but partly to an inherent property of the steroid itself.

2. Influence of dexamethasone pretreatment on female mouse adrenal weights

Groups of 5 female TO mice received dexamethasone (2 mg/kg) orally, daily, for 7 days. Control mice received the vehicle, 1% sodium carboxymethyl cellulose, orally, also for 7 days. On the eighth day the animals were killed and their adrenals removed and weighed. The average paired weights for test and control animals were calculated and compared. The average body weight of each group was also calculated (See Table 8). The average paired adrenal weight from dexamethasone pretreated mice weighed 35% less than the relevant control paired adrenal weight. The 5% difference in body weight of these animals could not account for this large decrease which must, therefore, be due to dexamethasone administration. This steroid depresses endogenous adrenocortical function probably by blocking ACTH formation in the adenohypothysis of the pituitary; sufficient to cause marked adrenal atrophy.

A similar reduction in adrenal weight was produced in female mice pretreated with a large dose of dexamethasone (10 mg/kg) for only 3 days. When a group of 5 female mice was pretreated with dexamethasone (10 mg/kg) subcutaneously for 4 days followed by a 3-day withdrawal, the reduced adrenal weight observed above was still obvious (See Table 9) whilst the effects upon the phenylquinone writhes (see above) were markedly potentiated.

SECTION II

1. Influence of dexamethasone (glucocorticoid), fludrocortisone (mainly mineralocorticoid) and adrenocorticotrophic hormone (ACTH) on morphine 'analgesia' in mice in various analgesia tests

Morphine was studied because it is a potent narcotic analgesic which is active in all three analgesia testing methods used. Also the ED50 of the drug differs somewhat depending upon the analgesia test chosen.

Dexamethasone (2 mg/kg) was administered orally to 40 female mice, daily, for 4 days. Control mice received the vehicle, 1% S.C.M.C., orally also for 4 days. On the fifth day the mice were divided into groups of 5 and each group received various doses of morphine hydrochloride subcutaneously 30 min before receiving 0.02% phenylquinone (0.25 ml/20 g). The control groups were similarly injected. The experiment was arranged so that one control and one test group, having received equal doses of morphine hydrochloride, were observed for phenylquinone writhes at the same time. In all, 6 doses of morphine hydrochloride were studied (0.1, 0.2, 0.5, 1.0, 3.0 and 10 mg/kg). At first glance (See Table 10 and fig. 13) there appears to be a definite potentiation of morphine analgesia especially at the lower dose levels studied. However, when it is remembered that dexamethasone itself reduces phenylquinone writhes then it becomes obvious that dexamethasone pretreatment does not directly influence morphine analgesia measured by this test.

Groups of 10 female TO mice were pretreated with dexamethasone (2 mg/kg) or fludrocortisone (2 mg/kg) subcutaneously in oily solution, daily, for 4 days; control mice received the oily vehicle only. On the fifth day, 18 hr after the last injection, the reaction time of mice subjected to the stimulus of a spring loaded clip applied to the tail was determined (by a scoring system outlined in the Methods, page 53). Subsequently each group of animals received morphine hydrochloride (5 mg/kg) sub-cutaneously and the degree of analgesia scored at various intervals after injection. The experiment was repeated in further groups of animals using a higher dose of morphine hydrochloride (10 mg/kg).

With the lower dose of morphine, after 15 min there was a significant antagonism of analgesia in the dexamethasone-pretreated animals (50% reduction) when compared with controls (See Table 11). In contrast, fludrocortisone-pretreatment was without significant effect. At the higher dose of morphine, there was a slight but insignificant increase in analgesia in both groups of steroid pretreated animals. (It is unfortunate that this method does not readily lend itself to conventional statistical analysis, but it was considered that changes of less than 30% were unimportant).

Groups of 10 male TO mice were pretreated with ACTH (20 i.u./mouse) intraperitoneally, hourly, for 3 hr. Controls received distilled water (0.1 ml/mouse) intra-peritoneally at similar times. Half an hour after the last pretreatment each mouse received a subcutaneous injection of morphine hydrochloride (7.5 mg/kg) and the tail-clip

reaction time scored at intervals after.

The results show clearly that ACTH pretreatment antagonises morphine analgesia to a marked extent (80%) (See Table 12 and Fig.14a). Since pretreatment with adrenocortical steroids in females failed to produce any marked antagonism to morphine analgesia it appeared that the ACTH was not mediated through adrenal corticosteroid release.

Groups of 10 female TO mice were pretreated with dexamethasone or fludrocortisone as above; control animals received the vehicle only. On the fifth day their reaction time in the tail-flick analgesic test was examined on three occasions at 15 min intervals. The average of the second and third readings for the group was taken as the normal reaction time. The prolonged reaction time with dexamethasone (previously discussed) was also seen with fludrocortisone-pretreated animals (5.75 sec and 4.9 sec compared with 5.0 sec and 4.45 sec for controls). The animals then received morphine hydrochloride at two dose levels (5.0 and 7.5 mg/kg) subcutaneously. The reaction time of each mouse was tested after using a cut-off time of 15 sec. The results are recorded in Table 13.

Fludrocortisone significantly potentiated the prolongation effect of both dose levels of morphine upon reaction time. At 30 and 60 min after 7.5 mg/kg morphine, fludrocortisone-pretreated animals showed an average reaction time between 3 and 4 sec longer than control animals ($P = 0.1 - 0.05$). The duration of action of the drug was also prolonged. However, if the difference in normal reaction time was taken into account the effects seen would not nearly be so marked. Dexamethasone prolongation was

found not to be significantly different from controls (possibly due to the large standard errors obtained when using a 15 sec cut-off time).

In order to reduce the standard errors of groups of 10 animals, the cut-off time was reduced from 15 to 10 sec for further experiments. This also reduced the risk of tissue damage due to prolonged exposure to radiant heat.

A group of 10 male mice was pretreated with ACTH as previously described, whilst control animals received saline (vehicle). The normal reaction time for each group was obtained in the tail-flick procedure. ACTH pretreatment tended to prolong the average normal reaction time above control levels (5.1 sec compared with 4.75 sec for controls). Each animal then received morphine hydrochloride (10 mg/kg) subcutaneously, and their reaction times measured at various intervals after.

The results (See Table 14) again demonstrate that ACTH pretreatment antagonises morphine 'analgesia' in mice. Between 30 and 60 min after morphine injection the average reaction times of the ACTH-pretreated animals were between 1 and 2 sec shorter than the controls (P = 0.1 - 0.05).Fig.14b.

SECTION III

Influence of lynestrenol (progestin) or mestranol (oestrogen) on morphine 'analgesia' and on pentazocine/morphine interaction in the 'tail-flick' test in female mice

Morphine: Groups of 10 female TO mice were pretreated with lynestrenol (5 mg/kg) or mestranol (1 mg/kg), subcutaneously

daily, for 4 days. Control animals received the vehicle only (5 ml/kg), daily. On the fifth day their average normal reaction time in the 'tail-flick' procedure was determined (See previous Section). Again, steroid-pretreatment tended to prolong the average reaction time above control levels. Each group of animals then received morphine hydrochloride at various dose levels (5.0, 7.5 or 10 mg/kg), subcutaneously, and their reaction times measured at various intervals after injection. The results are recorded in Table 15 and Figs.15a and 15b.

The results indicate that lynestrenol tends to potentiate the morphine prolongation of reaction time but the effects are not marked (between 0.5 and 1 sec depending on the dose of morphine). The reverse effects are seen in mestranol pretreated animals but again the changes are not very significant. It is difficult to separate the effects of the steroids upon normal reaction time from those upon reaction times prolonged by analgesic. However, when reaction times at 45 min after injection for each dose of the analgesic are plotted (for each pretreatment), then definite trends are seen. Thus, lynestrenol potentiation and mestranol antagonism of morphine-induced prolongation of reaction times are greater at the high dose of morphine and lower at the low dose of the drug. This indicates an interaction between the steroid and the analgesic drug rather than a direct steroid effect upon reaction times.

Pentazocine: Groups of 10 female mice pretreated with sex steroids, as above, were used to test the 'analgesic' effect of pentazocine in the 'tail-flick' procedure. Pentazocine sulphate (30 mg/kg) was injected subcutaneously into each mouse after the average normal reaction times

for the groups were obtained. The animals were tested at intervals for any prolongation of reaction time. Pentazocine failed to significantly increase the reaction times in either control or steroid pretreated animals. (See Table 16).

Pentazocine/morphine interactions: Since pentazocine does not prolong the reaction time in the 'tail-flick' test, any steroid-induced changes in its spectrum of activity in mice would not be revealed. However, it is known that the potency of pentazocine as a morphine antagonist in animals parallels closely the clinical efficacy of the drug as an analgesic in man. Thus, measurement of morphine antagonism by pentazocine in steroid pretreated animals might give an indication of possible steroid-induced changes in pentazocine analgesic potency in man.

The ability of pentazocine sulphate (20 and 60 mg/kg) to antagonise morphine hydrochloride (10 mg/kg) effects in groups of 5 steroid-pretreated mice (as above) were examined. The results were compared with the reaction times of sex steroid pretreated mice receiving morphine hydrochloride (10 mg/kg) only. The pentazocine was administered 15 min before the morphine and the reaction times were measured at 15, 30 and 45 min after the latter injection. Both drugs were injected subcutaneously.

In both mestranol and lynestrenol-pretreated animals, after 45 min pentazocine (20 mg/kg) antagonised the morphine-induced prolongation of reaction time by 2 sec; a significant antagonism. (See Table 17). In control animals, this dose of pentazocine failed to antagonise morphine 'analgesia'. The higher dose of pentazocine

obliterated the morphine prolongation in all 3 groups. However, since the steroid might alter the fate of either drug to varying extents, it is difficult to draw definite conclusions from these results.

DISCUSSION

Three classes of nociceptive stimuli were used in these experiments (chemical, mechanical and thermal), in order to test the merits of each. Rarely do two workers use the same methods but it is accepted that the criteria for an adequate 'analgesic' test should include fairly constant 'normal' responses to the nociceptive stimulus, from animal to animal and from day to day; they should also discriminate between graded doses of the analgesic drug and be suitable for statistical analysis. Obviously the stimulus should be constant and should produce little tissue damage. The animal response should be co-ordinated in the brain (i.e. not entirely a spinal reflex) since this is where narcotic analgesics are thought primarily to act clinically.

The 'phenylquinone writhing' test (Siegmund et al, 1957) was shown to discriminate between a wide range of low doses of morphine which was thought to be useful in detecting any small steroid-induced changes in morphine activity. However, this test suffered a number of serious disadvantages. The 'normal' number of writhes from a group of 5 mice varied considerably from day to day, and itself appeared to be markedly affected by the presence of steroids. Also, since a wide variety of non-analgesic drugs were known to be active in antagonising phenylquinone writhes (Siegmund et al, 1957; Hendershot & Forsaith, 1959), there is some doubt

whether brain stem mechanisms are involved. It was found difficult to analyse the results statistically unless large numbers of animals were used.

A spring loaded clip applied to the tail of the mouse was used to produce a mechanical noxious stimulus (Haffner, 1929; Bianchi & Franceschini, 1954) in mice. Because of the wide range of normal reaction times in mice it was found necessary to adopt a simple scoring system based on reaction times before and after the analgesic drug. It was difficult to establish a graded response to morphine by this method. The stimulus was not very reproducible because of the variety of tail diameters but the animals' response appeared to be perceptual, (they tried to remove the clip by biting).

The 'tail-flick' test (D'Amour & Smith, 1941) was found to give the most reproducible results. The 'normal' reaction time to the thermal stimulus did not vary a great deal from animal to animal nor from day to day. A graded response to fairly high doses of morphine was established and found to be reproducible. The criticism that the 'tail-flick' response is purely a spinal reflex was refuted by Winter and Flataker (1951) when they showed with an identical test in rats that spinal animals have a shorter reaction time than normal animals.

The results in this Chapter appear to indicate that pretreatment with any of the 4 synthetic steroids or ACTH produces a slight 'analgesia' in mice in at least 2 of the analgesia testing methods used. However, the antagonism of phenylquinone writhing by dexamethasone is probably due to a peripheral anti-inflammatory action of the steroid since

the writhing syndrome itself is thought to have a local inflammatory component (Winder, 1959). The prolongation of 'normal' reaction time in the 'tail-flick' analgesia test by dexamethasone, fludrocortisone and ACTH is difficult to correlate with their local anti-inflammatory action. It is well known that cortisone and ACTH exert profound effects upon the activity of the CNS; also the most frequently observed clinical responses to these hormones are euphoria and relief of pain. (See Introduction, page 30). Whether the steroids used in this work exert a similar analgesic action in mice is difficult to assess. Previously, however, when this hypothesis was tested in rats, pretreatment with cortisone or ACTH failed to prolong the 'normal' reaction time (Winter and Flataker, 1951). There is no evidence in the literature that the oestrogens and progestins have any analgesic activity but they do alter reaction thresholds to various stimuli, (See Introduction, page 20), which might explain the prolongation of normal reaction times by mestranol and lynestrenol in mice. These steroid-induced modifications of 'normal' reaction responses made it difficult to assess any changes in the spectrum of morphine and pentazocine activities after steroid pretreatment.

The apparent antagonism of morphine analgesia (low dose) by dexamethasone in the 'tail-clip' test could not be reproduced in the 'tail-flick' test. The latter finding confirms Paroli's observation (1957) that dexamethasone pretreatment did not affect the response of rats to morphine in the 'tail-flick' test compared with controls after receiving morphine. It appears that the cortisone-induced

antagonism of morphine observed by Winter and Flataker (1951) in rats does not occur with the synthetic glucocorticoid. However, the ACTH-induced antagonism ('tail-clip' and 'tail-flick' tests) and fludrocortisone-induced potentiation ('fail-flick' test) of morphine 'analgesia' in mice confirms the work of Winter and Flataker (1951) using ACTH and DOCA in rats. They agreed that the effects of cortisone upon morphine analgesia are due to a direct stimulation of the cerebrospinal axis and those of ACTH through an indirect stimulation, via adrenocorticoid release; DOCA is thought to depress the cerebrospinal axis (Winter & Flataker, 1951). Dexamethasone, though having a greater glucocorticoid activity than cortisone, appears to have less central activity; fludrocortisone, on the other hand, mimics the mineralocorticoid DOCA.

Lynestrenol potentiates whilst mestranol antagonises morphine 'analgesia' in the 'tail-flick' analgesia test. These results largely confirm the work of Paroli (1957) using less potent natural and synthetic steroids in rats. He maintained that hexoestrol, stilboestrol and oestradiol, while not influencing the analgesia produced by a single dose of morphine, markedly shortened the time of onset of tolerance, reduced the LD50 by 50% and increased the levels of unbound morphine. He pointed out that oestrogens altered the metabolic fate of morphine, and showed that anaesthetic doses of progesterone and its metabolites potentiated the analgesia of a given dose of morphine. A possible explanation of the effects seen with lynestrenol and mestranol, and those observed by Paroli, involves the consideration of the theory of morphine analgesia as

postulated by Beckett et al (1956).

As many narcotic analgesics are metabolized by N-dealkylation, Beckett, Casy, Harper and Phillips (1956) postulated that the dealkylated product (normorphine) is the actual analgesic compound. Morphine antagonism is explained by the premise that the larger the alkyl group of the antagonist the more strongly it is absorbed but less readily de-alkylated. Way and Adler (1960) discussed these theories in detail, but pointed out that certain large alkyl groups attached to morphine in fact produce potent analgesics. Intracisternal normorphine is a very potent analgesic whereas intraperitoneal normorphine is almost inactive. Further to this, Milthers (1962) obtained evidence that morphine and nalorphine are de-alkylated in the brain of rats. Beckett's postulate has stimulated much investigation but the role of N-dealkylation in the mechanism of action of potent analgesics is still uncertain. Nevertheless, if this theory is accepted then agents which depress metabolism by N-dealkylation would tend to antagonise morphine (normorphine) analgesia and those which increase production of normorphine would potentiate short-term analgesia. Oestrogens fit into the first category and progestins into the second. The fact that in mestranol-pretreated mice, the morphine antagonist pentazocine appeared to be more potent than in controls would support the contention that in these animals there is less central normorphine to be displaced by the antagonist. The increased potency of pentazocine in lynestrenol-pretreated animals remains to be explained, although the duration of action of morphine in lynestrenol-pretreated animals might be reduced - and be a simple explanation of the above

observation.

Further experiments to study the duration of morphine analgesia in steroid-pretreated animals are indicated.

Pretreatment	No. of writhes per group of 5 mice	Average (± S.E.)
Dexamethasone 2 mg/kg orally, daily for 2 days.	198, 189, 128	171.7 ± 21.9
Controls, vehicle 10 ml/kg, orally, daily, for 2 days	275, 269, 182	242 ± 30
Dexamethasone, 2 mg/kg orally, daily for 4 days.	182, 172, 180	178 ± 3.1 (P = 0.05-0.10)
Controls, vehicle 10 ml/kg, orally, daily for 4 days	208, 156, 227	197 ± 21.2
Dexamethasone, 2mg/kg orally, daily for 7 days.	133, 113, 150	*132 ± 10.7
Controls, vehicle 10 ml/kg, orally, daily for 7 days	170, 186, 165	173.7 ± 6.3

TABLE 4

Effect of various dexamethasone pretreatments on the response of female mice to phenylquinone. The mice received 0.02% phenylquinone (0.25 ml/20 g intraperitoneally) 18 hr after the last administration of steroid, and total group writhes counted during the next 20 min.

* Significantly different from relevant controls ($P < 0.05$)

Pretreatment	No. of writhes per group of 5 mice	Average (± S.E.)
Dexamethasone 10 mg/kg S.C. daily for 3 days.	71, 59, 106	*78.7 ± 14.1
Controls, vehicle, 5 ml/kg S.C., daily for 3 days.	171, 215, 195	193.7 ± 6.2
Dexamethasone 10 mg/kg S.C. daily for 5 days.	145, 109, 122	*125.3 ± 10.5
Controls, vehicle, 5 ml/kg S.C., daily for 5 days.	165, 157, 178	166.7 ± 6.1
Dexamethasone 10 mg/kg S.C. daily for 5 days, then 3-day rest.	245, 285	*265 ± 20
Controls, vehicle 5 ml/kg S.C., daily for 5 days, then 3-day rest.	193, 197	195 ± 2

TABLE 5

Effect of various dexamethasone pretreatments on the response of female mice to phenylquinone. The mice received 0.02% phenylquinone (0.25ml/20g intraperitoneally) 18 hr after the last administration of steroid, and total group writhes counted during the next 20 min.

*Significantly different from relevant controls ($P < 0.05$)

Pretreatment	Reaction time in sec.	Mean reaction time (± S.E.)
Dexamethasone 2 mg/kg, S.C. daily for 4 days.	1st Expt. 0.5, 5.6, 3.2, 9.6,13.0, 5.0, 1.0, 2.0, 0.5, 0.8.	4.1 ± 1.4
	2nd Expt. 5.0, 1.0, 2.0, 0.5, 0.8, 2.5, 2.5, 2.5, 0.5, 10.0	2.7 ± 0.9
Controls, vehicle, 5 ml/kg, S.C., daily for 4 days.	1st Expt. 10.5,16.2, 5.5, 5.2, 0.5, 1.8, 2.4, 4.0, 0.2, 1.0.	4.7 ± 1.6
	2nd Expt. 1.8, 2.4, 4.0, 0.2, 1.0, 0.5, 2.0, 0.5, 0.5, 1.0.	1.4 ± 0.4

TABLE 6

Tail-clip reaction time of female mice pretreated with dexamethasone. The animals were tested 18 hr after the last administration of steroid or vehicle.

Pretreatment	Average reaction times in sec	'Normal' reaction time in sec
Dexamethasone, 2 mg/kg, S.C., daily for 4 days.	1st Expt. 15' = 5.6 30' = 5.5 } 45' = 5.4 } 2nd Expt. 15' = 8.2 30' = 4.9 } 45' = 5.2 } 3rd Expt. 15' = 5.5 30' = 5.2 } 45' = 5.3 }	5.45 5.05 5.25
Controls, vehicle, 5 ml/kg S.C., daily for 4 days.	1st Expt. 15' = 5.0 30' = 4.5 } 45' = 4.6 } 2nd Expt. 15' = 6.9 30' = 5.0 } 45' = 5.0 } 3rd Expt. 15' = 4.8 30' = 4.9 } 45' = 4.8 }	4.55 5.0 4.85

TABLE 7

Tail-flick reaction time of female mice, pretreated with dexamethasone. The animals were tested 18 hr after the last administration of steroid or vehicle.

Test (2 mg/kg dexamethasone in 1% S.C.M.C. daily for 7 days)		Control (10 ml/kg 1% S.C.M.C. daily for 7 days)	
Mouse weight in g	Paired adrenal weight in mg	Mouse weight in g	Paired adrenal weight in mg
20	3.70	20.5	4.70
17	4.00	20.0	6.10
17	3.30	18.5	4.85
18.5	3.40	19.5	5.15
18.5	3.35	18.5	5.05
Average = 18.2 ± 0.6	Average = 3.55 ± 0.13	Average = 19.4 ± 0.4	Average = 5.17 ± 0.25

TABLE 8

Paired adrenal weights and body weights of female mice pretreated with dexamethasone. 18 hr after the last administration of steroid or vehicle, the animals were killed and the adrenals removed and weighed.

Test (10 mg/kg dexamethasone S.C. daily for 4 days, followed by 3-day withdrawal)		Control (5 ml/kg of the vehicle S.C., daily for 4 days followed by 3-day withdrawal.)	
Mouse weight in g	Paired adrenal weight in mg	Mouse weight in g	Paired adrenal weight in mg
17	5.2	18	5.80
17	4.1	18	7.80
18	4.4	17.5	5.30
15.5	4.1.	16	5.40
17	3.9	17	5.20
Mean = 16.9 ± 0.4	Mean = 4.3 ± 0.23	Mean = 17.3 ± 0.4	Mean = 5.9 ± 0.49

TABLE 9

Paired adrenal weights and body weights of female mice pretreated with dexamethasone. 18 hr after the last administration of steroid or vehicle the animals were killed and the adrenals removed and weighed.

Dose of morphine HCl, mg/kg S.C.	Total number of writhes in groups of 5 mice			
	Dexamethasone 2 mg/kg orally daily for 4 days		Controls, vehicle 10 ml/kg orally daily for 4 days	
		%		%
0	126	100	181	100
0.1	133		157	87
0.2	113	90	149	82
0.5	87	69	88	49
1.0	13	10	63	35
3.0	0	0	0	0
10	0	0	0	0

TABLE 10

Effect of dexamethasone pretreatment on the response of female mice to phenylquinone after various doses of morphine hydrochloride. The analgesic drug was administered on the fifth day, 18 hr after the last injection of steroid or vehicle, and phenylquinone was given intraperitoneally 30 min later.

Analgesic	Time after drug in min.	Total score for 10 animals		
		Pretreatment		
		Dexamethasone 2 mg/kg S.C. daily for 4 days.	Fludrocortisone 2 mg/kg S.C. daily for 4 days.	Controls Vehicle 5 ml/kg S.C.daily for 4 days.
Morphine hyd. 5 mg/kg S.C.	0	4	5	5
	15	11	17	21
	30	12	14	17
	60	8	6	11
	120	1	5	2
Morphine hyd. 10 mg/kg S.C.	0	1	4	2
	15	13	30	22
	30	27	25	23
	60	19	11	9
	120	1	8	3

TABLE 11

Effect of steroid pretreatment on the 'tail-clip' test 'analgesia' score of groups of 10 female mice after receiving morphine HCl at 2 dose levels.

Analgesic	Time after drug in min.	Total score for 10 animals	
		Pretreatment	
		ACTH, 20 u/mouse I.P., hourly for 3 hr.	Controls, distilled water 0.1 ml/mouse I.P. hourly for 3 hr.
Morphine Hyd. 7.5 mg/kg S.C.	0	0	0
	15	6	29
	30	9	23
	60	0	15
	120	o	1

TABLE 12

Effect of ACTH pretreatment in the 'tail-clip' test 'analgesia' score of groups of 10 male mice after receiving morphine HCl at 1 dose level.

Analgesic	Time after analgesic in min	Average reaction time in sec (± S.E.)		
		Pretreatment		
		Dexamethasone 2 mg/kg S.C. daily for 4 days	Fludrocortisone 2 mg/kg S.C. daily for 4 days	Controls vehicle 5 ml/kg S.C. daily for 4 days
Morphine hyd. 5 mg/kg S.C.	0	5.6	5.1	5.0
	0	5.5) 5.45 ±	4.7) 4.9 ±	4.5) 4.55 ±
	0	5.4) 0.2	5.1) 0.15	4.6) 0.16
	30	7.6 ± 0.9	* 8.5 ± 0.9	6.1 ± 1.0
	60	8.9 ± 1.4	*11.6 ± 1.1	8.4 ± 1.3
	90	7.5 ± 1.0	*10.7 ± 1.2	6.7 ± 1.0
	120	5.7 ± 0.3	* 6.8 ± 0.9	4.9 ± 0.5
Morphine hyd. 7.5 mg/kg S.C.	0	8.2	8.0	6.9
	0	4.9) 5.05 ±	5.8) 5.75 ±	5.0) 5.0 ±
	0	5.2) 0.3	5.7) 0.3	5.0) 0.25
	30	10.7 ± 1.4	*13.0 ± 1.1	9.1 ± 1.4
	60	11.0 ± 1.4	*12.7 ± 1.1	9.3 ± 1.5
	90	9.6 ± 1.4	12.4 ± 1.2	8.9 ± 1.7
	120	6.6 ± 0.7	9.7 ± 1.2	6.6 ± 1.4

TABLE 13

Effect of steroid pretreatment on average reaction times of groups of 10 female mice after receiving morphine HCl at 2 dose levels, in the 'tail-flick' test (cut-off time 15 sec).

* Level of significance compared with relevant controls

(P = 0.1 - 0.05)

Analgesic	Time after analgesic in min.	Average reaction time in sec (± S.E.)	
		Pretreatment	
		ACTH 20 i.u./mouse I.P. hourly for 3 hr.	Control, saline 5 ml/kg I.P. hourly for 3 hr.
Morphine hyd. 10 mg/kg S.C.	0	5.5	5.2
	0	5.1) 5.1	4.7) 4.75
	0	5.1)	4.8) ± 0.31
	15	6.4 ± 0.67	6.8 ± 0.71
	30	*7.1 ± 0.64	8.1 ± 0.62
	45	*7.0 ± 0.56	8.6 ± 0.57
	60	*6.6 ± 0.57	8.4 ± 0.67
	90	*5.9 ± 0.39	7.5 ± 0.77

TABLE 14

Effect of ACTH pretreatment upon average reaction times of a group of 10 male mice after receiving morphine HCl (10 mg/kg) in the 'tail-flick' test. (cut-off time 10 sec).

* Significantly different from controls (P = 0.1 - 0.05)

Analgesic	Time after drug in min.	Average reaction time in sec (± S.E.)		
		Pretreatment		
		Lynestrenol 5 mg/kg S.C. daily for 4 days	Mestranol 1 mg/kg S.C. daily for 4 days	Control Vehicle 5 ml/kg S.C.daily for 4 days
Morphine hyd. 5 mg/kg S.C.	0	4.9	5.3	5.0
	0	5.1) 5.15±	5.3) 5.2±	4.5) 4.55±
	0	5.2) 0.22	5.1) 0.25	4.6)
	30	6.9 ± 0.30	6.1 ± 0.17	6.1 ± 0.24
	45	6.5 ± 0.30	6.0 ± 0.17	6.4 ± 0.24
	60	6.3 ± 0.50	5.8 ± 0.54	5.7 ± 0.37
	120	5.9 ± 0.43	5.4 ± 0.54	4.9 ± 0.27
Morphine hyd. 7.5 mg/kg S.C.	0	4.4	4.3	4.6
	0	4.5) 4.5±	4.5) 4.5±	4.3) 4.45±
	0	4.5) 0.1	4.5) 0.1	4.6) 0.15
	15	5.5 ± 0.23	5.1 ± 0.30	5.6 ± 0.60
	30	7.4 ± 0.49	5.7 ± 0.54	6.9 ± 0.79
	45	7.7 ± 0.67	6.2 ± 0.55	6.8 ± 0.61
	60	7.0 ± 0.74	5.7 ± 0.37	6.5 ± 0.79
	90	6.2 ± 0.60	5.5 ± 0.53	5.8 ± 0.72
	120	5.8 ± 0.65	5.1 ± 0.22	5.1 ± 0.33
Morphine hyd. 10 mg/kg S.C.	0	5.7	5.2	4.5
	0	5.5) 5.5±	5.5) 5.35±	4.6) 4.65±
	0	5.5) 0.20	5.2) 0.27	4.7) 0.13
	15	6.1 ± 0.21	6.0 ± 0.33	5.8 ± 0.53
	30	8.4 ± 0.56	8.1 ± 0.61	7.7 ± 0.79
	45	9.4 ± 0.37	8.1 ± 0.63	8.4 ± 0.75
	60	9.4 ± 0.43	7.3 ± 0.58	8.0 ± 0.78
	120	7.1 ± 0.59	7.0 ± 0.68	5.4 ± 0.45

TABLE 15

Effect of sex steroid pretreatment upon average reaction times of groups of 10 female mice after receiving various doses of morphine HCl, in the 'tail-flick' test. (cut-off time 10 sec).

Analgesic	Time after drug in min	Average reaction time in sec (± S.E.)		
		Pretreatment		
		Lynestrenol 5 mg/kg S.C. daily for 4 days	Mestranol 1 mg/kg S.C. daily for 4 days	Control vehicle 5 ml/kg S.C. daily for 4 days
Pentazocine 30 mg/kg S.C.	0	5.4	4.9	4.1
	0	5.4) 5.4) ±	4.9) 4.9) ±	4.1) 4.35) ±
	0	5.4) 0.23	4.9) 0.21	4.6) 0.16
	15	5.5 ± 0.37	5.1 ± 0.28	4.3 ± 0.22
	30	5.5 ± 0.34	5.6 ± 0.14	4.5 ± 0.12
	45	5.8 ± 0.29	5.4 ± 0.21	4.3 ± 0.16
	60	6.1 ± 0.22	5.2 ± 0.17	4.4 ± 0.15
	120	5.4 ± 0.25	4.8 ± 0.26	4.1 ± 0.11

TABLE 16

Effect of sex steroid pretreatment upon average reaction times of groups of 10 female mice after receiving pentazocine sulph. in the "tail-flick" test.

Analgesic	Time after drug in min.	Average reaction time in sec (± S.E.)		
		Pretreatment		
		Lynestrenol 5 mg/kg S.C. daily for 4 days	Mestranol 1 mg/kg S.C. daily for 4 days	Control vehicle 5 ml/kg S.C. daily for 4 days
Morphine hyd. 10 mg/kg S.C.	15	6.1 ± 0.21	6.0 ± 0.33	5.8 ± 0.53
	30	8.4 ± 0.56	8.1 ± 0.61	7.7 ± 0.79
	45	9.4 ± 0.37	8.1 ± 0.63	8.4 ± 0.75
Pentazocine sulph 20mg/kg S.C. + Morphine hyd. 10 mg/kg S.C.	15	6.5 ± 0.55	6.3 ± 0.60	6.0 ± 0.52
	30	*6.7 ± 0.89	*6.4 ± 0.60	7.6 ± 1.20
	45	*7.4 ± 0.69	*5.8 ± 0.27	9.3 ± 0.70
Pentazocine sulph 60 mg/kg S.C. + Morphine hyd. 10 mg/kg S.C.	15	5.1 ± 0.43	6.2 ± 0.53	4.1 ± 0.52
	30	*5.1 ± 0.37	*5.9 ± 0.33	*4.7 ± 0.17
	45	*6.4 ± 0.69	*5.5 ± 0.26	*5.2 ± 0.57

TABLE 17

Effect of steroid pretreatment on pentazocine antagonism of morphine 'analgesia' in groups of five female mice in the 'tail-flick' test. (cut-off time = 10 sec).

* Significantly different from relevant morphine controls (P = 0.1 - 0.05)

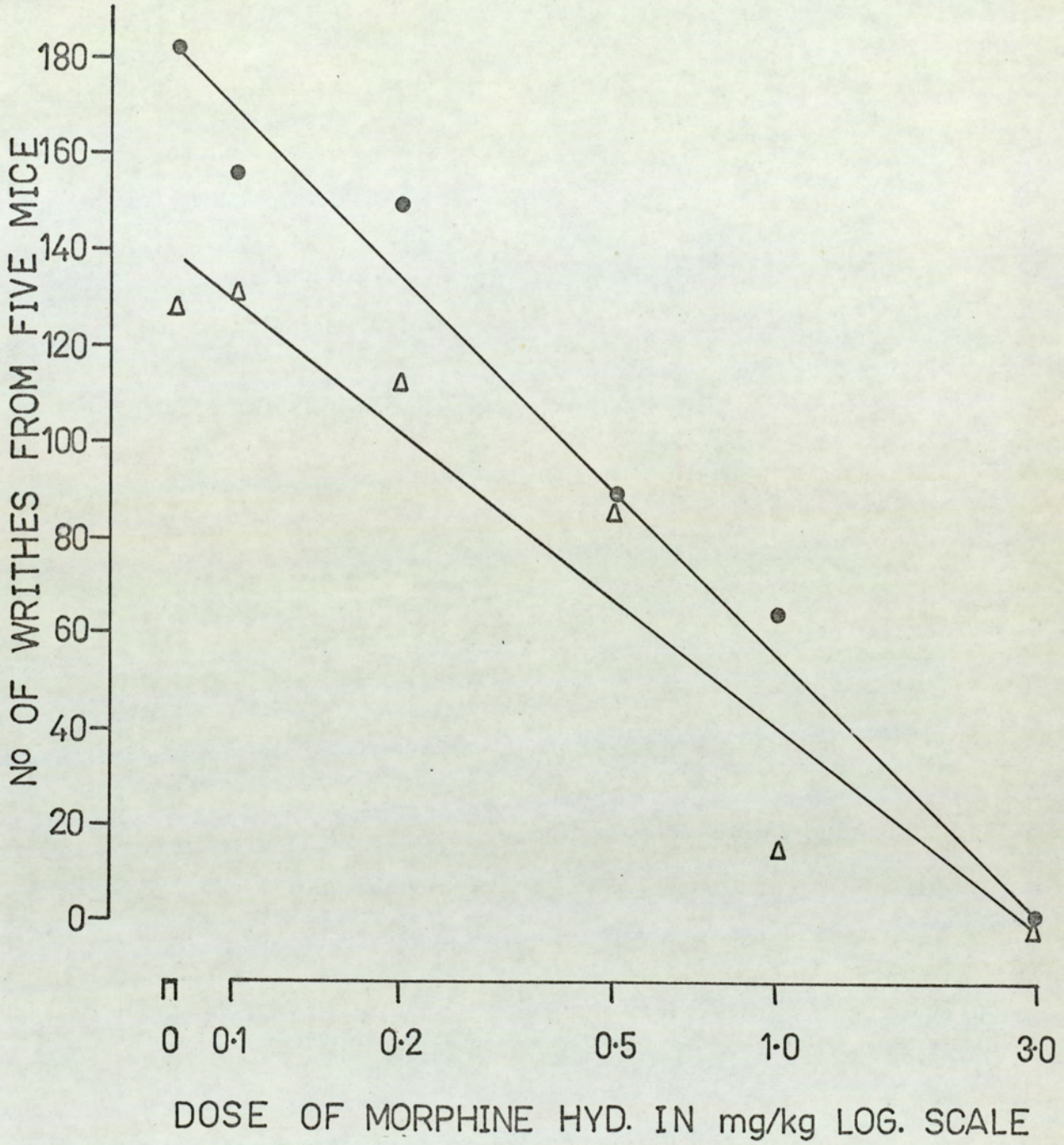


Fig. 13

Effect of pretreatment with dexamethasone on the response of female mice to phenylquinone (0.25 ml/20g), administered intraperitoneally. Animals were injected subcutaneously with dexamethasone (2.0 mg/kg) daily for 4 days (Δ——Δ), or the vehicle only (5.0 ml/kg) daily for 4 days (●——●).

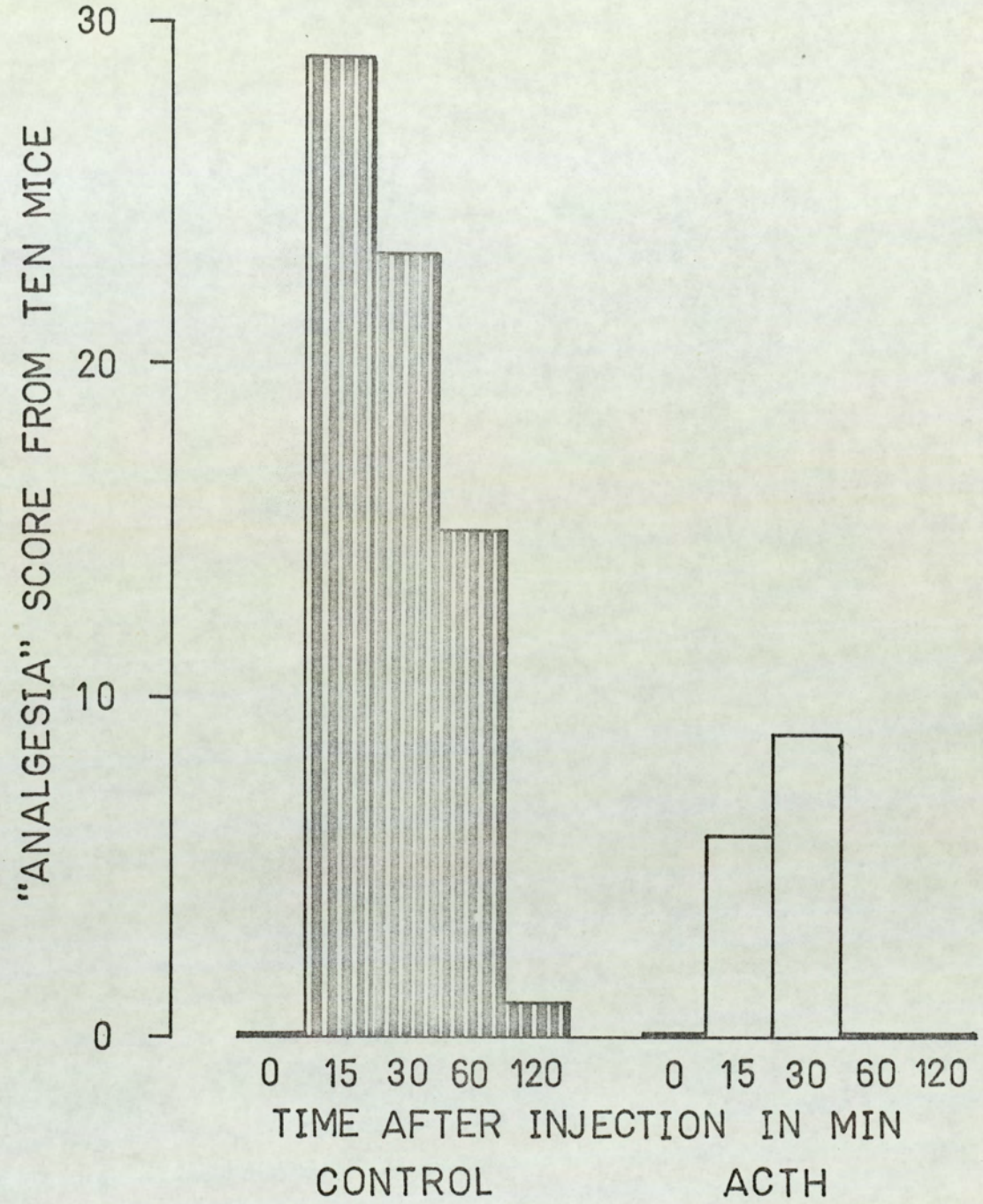


Fig. 14a

Effect of pretreatment with ACTH on the 'analgesia' score of male mice in the 'tail-clip' test at various times after morphine hyd. (7.5 mg/kg), injected subcutaneously. Animals were pretreated with ACTH (20iu/mouse) intraperitoneally, hourly for 3 hr (open bars); controls (closed bars) received the saline vehicle (5.0 ml/kg).

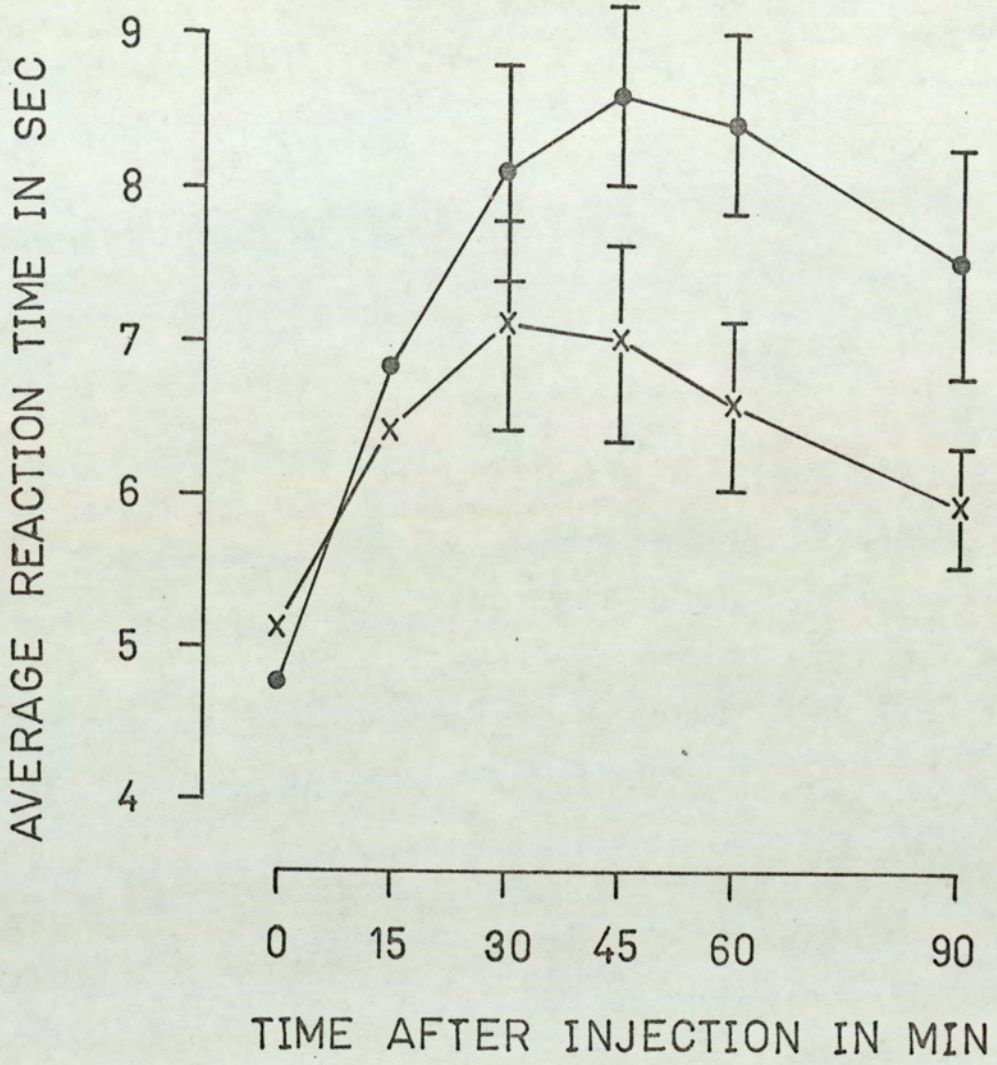


Fig. 14b

Effect of pretreatment with ACTH on the reaction times of male mice in the 'tail-flick' test at various times after morphine hyd. (7.5 mg/kg), injected subcutaneously. Animals were pretreated with ACTH (20 iu/mouse) intraperitoneally, hourly for 3 hr (X—X); controls (●—●) received the saline vehicle (5.0 ml/kg).

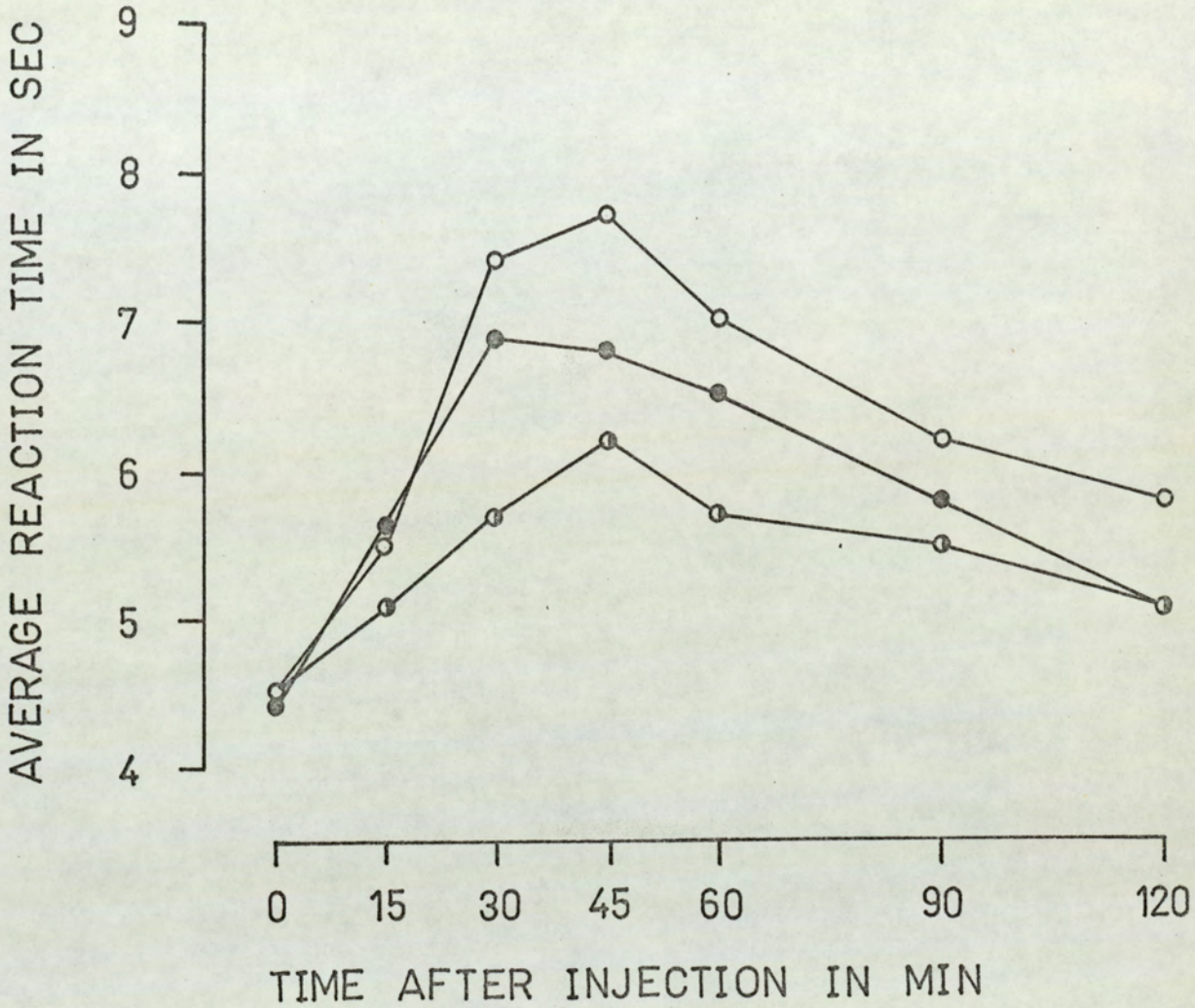


Fig. 15a

Effect of pretreatment with sex steroids on the reaction times of female mice in the 'tail-flick' test at various times after morphine hyd. (7.5 mg/kg), injected subcutaneously. Animals were pretreated as follows: mestranol (1.0 mg/kg), subcutaneously, for 4 days (◐——◐); or lynestrenol (5.0 mg/kg), subcutaneously, for 4 days (○——○); or the vehicle only (5.0 ml/kg), for 4 days (●——●).

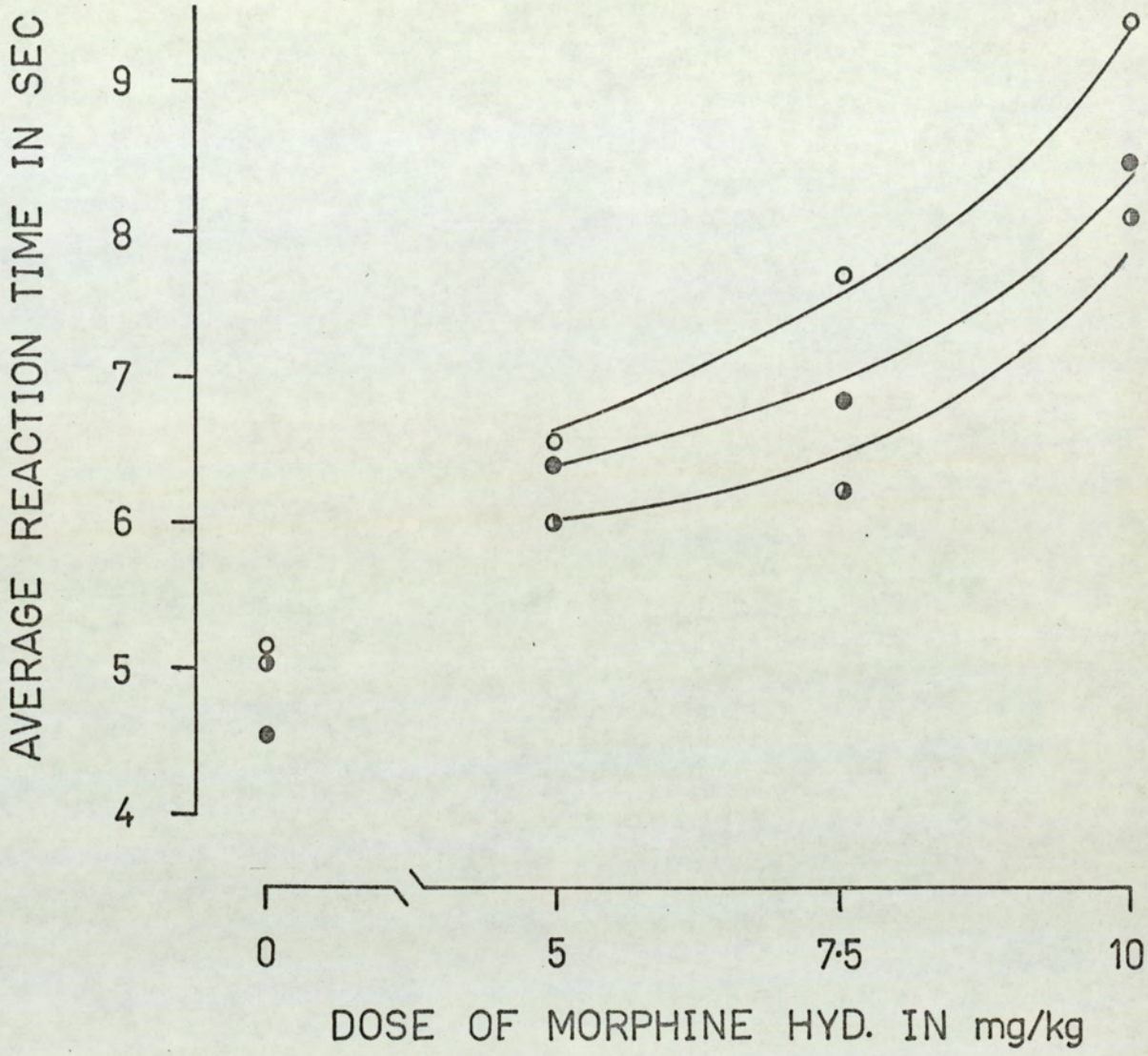


Fig. 15b

Effect of pretreatment with sex steroids on the reaction times of female mice in the 'tail-flick' test, 45 min after various doses of morphine hyd. (5.0, 7.5, or 10 mg/kg), injected subcutaneously. Animals were pretreated as follows: mestranol (1.0 mg/kg) subcutaneously, for 4 days (◐——◐); or lynestrenol (5.0 mg/kg) subcutaneously, for 4 days (○——○); or the vehicle only (5 ml/kg), for 4 days (●——●).

CHAPTER II - STEROIDS AND BARBITURATES

Introduction

It has long been recognised that there exists a sex difference in the response of laboratory animals to certain drugs, particularly the barbiturates (Nicholas & Barron, 1932; Holck, Kanan, Mills & Smith, 1937; Homberger, Efstein & Himwich, 1947). Since then the effects of ovariectomy and sex steroid pretreatment upon the duration of barbiturate anaesthesia in many species of animal have been well documented, (See Introduction, page 38) female sex hormone administration in most cases being associated with prolongation and male sex hormone administration with shortening of barbiturate anaesthesia. Before the present study, work with the potent synthetic oestrogens, mestranol and ethinyl oestradiol, alone or in combination with the progestational 19-nor-testosterone derivatives, upon barbiturate anaesthesia had been neglected.

The effects of stress, adrenalectomy and the adrenocortical steroids upon barbiturate anaesthesia have been thoroughly investigated in the past. Circumstances which lead to increased levels of glucocorticoids in the blood, for example stress, administration of ACTH or cortisone usually produce a shortening of barbiturate anaesthesia. Conversely, adrenalectomy and sometimes DOCA produce a prolongation of barbiturate anaesthesia, (See Introduction, page 38). Potent synthetic gluco- andmineralo-corticoids

such as dexamethasone (glucocorticoid) and fludrocortisone (mainly mineralocorticoid) which are used clinically for anti-inflammatory or replacement therapy so far have not been investigated.

Throughout the following experiments the various environmental and intrinsic factors which affect barbiturate sleeping-time, as discussed in detail in the review of Sethy and Sheth (1968), were borne in mind. For instance, many workers have studied the effects of age, weight and species upon barbiturate anaesthesia. Homburger et al (1947) demonstrated that the spectrum of resistance is lowest in the newborn rats, maximum in rats weighing 50-200 g, and intermediate in rats advanced in age and weight. Similar reports have appeared in the literature with respect to guinea pigs, rabbits and humans. Other factors, such as environmental and body temperature, development of tolerance and rate of barbiturate metabolism by the liver, are dealt with in the discussion at the end of the chapter. In recent years it has become apparant that urinary excretion is of minor importance in limiting the duration of action of drugs and that most therapeutic agents cannot be excreted until they undergo chemical modification. This report includes work with the potent enzyme inhibiter SKF 525A. B-Diethylamino-diphenylpropylacetate (SKF 525A) has little pharmacological activity of its own but prolongs the activity of various drugs by inhibiting their rate of metabolism (Axelrod et al, 1954). Brodie et al (1954) demonstrated a dose-dependent potentiation of hexobarbitone narcosis in mice and rats after SKF 525A. These workers demonstrated that the inhibitor retarded the duration of action and

inhibited the biotransformation of hexobarbitone, pentobarbitone, meperidine, aminopyrine and ephedrine. In a later paper (Cooper et al, 1954) they showed that SKF 525A exerts action by inhibiting enzyme systems in the liver which oxidise the sidechains of pentobarbitone and hexobarbitone; dealkylate meperidine and aminopyrine; deaminate amphetamine; cleave the ether linkage in codeine; and conjugate morphine. The diversity of metabolic pathways inhibited by SKF 525A suggests that the potentiating agent is acting on a factor or factors which are common to a number of enzyme systems involved in the metabolism of drugs. B-Diethylamino-diphenylpropylacetic acid has no effect on intermediate metabolism such as TPN-cytochrome C reductase or cytochrome oxidase which points to a direct action on the drug enzyme or some other hydrogen transport system.

Cooper states (unpublished data) both SKF 525A and its acid inhibits various oxidations of tricarboxylic acid cycle intermediates, but the importance he does not discuss. In general, the inhibitor has a predilection for enzyme systems that metabolise foreign compounds and catalyze pathways as diverse as oxidation, reduction and conjugation (Review of Brodie et al, 1958). A large number of other compounds which interfere with microsomal enzymes has been shown to prolong the activity of barbiturates, for instance iproniazid, isoniazid, chloramphenical imipramine, etc. (Review of Sethy & Sheth, 1968).

A number of mechanisms have been proposed for the hypnotic action of barbiturates. They may cause :-

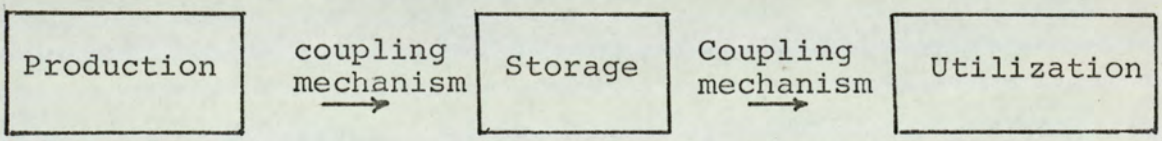
(1) depression of brain respiration;

(2) uncoupling of oxidative phosphorylation;

(3) lack of utilization of energy stores; and

(4) depression of excitability of neuronal membranes.

In 1951 Brody and Bain showed that thiopentone, pentobarbitone and amylobarbitone uncouple oxidative phosphorylation in vitro at concentrations which approximate to those attained in vivo during barbiturate anaesthesia. During the breakdown of glucose (and other substrates) there is a transfer of energy to a storage system within the cell. It is generally accepted that this comprises high energy phosphate bonds of NADP (See Introduction, page 35) and phospocreatinine. During oxidation of the substrate through the cytochrome chain electrons are transferred in a stepwise fashion with the concomitant appearance of high energy phosphate bonds. There exist labile enzyme systems which control these transferences. The following scheme illustrates the point :-



(Bain, 1952)

Agents which dissociate oxidation of energy-rich sugars by a coupling mechanism will not depress oxygen uptake but will decrease storage and utilization of high energy phosphate compounds. Brody and Killam (1952) demonstrated that 2, 4-dinitrophenol potentiates barbiturate anaesthesia, and itself produces depression in near toxic doses. Recently Doggett and Spencer (1970) have shown that intra-cerebral injection of 2, 4-dinitrophenol produces anaesthesia in mice. The locus of action of the barbiturate, anylobarbitone, appears to be an inhibition of the reduced nicotinamide adenine dinucleotide (NADH) chain of the electron

transport system (Grieg, 1946; Brody & Bain, 1951). Recently, Spiegel and Wainio (1969) have shown that inhibition of the NADH-cytochrome oxidoreductase enzyme by barbiturates is between the enzyme flavoprotein and a coenzyme (Q1).

If uncoupling of oxidative phosphorylation occurs during barbiturate anaesthesia then one would expect an increase in inorganic phosphate and a decrease in NADP and phosphocreatinine. In fact McIlwain in 1962 pointed out that the reverse occurs during barbiturate anaesthesia and the increase in NADP and phosphocreatinine is due to lack of utilization of these compounds under these conditions. However, it could be argued that uncouplers of oxidative phosphorylation, in addition to reducing the synthesis of energy-rich compounds, may also inhibit release of energy from NADP so causing a rise in tissue levels of this nucleotide.

PART I

STEROIDS IN MICE

SECTION I

(1) Duration of barbiturate-induced sleep in mice pretreated with oestrogens, protestins, or a combination of the two

Mice were injected subcutaneously with oestrogen (mestranol, 0.5 mg/kg daily) or with progestin (lynestrenol, 5.0 mg/kg daily) for four days; the duration of sleep after intravenous injection of pentobarbitone sodium (50 mg/kg), of hexobarbitone sodium (60 mg/kg), or of barbitone sodium

(300 mg/kg) was then determined on the fifth day. The results are summarized in Table 18. Mestranol prolonged(2x) whilst lynestrenol shortened the duration of sleep induced by pentobarbitone or hexobarbitone, whilst the effects of barbitone were not significantly altered by either type of steroid. There was no sex difference in the response of mice to pentobarbitone and the effects of lynestrenol or mestranol on pentobarbitone sleeping-time were similar in both male and female mice.

Since these effects on barbiturate sleeping time were quite marked it was decided to investigate the effects of graded doses of either mestranol or lynestrenol on the duration of pentobarbitone sleep in female mice. Groups of ten mice were injected on four successive days with mestranol at various dose levels (0.03, 0.1, 0.3 and 1.0 mg/kg daily) or with lynestrenol at various dose levels (0.3, 1.0, 3.0 and 10 mg/kg daily); or vehicle alone (controls). On the fifth day, each mouse received pentobarbitone sodium (50 mg/kg) intravenously and the duration of sleep measured. The effects of both mestranol and lynestrenol were dose-dependent, and an approximately linear relationship was observed between the duration of sleep and the log. dose of the hormone. See Fig. 16.

A number of oestrogens and progestins are used clinically in combination as anti-fertility agents, so it was of interest to study the effects of four commercially available oral contraceptive preparations in mice. The tablets from packs of "Norlestrin"*, "Volidan"*, "Conovid"* and "Lyndiol"* were crushed and suspended in a 2% acacia solution, the

(* for constituents, see Table 19)

equivalent of 1/5th of a tablet being administered orally to each female mouse, daily, for four days. The duration of sleep after intravenous pentobarbitone sodium (50 mg/kg), was determined on the fifth day. The effects of the equivalent of 1/15th and 1/50th of a tablet of "Lyndiol" on pentobarbitone sleeping-time were similarly determined. See Table 19. All six preparations of oestrogen/progestin combinations produced a shortening of pentobarbitone sleeping-time indicating that the progestin effect was predominant in these preparations.

The ratio of progestin to oestrogen in most oral contraceptives is approximately 50:1 and it was, therefore, not surprising that the progestin effect was predominant. It was decided to investigate the effects of such a combination using various ratios of progestin to oestrogen. The duration of pentobarbitone sleep in female mice pretreated, subcutaneously, for four days with various combinations of mestranol and lynestrenol is shown in Table 20. On a weight for weight basis, mestranol was the more potent, and the effects of the two steroids were cancelled out when they were combined lynestrenol:mestranol, 5:1.

(2) Onset and duration of effects of sex steroids on barbiturate sleeping-time

Groups of 10 female mice received two daily injections of mestranol (0.5 mg/kg) or lynestrenol (5.0 mg/kg) for 1, 2, 3 or 4 days; controls received the vehicle only (5.0 ml/kg). The mice received pentobarbitone sodium (50 mg/kg), intravenously, after the 2nd, 4th, 6th or 7th injection of steroid. The duration of sleeping-time and the oesophageal temperature at 0, 30, 60 and 120 min were measured. The degree of

hypothermia paralleled the depth of anaesthesia. The antagonism by lynestrenol (progestin) or the potentiation by mestranol (oestrogen) of the barbiturate anaesthesia and hypothermia, reached a maximum after 3 - 4 days' pretreatment with the steroid. See figs. 17 and 18.

(3) The effect of pregnancy, gonadotrophin or progesterone on pentobarbitone sleeping-time

A group of 16 female mice showing obvious signs of pregnancy after 16 to 18 days received pentobarbitone sodium (50 mg/kg) intravenously and their sleeping-time measured; another group of 16 female mice kept separately from males received the same dose of pentobarbitone sodium and their sleeping-time was measured and compared. During anaesthesia the degree of hypothermia in the two groups was measured. The initial body temperatures of the pregnant mice were, on average, 1°C above the controls. Further, during anaesthesia their hypothermia was not so marked and sleeping-time was slightly less than that of controls. See fig. 19.

Next, groups of 10 female mice received either gonadotrophin (10 u/day) daily for 6 days or saline (5 ml/kg) daily for 6 days. On the 7th day they received pentobarbitone sodium (50 mg/kg) intravenously and their sleeping-time recorded. The experiment was repeated but on the 7th day the test mice received gonadotrophin (25 u/hr) hourly for 3 hr prior to the pentobarbitone sodium. On neither occasion was the pentobarbitone sleeping-time modified by gonadotrophin pretreatment. See Table 21.

Finally, groups of 10 female mice received either

progesterone (20 mg/kg) daily for 4 days or oily vehicle (5 ml/kg) daily for four days. On the 5th day they received pentobarbitone sodium (50 mg/kg) intravenously, and their sleeping-time measured. Progesterone pretreatment produced a reduction (35%) in sleeping-time compared with controls, but even at this high dose the effects were not nearly so marked as with the synthetic progestins. See Table 22.

(4) Possible involvement of body temperature changes as an explanation of the observed changes in barbiturate response in steroid pretreated mice

It was observed frequently that mice pretreated with lynestrenol had slightly elevated oesophageal temperatures, whilst animals pretreated with mestranol were slightly hypothermic. It is well known that small changes in deep body temperature are associated with marked alterations in the rate of metabolism of certain drugs (see, for example, Fuhrman & Fuhrman, 1961), and it was possible, therefore, that the changes observed in this work might be due to alterations in body temperature. The potentiation by mestranol, and antagonism by lynestrenol of pentobarbitone sodium induced hypothermia has already been discussed (See page 130 and figs. 17 and 18). It was not possible from this experiment alone to determine whether the different degrees of hypothermia were responsible for differences in duration of pentobarbitone effect, or vice versa. Therefore, a study of sleeping-times was carried out at an elevated room temperature - specifically in an enclosed environment of 30°C instead of 22°C. A comparison of the effects of

intravenous pentobarbitone sodium (50 mg/kg) at these two temperatures in steroid pretreated mice is summarised in Table 23.

At 30°C pentobarbitone failed to induce significant hypothermia in any of the groups of mice, irrespective of their pretreatment. Examination of Table 23 shows that under these conditions the sleeping-time of controls was only one quarter that of controls allowed to become hypothermic. Similarly, sleeping-time in mestranol pretreated mice was reduced from nearly 4 hr (at 22°C) to 40 min when hypothermia was prevented. With lynestrenol pretreated animals, there was a slight reduction in the already short sleeping-time, but now the duration of sleep was identical with that of controls. Despite the marked reduction in the degree of potentiation, mestranol still produced a significantly longer sleeping-time under the raised environmental temperature than that seen in the controls.

(5) Effects of SKF 525A on the duration of pentobarbitone sleep in female mice, pretreated with mestranol or lynestrenol

It was possible that mestranol was producing its effects mainly through an inhibition of microsomal enzymes which metabolise certain barbiturates. SKF 525A is an inhibitor of hepatic microsomal enzymes and known to interfere with the metabolism of barbiturates (Axelrod, Reichenthal & Brodie, 1954); therefore, it was of interest to study its effects in mice pretreated with mestranol or lynestrenol. Mice were pretreated for 4 days with mestranol (0.5 mg/kg) or lynestrenol (5.0 mg/kg); on the 5th day, each mouse

received an intraperitoneal injection of SKF 525A (50 mg/kg) 90 min before an intravenous injection of pentobarbitone sodium (35 mg/kg). The duration of pentobarbitone sleep in these mice is recorded in Table 24.

SKF 525A increased the duration of pentobarbitone sleep in all mice, irrespective of whether they were steroid-pretreated or controls. Thus, the effects of lynestrenol were largely overcome, whilst those of mestranol were greatly potentiated (6x). In fact the duration of sleep in mice pretreated with both mestranol and SKF 525A was far greater than was expected from a simple combination of the effects of the two drugs. It is possible that the potentiation of barbiturate sleep induced by mestranol and SKF 525A is by different mechanisms and/or at different sites.

SECTION II

Blood and plasma levels of pentobarbitone in female mice pretreated with lynestrenol, mestranol or SKF 525A

(a) Blood levels of pentobarbitone sodium (obtained by the initial assay, see Methods, page 62) :

Mice were decapitated immediately or at various intervals up to 120 min after intravenous pentobarbitone sodium (50 mg/kg). 0.5 ml of the whole blood obtained from each mouse was assayed for pentobarbitone. Internal standards were made by adding 100 ug of pentobarbitone to untreated mouse blood and then subjecting this to the assay procedure. Blanks were made by subjecting the whole blood to the extraction procedure. Blood concentrations of

pentobarbitone were calculated from a graph constructed from absorbance measurements vs. standard solutions of pentobarbitone; Fig. 9. (See Methods, page 66). Pentobarbitone concentrations were obtained from four mice for each interval of time after injection. Absorbance readings in test animals were uncorrected for blank absorbance or percentage recovery as these tend to cancel each other out.

$$\% \text{ recovery} = \frac{\text{internal standard absorbance - blank absorbance}}{\text{standard absorbance at the same concentration}}$$

In control mice, the blood concentrations of pentobarbitone fall rapidly for the first 5 min after injection and then more slowly from 10 min onwards, (See Fig. 10).

Female mice were injected subcutaneously with lynestrenol (5.0 mg/kg) or mestranol (0.5 mg/kg) for 4 days, and pentobarbitone sodium (50 mg/kg) administered intravenously on the 5th day. The mice were decapitated immediately upon wakening and 0.5 ml of their blood assayed for pentobarbitone. Internal standards and blanks were constructed as described previously. Test mice pretreated with either hormone tended to wake at blood concentrations of pentobarbitone slightly lower than control mice. See Table 25.

However, the results show that with whole blood the assay blanks obtained were usually high and fluctuated wildly and, therefore, not truly negligible when calculating the results. It was decided to repeat the experiment using a modified assay procedure and plasma instead of blood.

(b) Plasma levels of pentobarbitone sodium (obtained by the final assay, see Methods, page 66):

Female mice were pretreated with lynestrenol (5.0 mg/kg) or mestranol (0.5 mg/kg) for 4 days, and pentobarbitone sodium (50 mg/kg) administered intravenously on the 5th day. Alternatively, mice received SKF 525A (50 mg/kg) intraperitoneally 90 min before the barbiturate. The mice were decapitated immediately or at various intervals up to 90 min after the barbiturate injection. Plasma from groups of 4 mice was pooled, and assayed for pentobarbitone. Blanks and internal standards were constructed using plasma instead of blood. The results from three experiments were averaged and are summarized in Table 26 and Fig. 20.

The Table illustrates that the blanks and test readings in this assay procedure are far more consistent. Each line is approximately linear from 10 min onwards when the log. of plasma barbiturate levels is plotted as a function of time. The slope of each line was examined by regression analysis and the significance of difference calculated. Mestranol and SKF 525A caused a significant reduction in the rate at which pentobarbitone disappeared from mouse plasma, whilst lynestrenol caused a significant increase in this disappearance.

SECTION III

Oxygen consumption in female TO mice pretreated with lynestrenol or mestranol

Groups of 15 female TO mice were pretreated with lynestrenol (5.0 mg/kg) or mestranol (0.5 mg/kg) subcutaneously

for 4 days. Controls received the vehicle only (5 ml/kg). On the 5th day the time for consumption of 3 cc of oxygen was measured in individual mice as described under Methods.

Mestranol reduced (10% approximately) while lynestrenol increased (10% approximately) the rate of oxygen consumption, (See Table 27 and Fig. 21).

SECTION IV

Duration of barbiturate-induced sleep in mice pretreated with dexamethasone or fludrocortisone

Groups of 16 female mice were pretreated with glucocorticoid (dexamethasone, 1.0 mg/kg daily) or with mineralocorticoid (fludrocortisone, 1.0 mg/kg daily) for 4 days; controls received the oily vehicle for 4 days (5 ml/kg daily). The duration of sleep after intravenous injection of pentobarbitone sodium (50 mg/kg) or hexobarbitone sodium (60 mg/kg) was determined on the 5th day. Neither dexamethasone nor fludrocortisone pretreatment influenced the sleeping-time to either barbiturate when compared with controls, (See Table 28).

DISCUSSION

The data presented in these results show clearly that in mice, mestranol and lynestrenol, two synthetic female sex hormones with respectively oestrogenic and progestogenic activity (World Health Technical Report, 1965), greatly altered the duration of action of pentobarbitone and hexobarbitone, but not that of barbitone. These effects

occurred in both female and male mice, were dose-related, and were persistent, their effects continuing for as long as their administration was continued. Yet, peak effects with either steroid were produced after only 4 days of pretreatment, a period approximately equal to the mouse's oestrus cycle. When relatively large doses of 4 contraceptive preparations were administered orally to female mice the progestational effects upon pentobarbitone sleeping-times were dominant. The relative potencies of the 4 preparations in reducing pentobarbitone sleeping-time ran as follows, the most effective first: Lyndiol, Norlestrin, Volidan and Conovid (the ratios of progestin to oestrogen are approximately 50:1 in all preparations, but the doses of each type of steroid vary with each preparation). The effects of a laboratory combination of oestrogen with progestin were also studied; thus, when a number of oestrogen/progestin combinations were examined, their effects were determined by the ratio of mestranol to lynestrenol; at a ratio of 1:5, the effects of both steroids were effectively cancalled out by one another.

The effect of pregnancy, gonadotrophin or progesterone on pentobarbitone was examined. During pregnancy, blood levels of both oestrogen and progestin rise in mammalian species. The reduction in sleeping-time seen in the pregnant mice might, therefore, be explained by the increased steroid levels, with the progestin effect dominant. It was also noted, however, that the body core temperatures of the pregnant mice were approximately $1^{o}C$ above that of the controls. Both environmental temperature and body temperature are known to influence the rate of metabolism of

drugs - a rise in temperature being associated with an increase, and a fall in a decrease in the rate of metabolism. This is discussed at greater length later in this Discussion. Therefore, at 1°C hyperthermia might possibly be sufficient to produce the slight reduction in sleeping-time.

Gonadotrophin pretreatment sufficient to produce ovarian hyperplasia and the appearance of Graafian follicles (Best, personal communication) and presumably large increases in the levels of circulating oestrogen did not produce any change in the duration of pentobarbitone sleeping-time. It appears that the levels of oestradiol produced by this pretreatment were not great enough, or that oestradiol was not potent enough, to produce a reduction in pentobarbitone sleeping-time.

Progesterone decreases brain excitability and in large doses produces anaesthesia, (Selye, 1941; Wahlstrom, 1968), and may be an acute and direct depression of neural pathways. The effect of chronic pretreatment with progesterone upon barbiturate anaesthesia in animals has not been studied to any extent, and the few reports available state that progesterone mimics the oestrogens in their effect upon barbiturate anaesthesia, (Robillard & D'Iono, 1954). In the present experiments 4 days' pretreatment of female mice with progesterone produced a reduction in pentobarbitone sleeping-time. The effect was not as marked as that produced by the synthetic progestins, even though the dose of progesterone was 4 times greater.

There are several explanations of these steroid-induced changes in the sensitivity of the mouse to certain barbiturates; there may be changes in the inherent sensit-

ivity of the cerebrospinal axis to these drugs; or there may be changes in the tissue distribution or plasma binding of the barbiturates; renal handling of the barbiturates may be affected; or, finally, there may be changes in the ability of the mouse to metabolise barbiturates. Each of these factors is known to influence the duration of barbiturate anaesthesia. The inability of oestrogens or progestins to alter the sensitivity of the mouse to barbitone largely excludes the first three possibilities, since the modes of action and physico-chemical properties of the three barbiturates studied are qualitatively the same. One area in which these barbiturates are known to differ is in their susceptibilities to biotransformation; barbitone remains largely un-metabolised in most mammalian species (Maynert & Van Dyke, 1950), so that little or no change in the effects of barbitone would be anticipated from a quantitative change of the rate of hepatic metabolism. Previous workers' findings confirm this conclusion: thus, long-term treatment of mice with testosterone shortened the duration of hexobarbitone but not that of barbitone hypnosis (Gessner et al, 1967). The same workers observed that oestrogens prolonged the action of hexobarbitone, a drug known to be extensively metabolised by the liver in both male and female animals (Quinn, Axelrod & Brodie, 1954; Noordhoek, 1968).

The actions of the microsomal enzyme inhibitor, SKF 525A, on pentobarbitone hypnosis were also studied. Whilst the effects of lynestrenol were completely reversed by SKF 525A, those of mestranol were greatly prolonged. The prolongation of pentobarbitone anaesthesia by a combination

of mestranol plus SKF 525A was far greater than could be predicted from a summation of their individual effects, and also far greater than that achieved with the largest doses of mestranol used in the earlier dose-response experiment. It appears, therefore, that the loci and/or mechanisms by which mestranol and SKF 525A inhibit pentobarbitone metabolism are different.

A study of plasma pentobarbitone levels supports the conclusion that these steroids, like SKF 525A, alter the rate of metabolism of pentobarbitone. Noordhoek (1968) stated that the elimination of a barbiturate, (hexobarbitone in his study), proceeds essentially by diffusion into tissues, by liver metabolism and renal excretion. A similar picture can be assumed for pentobarbitone. In this study, not only the controls, but also animals pretreated with mestranol, lynestrenol or SKF 525A exhibited an approximately linear relationship between the logarithm of plasma pentobarbitone concentration and time, from about 10 min after injection. This suggests that diffusion processes from plasma into other compartments had ceased by this time (Dost, 1953), and that only hepatic and renal sites of removal would remain. Little is known about the renal handling of barbiturates. In the dog excretion of unchanged hexobarbitone is negligible (Bush et al, 1953), whilst barbitone is known to be reabsorbed in the renal tubule (Giotti & Maynert, 1951). It is concluded that an effect on hepatic metabolism, admittedly at perhaps different sites, is a common explanation for the changes in plasma clearance of pentobarbitone seen in animals pretreated with oestrogen, progestin or SKF 525A.

The hepatic sites at which the steroids and SKF 525A

interact are unknown, but there is increasing evidence that steroids are endogenous substrates for drug-metabolising enzymes in mammals (Kuntzman et al, 1964; Jacobson & Kuntzman, 1968; Levin et al, 1967 and for others see Introduction, page 35). Also Kato et al (1968) showed that in rats, where a sex difference in their response to hexobarbitone occurred, there existed differences in the NADPH-linked electron transporting system, the cytochrome P-450 content of rat liver microsomes; and the terminal oxidase enzyme; whereas in mice and rabbits where no sex difference in response to hexobarbitone occurred, no such differences existed. In rats the activity of the drug-oxidation in liver microsomes is regulated by anabolic action of male sex hormones (Kato et al, 1962) and it is thought by Kato and co-workers (1968) that it is the androgen which exerts this influence on the drug-metabolising enzymes (See Introduction, page 35). It appears likely that administration of lynestrenol and mestranol (and the steroid combinations) may produce their effects in mice at a similar site to those mentioned above. It is interesting to note that the most recent theory of barbiturate anaesthesia implicates barbiturates with inhibitor of the NADH cytochrome oxidoreductase enzyme system at a point between the enzyme flavoprotein and a coenzyme (Q1) (Spiegel & Waino, 1969) which is unaffected by androgen (Kato et al, 1968). Thus barbiturates inhibit the production of energy stores whilst steroids interfere in the utilization of energy rich compounds necessary for barbiturate metabolism.

If SKF 525A does act at a different site from the sex steroids, as suggested above, then this might explain why

mestranol plus SKF 525A was so potent in prolonging pentobarbitone sleeping-time, since all three drugs inhibit metabolism at some point.

Steroids do influence the brain levels of oxygen and oxidative metabolism and it has been suggested that the anaesthetic steroids - like the barbiturates - uncouple oxidative phosphorylation (See Introduction, page 127). Lynestrenol pretreatment produced a slight increase in oxygen consumption in female mice, whilst mestranol had the reverse effect. Thus stimulation in the former case, and inhibition in the latter, of general body metabolism may be other causal factors in the observed changes in barbiturate anaesthesia.

There may be one further explanation of the altered rates of metabolism observed in steroid pretreated mice. It is well known that changes in the ambient temperature, and more specifically in the animal's core temperature, can markedly alter the rate of metabolism of certain drugs (See, for example, Fuhrman & Fuhrman, 1961; Morris, 1963). Specifically, Morris (1963) observed that the duration of pentobarbitone sleep was reduced whilst that of barbitone sleep was unaffected, when the ambient temperature was raised $10^{o}C$; although no direct measurement of body temperature was made in that study, the mice were taken well above their critical temperature so that at least hypothermia would have been prevented. As well as sleep, but perhaps a consequence of it, pentobarbitone and other medium-to-long-acting barbiturates may produce marked falls in body temperature, and it is likely such a fall in body temperature will drastically affect the rate of metabolism of the pentobarbitone.

It has also been observed that progestin raises whilst oestrogen depresses the human body temperature (Israel & Schneller, 1950) and in this present study slight, and statistically not significant but nevertheless consistent, falls or rises in resting body temperatures were observed in mice given only mestranol or lynestrenol. It was, therefore, of interest to see whether or not pentobarbitone sleep was accompanied by greater or smaller degrees of hypothermia in steroid-pretreated mice. The speed of onset of hypothermia was not affected by steroid pretreatment, but the final depth and duration of hypothermia were significantly altered (See Fig. 18), suggesting that the actual duration of pentobarbitone sleep might be significantly affected by the hypothermia. To test this hypothesis, the experiment was repeated at a laboratory temperature of 30°C instead of the usual 22°C. At this temperature, pentobarbitone did not induce hypothermia, and control animals slept for only a short period of time which was identical to that of lynestrenol-pretreated mice. The effects of mestranol were also greatly reduced although there was still a significantly longer sleeping-time in these animals than in the controls. In some way, probably by a reduction in basal metabolic rate, mestranol increases the duration of pentobarbitone hypothermia in mice, but this does not completely explain the observed reduction in the rate of pentobarbitone metabolism observed in mestranol-pretreated mice, and it seems likely that some direct effect upon the liver is involved.

PART II

STEROIDS IN RATS

SECTION I

Many workers have shown the existence of a sex difference in the response of rats to barbiturates (See Introduction, page 39). More recently, Fujii et al (1965) showed the existence of a sex difference in the development of tolerance to barbiturates in rats. They also pointed out that immature male and female rats more rapidly developed tolerance to phenobarbitone. It was decided to investigate the effect of age, weight, ovariectomy and sex steroids on the duration of sleep and the development of tolerance to pentobarbitone in female rats.

(1) Effects of sex steroids and ovariectomy on pentobarbitone sleeping-time in mature female rats:

Groups of 5 female rats weighing approximately 200 g received mestranol (0.5 mg/kg twice daily) or lynestrenol (5.0 mg/kg twice daily) for 4 days; control rats received the oily vehicle alone (2.5 ml/kg twice daily) for 4 days. A fourth group of rats was ovariectomised. On the 5th day, all the rats received an intraperitoneal injection of pentobarbitone sodium (25 mg/kg) and their individual sleeping-times recorded. Sex steroid pretreatment (both oestrogen and progestin) produced a 15% increase ($P = < 0.05$) in sleeping-time whilst ovariectomy produced no effect when compared with controls. See Table 29.

(2) The effects of sex steroids and ovariectomy on the duration of sleep and onset of tolerance to a low dose of pentobarbitone in 6-week old female rats

3 groups of 6 young female rats (weighing approximately 100-120 g) were ovariectomised 4 days prior to receiving mestranol (0.5 mg/kg, twice daily) or lynestrenol (5.0 mg/kg, twice daily) subcutaneously for four days; control rats received the oily vehicle only (2.5 ml/kg twice daily) for four days. 3 groups of sham-operated female rats were similarly pretreated. On the 5th day they all received pentobarbitone sodium (30 mg/kg) intraperitoneally and their duration of sleep measured. On the 6th and 7th days they all received further injections of pentobarbitone sodium (30 mg/kg) intraperitoneally and their sleeping-times measured again on each occasion. The results are recorded in the histograms, Fig. 22.

With the initial dose of pentobarbitone, ovariectomy reduced the sleeping-time of young female rats, whilst sex steroid pretreatment of the ovariectomised rats produced no additional effect. However, steroid pretreatment (both mestranol and lynestrenol) of sham-operated female rats produced a 25% reduction in sleeping-time to pentobarbitone. These results tend to be opposite to those obtained in mature female rats. Development of tolerance was rapid (30% reduction within the space of 2 days), but was not markedly affected by pretreatment in any of the groups.

(3) Effects of sex steroids and ovariectomy on the duration of sleep and onset of tolerance to a high dose of pentobarbitone in 6-week old female rats

6 groups of 8 female rats, either ovariectomised or

sham-operated, were pretreated with sex steroids as above. On the 5th day they all received pentobarbitone sodium (50 mg/kg), intraperitoneally; similarly on both the 6th and 7th days pentobarbitone was given again and sleeping-times measured on all three occasions. The reduction in pentobarbitone sleeping-time due to ovariectomy and ovariectomy plus sex steroid pretreatment are similar but less than those obtained with the low dose of pentobarbitone. The onset of tolerance was rapid and more pronounced than previously observed at the lower doses of the barbiturate (50% reduction within the space of 2 days). Neither ovariectomy nor sex steroids pretreatment appreciably altered the onset or degree of pentobarbitone tolerance. See histograms, Fig. 23.

A few days later the adrenal glands were removed from each animal and weighed. The average paired adrenal weight from each group was compared, see Table 30). When the mean paired adrenal weights are compared on a mg/100 g body weight basis mestranol pretreatment produced a 25% increase in weight in sham-operated rats and a 50% increase in ovariectomised animals compared with controls.

(4) Effects of ovariectomy and sex steroids on the rate of growth of 6-week old female rats

Six groups of 6 female rats, three groups ovariectomised and three groups sham-operated, were pretreated with sex steroids or the vehicle for a period of 10 days. At similar times in the afternoon of each day during that period the body weight of each animal was recorded. The effects of ovariectomy and sex steroid pretreatment on the average

body weights of the normally rapidly growing rats are recorded in Fig. 24.

During this period ovariectomy nearly doubled the rate of growth compared with the sham-operated animals, while lynestrenol arrested growth and mestranol caused actual loss of weight in both ovariectomised and sham-operated rats.

SECTION II

Recent evidence has suggested that stress and corticosteroids influence barbiturate anaesthesia (See Introduction, page 38). It was decided to investigate the effects of adrenalectomy and synthetic corticosteroids on pentobarbitone induced sleep in female rats.

(1) Effect of corticosteroids and adrenalectomy on the duration of sleep after injection of pentobarbitone in female rats

Three groups of 8 female rats weighing between 130 and 150 g were adrenalectomised 3 days prior to receiving glucocorticoid (dexamethasone, 1.0 mg/kg daily) or mineralocorticoid* (fludrocortisone 1.0mg/kg daily) subcutaneously for 4 days; three groups of sham-operated female rats were similarly pretreated. On the 5th day they all received pentobarbitone sodium (35 mg/kg) intraperitoneally and their sleeping-times recorded. This dose of barbiturate was chosed in order to produce a sleeping-time of approximately 3 hr duration. The results are recorded in Fig. 25.

*fludrocortisone is a potent mineralocorticoid but has glucocorticoid activity.

Adrenalectomy prolonged sleeping-time to pentobarbitone (by 20%). Fludrocortisone, possibly acting as a replacement hormone, nullified the effects of adrenalectomy upon pentobarbitone sleeping-time but produced no effect in sham-operated rats. Dexamethasone pretreatment significantly decreased sleeping-times in both adrenalectomized (by 40%) and sham-operated (by 35%) female rats.

(2) Effects of adrenalectomy and synthetic corticosteroids on the body weight of female rats

During the 4-day pretreatment of the six groups of rats in experiment (1) above, the body weights of each animal was recorded daily. The effects of adrenalectomy and corticosteroid pretreatment on the average weights of the six groups of animals is recorded in Fig. 26.

Neither adrenalectomy nor fludrocortisone pretreatment markedly affected the weight of the rats, but dexamethasone (glucocorticoid) caused a pronounced loss in weight. A possible explanation could be a loss of protein by gluconeogenesis produced by the glucocorticoid.

Since high blood sugar levels during dexamethasone pretreatment might influence the course of pentobarbitone anaesthesia, it was decided to test the effects of hyperglycaemia on pentobarbitone sleeping-time.

(3) Effect of hyperglycaemia on pentobarbitone sleeping-time in female rats

15 days after the previous experiment the control and dexamethasone pretreated sham-operated groups were tested

again. Each group was divided into 2 sub-groups of 4 rats each. Hyperglycaemia was produced in one sub-group of each main group by the method of Adamkiewicz and Adamkiewicz (1960). Glucosuria was obtained by injecting 2.0 ml of a 25% $^{w}/_{v}$ hypertonic solution of glucose in water subcutaneously into the backs of the rats. This procedure was repeated 60 min and 150 min later, whilst hypertonic sodium chloride solution (8.5% $^{w}/_{v}$) was injected in controls. Half an hour after the last injection, pentobarbitone sodium (35 mg/kg) was injected intraperitoneally into the rats of all four groups and their sleeping-times measured. The results are shown in Table 31. The results demonstrate that high blood glucose levels (Clinitest strips positive for glucosuria) produced no effect upon pentobarbitone sleeping-time. Sleeping-time reduction due to dexamethasone disappeared when administration of the steroid was discontinued.

Fludrocortisone appeared to act as a replacement hormone since the effects of adrenalectomy on pentobarbitone anaesthesia in rats were returned to control levels by fludrocortisone pretreatment. It was decided to examine the influence of deoxycortisone acetate (DOCA), which possesses almost entirely mineralocorticoid activity. The effects of adrenalectomy and dexamethasone on pentobarbitone sleeping-time were re-examined.

(4) Effects of adrenalectomy, dexamethasone and deoxycorticosterone acetate on the duration of sleep after injection of pentobarbitone

Three groups of 8 female rats (weighing between 140 and

170 g) were adrenalectomised 2 days prior to receiving glucocorticoid (dexamethasone, 1 mg/kg, daily) or mineralocorticoid (deoxycorticosterone acetate, 5.0 mg/kg, daily) for 4 days; controls received the oily vehicle only (1.0 ml/kg, daily) for 4 days. Three groups of sham-operated female rats were similarly pretreated. On the 5th day they all received pentobarbitone sodium (35 mg/kg) intraperitoneally and their sleeping-times recorded. See Fig. 27.

Adrenalectomy again significantly prolonged sleeping-time (by 25%) but DOCA pretreatment in adrenalectomised animals was without effect. However, in sham-operated animals DOCA prolonged the anaesthesia nearly to the levels produced by adrenalectomy. In both sham-operated and adrenalectomised groups, dexamethasone produced a marked and similar reduction in pentobarbitone sleeping-time (by 40-50%).

(5) Effects of adrenalectomy, dexamethasone and deoxycorticosterone acetate (DOCA) on the body weight of female rats

During the pretreatment of the six groups of rats in experiment (4) above, the body weights of each animal were measured and recorded daily. The effects of adrenalectomy and steroid pretreatment are recorded in Fig. 28.

Dexamethasone (glucocorticoid) again produced a substantial loss in weight which was attributed to loss of protein. Adrenalectomy did not markedly affect the body weight of the animals when compared with those of the sham-operated group. DOCA, however, produced a significant

increase in the average body weight of adrenalectomised rats, whilst it produced little or no effect in the sham-operated group. This great increase (15%) in weight of these rats was most likely due to oedema resulting from sodium retention which was exacerbated as these animals received 0.9% sodium chloride in their drinking water. (See Methods, page 52).

6 days after withdrawal of the steroid treatment the body weights of all six groups of rats tended towards a mean of 175-180 g. Thus the steroid effects upon body weight in rats are reversible.

DISCUSSION

In mature female rats ovariectomy failed to produce any change in pentobarbitone sleeping-time whilst mestranol and lynestrenol pretreatment produced a slight prolongation. In young immature females ovariectomy reduced pentobarbitone sleeping-time compared with intact control animals; both mestranol and lynestrenol reduced the sleeping-time in intact young females but produced no difference in ovariectomised rats. The onset and degree of tolerance to both high and low doses of pentobarbitone remained largely unaltered by ovariectomy or sex steroid pretreatment. Maximum tolerance to pentobarbitone was reached rapidly by the second day, which largely confirms the results obtained by Fujii et al (1965) using 40-day old rats.

The paired adrenal weights of oestrogen-treated rats were significantly larger than those of the relevant control groups in experiments using immature rats. This confirms

results obtained by past workers (Selye & Collip, 1936; Gemzell, 1952) and the more recent work of Gibson et al (1967). Gibson and co-workers showed that chronic pretreatment of both male and female rats with any of three synthetic oral oestrogens produced adrenal enlargement. Both Selye and Collip (1936) and Gemzell (1952) maintained that oestrogen-induced adrenal growth is mediated through the pituitary. These results point to an oestrogen-stimulated adrenal hyperactivity and release of adrenocorticosteroids, certain of which are known to shorten pentobarbitone sleeping-time (Tureman et al, 1952; Winter & Flataker, 1952; Robillard & D'Iorio, 1954: Bousquet et al, 1965). Robillard and D'Iorio (1954) postulated that testosterone-induced shortening of pentobarbitone sleeping-time is mediated through adrenal release.

Unlike the results obtained with mice, lynestrenol (progestin) mimicked the action of mestranol (oestrogen) upon pentobarbitone anaesthesia in both mature (prolongation of pentobarbitone sleeping-time) and immature (shortening of pentobarbitone sleeping-time) female rats. Other workers have obtained similar results (Robillard & D'Iorio, 1954), both oestrogen and progestin producing a prolongation of pentobarbitone anaesthesia in rats.

During their experiments on the effects of sex upon development of barbiturate tolerance in rats, Fujii et al (1965) noted that certain metabolic enzymes were more active in female than in male rats at 40 days of age. This underlines the importance of age on metabolic pathways and may explain the opposite results obtained in mature and immature female rats in these experiments. Also, it

appears that in fast growing rats tolerance to barbiturates is more rapidly established due to the facility with which the liver can increase in size and weight and the concomitant increase in microsomal enzyme activity, (Conney et al, 1960). Ovariectomised rats were shown to grow twice as quickly as intact animals, and this too may reflect a greater metabolic activity with the observed resultant shortening of drug action.

Mestranol caused a loss in body weight in immature female rats which are normally rapidly growing. This phenomenon has previously been shown to occur in rats chronically pretreated with oestrogen (Sullivan & Smith, 1957; Gibson et al, 1967) and is thought to be mediated through an effect on the pituitary growth hormone (See review Reichlin, 1966) i.e. a hormone-induced anorexia. Lynestrenol slowed down the rate of growth in both intact and ovariectomised immature rats and this is possibly due to an increased turnover of amino acids since progesterone is known to increase nitrogen excretion (See Introduction, page 16). It should be pointed out that pentobarbitone was administered as a body weight-related dose to rats of equal age and presumably equal central sensitivity but having vastly different body weights due to steroid pretreatment. The extent to which pentobarbitone sleeping-time is thereby affected is difficult to assess since ovariectomised rats which received a higher dose of hypnotic due to an excess body weight over intact animals, slept for a shorter period than the intact animals.

In conclusion, the minor changes in pentobarbitone sleeping-time induced by ovariectomy and sex steroid

pretreatment appear to be due to differences in rates of drug metabolism. The differences may be due to a direct action of the pretreatment or more likely an action mediated through the adrenals. The issue is further complicated by vast weight differences in the steroid pretreated animals.

Adrenalectomy and DOCA administration induced a prolongation of pentobarbitone sleeping-time in female rats. Dexamethasone (glucocorticoid) pretreatment produced a shortening of pentobarbitone sleeping-time in both intact and adrenalectomised female rats. Fludrocortisone (mainly mineralocorticoid) induced no change in pentobarbitone response to intact animals but reduced the sleeping-time of adrenalectomised animals to control (intact animal) levels. It appears, therefore, that it acts as a replacement hormone, counteracting the effects of adrenalectomy.

Previous workers have shown that adrenalectomy produces a prolongation of barbiturate response in a number of species, including rats (Tureman et al, 1952; Robillard & D'Iorio, 1954; Bousquet et al, 1965) and mice (Winter and Flataker, 1952). The results with dexamethasone (glucocorticoid) to a large extent confirm the results obtained by a number of workers using cortisone. DOCA may exert its action in prolonging pentobarbitone sleeping-time in intact animals indirectly by preventing the release of ACTH and thus lowering the levels of endogenous glucocorticoid. This seems probable since DOCA produced no further prolongation in adrenalectomised animals. Remmer (1958) showed that adrenalectomy of either male or female rats decreased the activity of liver microsomal enzymes that oxidise hexobarbitone. This would explain the results obtained in adrenalectomised

female rats using pentobarbitone. Glucocorticoids are known to induce hepatic enzymes (See Introduction, page 33) and it appears likely this is the mechanism whereby dexamethasone produces a shortening of pentobarbitone sleeping-time.

However, as with the sex steroid pretreatment, corticosteroids also produce vast changes in body weight which then alter the total dose of barbiturate administered to rats of equal age. Dexamethasone produced a great loss in weight in both adrenalectomised and intact animals, but it seems unlikely that this fact alone could account for the 50% reduction in pentobarbitone sleeping-time.

In conclusion, adrenalectomy and DOCA administration produce prolongation of pentobarbitone sleeping-time by lowering the endogenous glucocorticoid levels, directly in the former case and indirectly in the latter. Administration of dexamethasone reduces pentobarbitone sleeping-time probably by inducing hepatic microsomal enzymes which oxidise the drug. Fludrocortisone acts largely as a replacement hormone since it possesses both gluco- and mineralocorticoid activity.

Pretreatment	Group mean sleeping-time (min ± S.E.) after			
	Pentobarbitone		Hexobarbitone	Barbitone
	(females)	(males)	(females)	(females)
Vehicle only (controls)	102 ± 9.6	96.6 ± 5.8	19.6 ± 1.9	111 ± -8
Mestranol (oestrogen)	*201 ± 23	*237.1 ± 14.9	*51.7 ± 3.5	109 ± 17
Lynestrenol (progestin)	*37.3 ± 2.3	*75.4 ± 5.7	*8.6 ± 0.5	109 ± 20

TABLE 18

Duration of barbiturate sleep in male and female mice pretreated with sex steroids. Pentobarbitone sodium (50 mg/kg), hexobarbitone sodium (60 mg/kg) or barbitone sodium (300 mg/kg) were injected intravenously 18 hr after the last of four daily subcutaneous injections of mestranol (0.5 mg/kg), lynestrenol (5.0 mg/kg) or vehicle (5.0 ml/kg).

*Significant difference from controls ($P = < 0.05$)

Pretreatment (Proprietory name of tablet and its constituents)	Doses given daily to each mouse		Pentobarbitone sleeping-time Group mean (min ± S.E.)
	Tablets per day	Equivalent mg/kg (orally)	
NORLESTRIN: ethinyloestradiol 0.05mg, norethisterone acetate 2.5mg.	1/5th	0.5 25	26.5 ± 1.8 *
VOLIDAN: ethinyloestradiol, 0.05mg, megestrol acetate, 4 mg.	1/5th	0.5 40	53.9 ± 5.1 *
CONOVID: mestranol, 0.075 mg, norethynodrel 5 mg.	1/5th	0.75 50	62.1 ± 7.9 *
LYNDIOL: mestranol, 0.15 mg lynestrenol 5 mg.	1/5th	1.5 50	16.9 ± 1.0 *
"	1/15th	0.5 16.7	18.2 ± 3.1 *
"	1/50th	0.15 5.0	27.2 ± 2.8 *

TABLE 19

Duration of pentobarbitone sleep in female mice pretreated with four commercially available oral contraceptive preparations. Tablets were crushed, suspended in 2% acacia solution, and the equivalent of 1/5th, 1/15th or 1/50th of a tablet administered to each mouse, daily, orally for 4 days. The response to pentobarbitone sodium (50 mg/kg) administered intravenously, was determined on the 5th day.

*Significant difference from controls ($P = < 0.05$)

Pretreatment	Ratio	Pentobarbitone sleeping-time Group mean (min ± S.E.)
Vehicle only (Controls)	-	98.7 ± 6.4
Lynestrenol (10mg/kg) + mestranol (1.0mg/kg)	10:1	68.9 ± 3.9 *
Lynestrenol (5.0mg/kg) + mestranol (1.0mg/kg)	5:1	102.3 ± 6.0
Lynestrenol (1.0mg/kg) + mestranol (1.0mg/kg)	1:1	156.1 ± 9.0 *
Lynestrenol alone (5 mg/kg)	-	32.4 ± 4.0 *
Mestranol alone (1 mg/kg)	-	215 ± 18 *

TABLE 20

Duration of pentobarbitone sleep in female mice pretreated with various combinations of mestranol and lynestrenol. Pentobarbitone sodium (50 mg/kg) was injected intravenously 18 hr after the last of four daily subcutaneous injections of steroid or vehicle (5.0 ml/kg).

* Significant difference from controls ($P = < 0.05$)

Pretreatment	Pentobarbitone sleeping-time Group mean (min ± S.E.)
Saline only (controls)	103.1 ± 10.0
Human chorionic gonadotrophin (HCG) 10u daily for 6 days	117.1 ± 11.1
Saline only (controls)	97.8 ± 13.9
Human chorionic gonadotrophin (HCG) 10u daily for 6 days and on the 7th day, 25 u hourly for 3 hr.	103.4 ± 9.0

TABLE 21

Duration of pentobarbitone sleep in female mice pretreated with gonadotrophin. The animals were injected subcutaneously for six or seven days with gonadotrophin and on the seventh day they received an intravenous injection of pentobarbitone sodium (50 mg/kg).

Pretreatment	Pentobarbitone sleeping-time Group mean (min ± S.E.)
Vehicle only (controls)	102.8 ±
Progesterone (20 mg/kg)	62.5 ± *

TABLE 22

Duration of pentobarbitone sleep in female mice pretreated with progesterone. Pentobarbitone sodium (50 mg/kg) was administered intravenously 18 hr after the last of four daily subcutaneous injections of progesterone (20 mg/kg) or vehicle (5.0 ml/kg).

* Significant difference from controls ($P = < 0.05$).

Pretreatment	Pentobarbitone sleeping-time Group mean (min ± S.E.) at:	
	22°C	30°C
Vehicle only (controls) daily for 4 days	98.7 ± 9.2	24.9 ± 1.6
Mestranol (0.5 mg/kg) daily for 4 days	237.7* ± 19.7	40.7* ± 2.7
Lynestrenol (5.0 mg/kg) daily for 4 days	37.6* ± 3.4	24.9 ± 1.3

TABLE 23

Duration of pentobarbitone sleep in female mice pretreated with lynestrenol or mestranol at an environmental temperature of 22°C or 30°C. Pentobarbitone sodium (50 mg/kg) was injected intravenously 18 hr after the fourth subcutaneous injection of steroid.

* Significant differences from relevant controls ($P = < 0.05$).

Pretreatment	Pentobarbitone sleeping-time Group mean (min ± S.E.) at:	
	No further treatment	SKF 525A
Vehicle only (controls) daily for 4 days	51.4 ± 8.0	260 ± 22
Mestranol (0.5 mg/kg) daily for 4 days	124* ± 6.2	Approx. 600*
Lynestrenol (5.0 mg/kg) daily for 4 days	15.0* ± 3.1	117* ± 24

TABLE 24

Duration of pentobarbitone sleep in female mice pretreated with mestranol or lynestrenol, plus SKF 525A; Pentobarbitone sodium (35 mg/kg) was administered on the fifth day of experiment, 18 hr after the fourth injection of steroid and 90 min after an intraperitoneal injection of SKF 525A (50 mg/kg).

* Significant differences from relevant controls ($P = < 0.05$)

ABSORBANCE at 240 mU								
Standard	Control	Mestranol	Control	Mestranol	Control	Mestranol	Control	Lynestranol
25	0.204	0.204	0.202	0.202	0.212	0.212	0.174	0.174
Blank	0.040	0.086	0.027	0.067	0.037	0.047	0.054	0.028
E_1	0.128	0.125	0.104	0.132	0.162	0.135	0.126	0.123
E_2	0.131	0.107	0.087	0.102	0.072	0.105	0.120	0.075
E_3	0.093	0.092	0.140	0.096	0.132	0.096	0.096	0.066
E_4	0.153	0.092	0.157	0.138	0.132	0.126	0.117	0.132
Av.E	0.126	0.104	0.122	0.117	0.125	0.115	0.115	0.99
I.S.25	0.186	0.240	0.198	0.231	0.174	0.192	0.159	0.189
Concn 0.5 ml	14	12	14	13	14	13	13	11
Concn 1.0 ml	28	24	28	26	28	26	26	22

TABLE 25

Concentration of pentobarbitone sodium (µg/ml) in mouse whole blood at waking after receiving pentobarbitone sodium (50 mg/kg) intravenously.

B - Blank

E - Extraction

I.S. - Internal Standard.

ABSORBANCE at 240 mU																		
	Co ntrols					Mestranol					Lynestrenol					SKF 525A			
	1	2	3	Av	µg/ml	1	2	3	Av	µg/ml	1	2	3	Av	ug/ml	1	2	Av	ug/ml
50	0.452	0.452	0.472	-		0.470	0.450	0.458	-		0.480	0.470	0.437	-		0.448	-	0.459	
100	0.910	0.918	0.920	-		0.940	0.880	0.910	-		0.910	0.920	0.900	-		0.905	-	0.912	
B	0.023	0.035	0.020			0.035	0.004	0.040			0.029	0.002	0.025			0.042	0.019		
E0	0.952	0.767	0.788	0.896	96	0.732	0.740	0.789	0.754	83	1.036	0.868	0.781	0.895	98	0.934	0.792	0.863	95
E2.5	0.440	0.457	0.392	0.430	47	0.440	0.469	0.464	0.458	50	0.452	0.408	0.504	0.455	50	0.428	0.444	0.436	48
E5	0.328	0.385	0.348	0.354	39	0.397	0.400	0.393	0.397	44	0.412	0.320	0.224	0.319	35	0.368	0.412	0.390	43
E10	0.292	0.287	0.280	0.286	31	0.360	0.345	0.347	0.351	38	0.304	0.272	0.220	0.265	30	0.358	0.348	0.353	39
E20	0.252	0.271	0.244	0.256	28	0.291	0.331	0.317	0.313	34	0.240	0.212	0.167	0.206	22	0.332	0.328	0.330	36
E40	0.192	0.213	0.204	0.203	22	0.253	0.299	0.291	0.281	31	0.200	0.124	0.181	0.168	18	0.308	0.312	0.310	34
E60	0.164	0.185	0.180	0.176	19	0.252	0.264	0.237	0.251	27	0.112	0.104	0.149	0.122	13	0.300	0.276	0.286	31
E90	0.152	0.167	0.152	0.157	17	0.209	0.224	0.237	0.223	24	0.092	0.072	0.071	0.078	8	0.260	0.248	0.254	28
%R.	97.3	97.8	94.6			104.1	96.8	101.8			102.1	97.0	103			98.2	100		
I.S.	0.800	0.900	0.868			0.958	0.881	0.896			0.960	0.892	0.892			0.884	0.912		

TABLE 26

Contentration of pentobarbitone sodium (ug/ml) in mouse plasma at various times after receiving pentobarbitone sodium (50 mg/kg) intravenously.

B - Blank
E - Extraction
I.S. - Internal standard.

Pretreatment	Average time for consumption of 3 cc O_2 in sec (± S.E.)
Control (5.0 ml/kg, 4 days)	93 ± 4.4
Lynestrenol (5.0 mg/kg, 4 days)	81 ± 3.8 *
Mestranol (0.5 mg/kg, 4 days)	105 ± 3.1 *

TABLE 27

Oxygen consumption of groups of 15 female mice pretreated with lynestrenol or mestranol, subcutaneously, for 4 days.

* Significantly different from relevant controls ($P=<0.05$)

Pretreatment	Sleeping-time (± S.E.)	
	Hexobarbitone sodium 60 mg/kg I.V.	Pentobarbitone sodium 50 mg/kg I.V.
Dexamethasone 1 mg/kg S.C.	26.3 ± 2.5	108.4 ± 6.6
Fludrocortisone 1 mg/kg S.C.	21.8 ± 2.1	117.8 ± 11.4
Vehicle (controls) 5 ml/kg	26.1 ± 2.8	113.5 ± 8.6

TABLE 28

Barbiturate sleeping-time in female mice pretreated with synthetic corticosteroids for 4 days.

Pretreatment	Doses given twice daily to each rat	Pentobarbitone sleeping-time (min ± S.E.)
Controls	Vehicle only 2.5 ml/kg	85.2 ± 6.0
Mestranol	0.5 mg/kg	100.6 ± 7.3 *
Lynestrenol	5.0 mg/kg	105.6 ± 5.5 *
Ovariectomy	-	85.4 ± 3.0

TABLE 29

Duration of pentobarbitone sleep in MATURE female rats pretreated twice daily for 4 days with lynestrenol, mestranol, or vehicle only; a fourth group was ovariectomised. On the fifth day they all received pentobarbitone sodium (25 mg/kg, intraperitoneally) and their sleeping-times measured.

* Significantly different from relevant controls ($P \leq 0.05$)

OVARIECTOMISED

CONTROL		MESTRANOL		LYNESTRENOL	
Body weight g	Paired adrenal weight mg	Body weight g	Paired adrenal weight mg	Body weight g	Paired adrenal weight mg
180	72	110	52.5	133	57
176	64.5	111	57.5	135	52
173 (5)	63	110 (6)	63.5	170 (6)	55
157	59.5	123	58.5	148	49
181	57.5	116	73	130	50.5
		114	72.5	150	56.5
mean 173.4	mean 63.3	mean 114	mean 62.9	mean 144.3	mean 53.3
	36.5 mg/100g		55.2 mg/100g		36.9 mg/100

SHAM-OPERATED

CONTROL		MESTRANOL		LYNESTRENOL	
Body weight g	Paired adrenal weight mg	Body weight g	Paired adrenal weight mg	Body weight g	Paired adrenal weight mg
156	52.5	108	58.5	130	56
152	54.5	100	46.5	140	62.5
132 (5)	52	117	53.5	136	52
150	52	121 (7)	57	131 (7)	56.5
143	56.5	137	52	121	54.5
		114	44.5	134	51
		124	58	132	53.5
mean 146.6	mean 53.5	mean 117.3	mean 53.4	mean 132	mean 55.1
	36.5 mg/100g		45.5 mg/100g		41.6 mg/100g

TABLE 30

Adrenal and body weights of female rats pretreated with synthetic sex steroids administered subcutaneously (twice daily) for 4 days.

	Pretreatment	Pentobarbitone sleeping-time (min ± S.E.)
Controls	25% $^{W}/_{V}$ Glucose 2 ml S.C. at 0, 1 and 2½ hr	220 ± 18
	Hypertonic Saline 2 ml S.C. at 0, 1 and 2½ hr	237 ± 11
Dexamethasone	25% $^{W}/_{V}$ Glucose 2 ml S.C. at 0, 1 and 2½ hr	202 ± 19
	Hypertonic Saline 2 ml S.C. at 0, 1 and 2½ hr	231 ± 35

TABLE 31

Duration of pentobarbitone sleeping-time in groups of four female rats, 15 days after a course of steroid or vehicle pretreatment. Each rat received pentobarbitone sodium (35 mg/kg, intraperitoneally) and their sleeping-times measured.

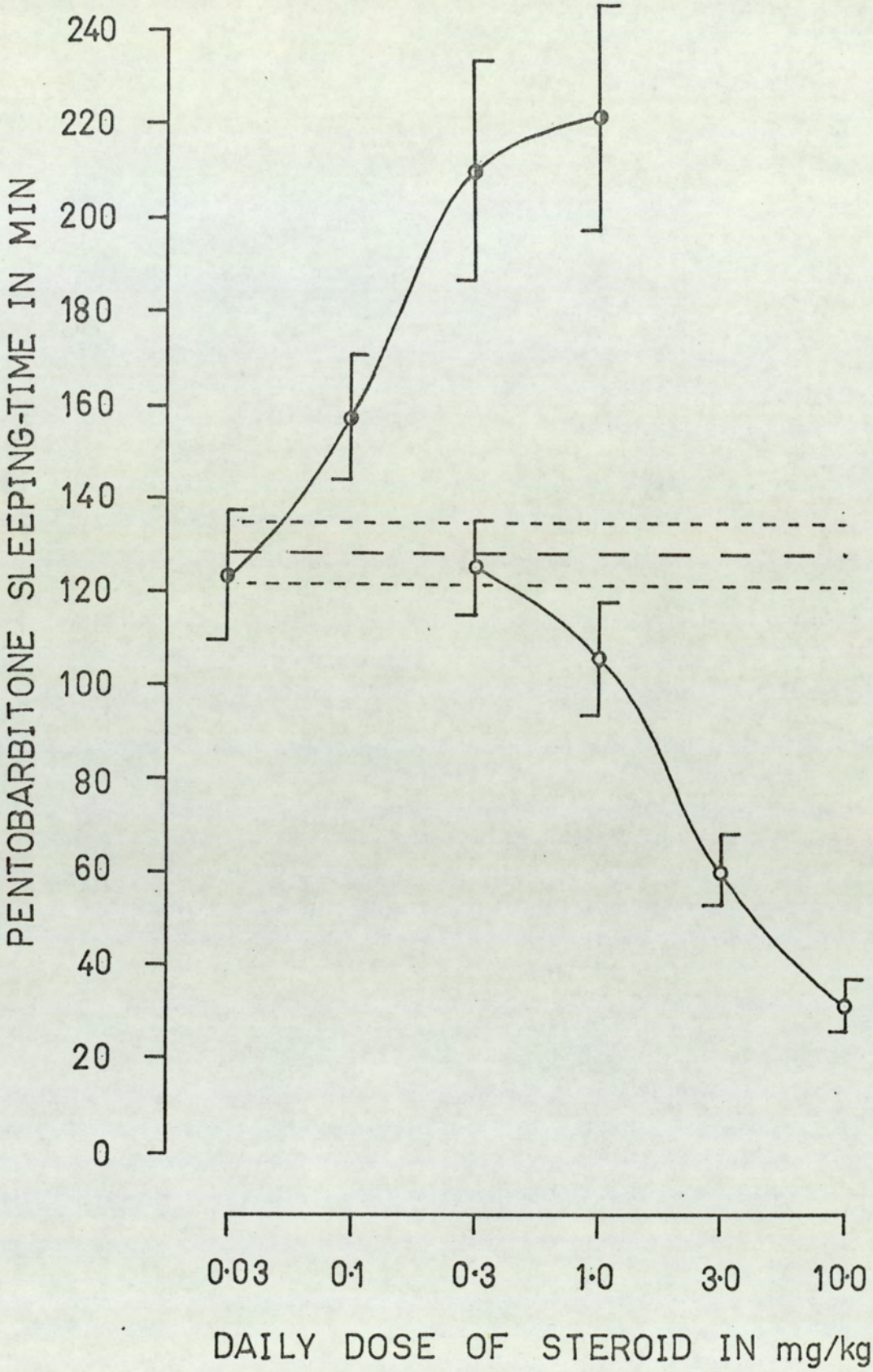


Fig. 16

Duration of pentobarbitone sleep in female mice after pretreatment with various doses of mestranol or lynestrenol. Animals were injected subcutaneously with mestranol (●—●) or lynestrenol (○—○) for 4 days; on the fifth day they received pentobarbitone sodium (50 mg/kg) intravenously. The dotted lines represent the response in control (vehicle pretreated) animals, group mean ± S.E.

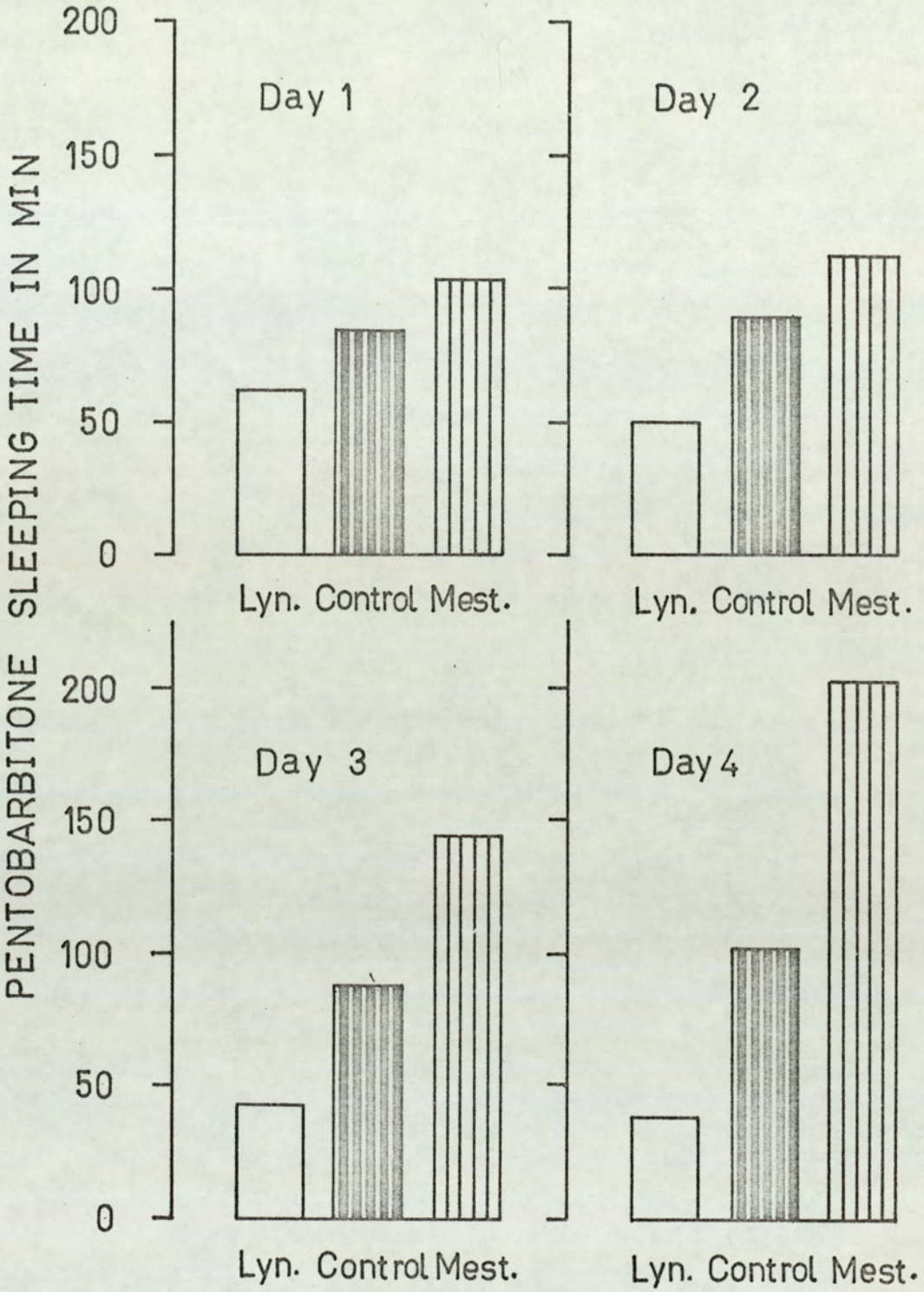


Fig. 17

Duration of pentobarbitone sleep in female mice after pretreatment with mestranol or lynestrenol. Animals were injected subcutaneously with mestranol (0.5 mg/kg) for 1, 2, 3 or 4 days (vertical lines) or lynestrenol (5.0 mg/kg) for 1, 2, 3 or 4 days (open bars); controls received the vehicle only (5 ml/kg, vertical bars). 18 hr after the last steroid injection they received an intravenous injection of pentobarbitone sodium (50 mg/kg).

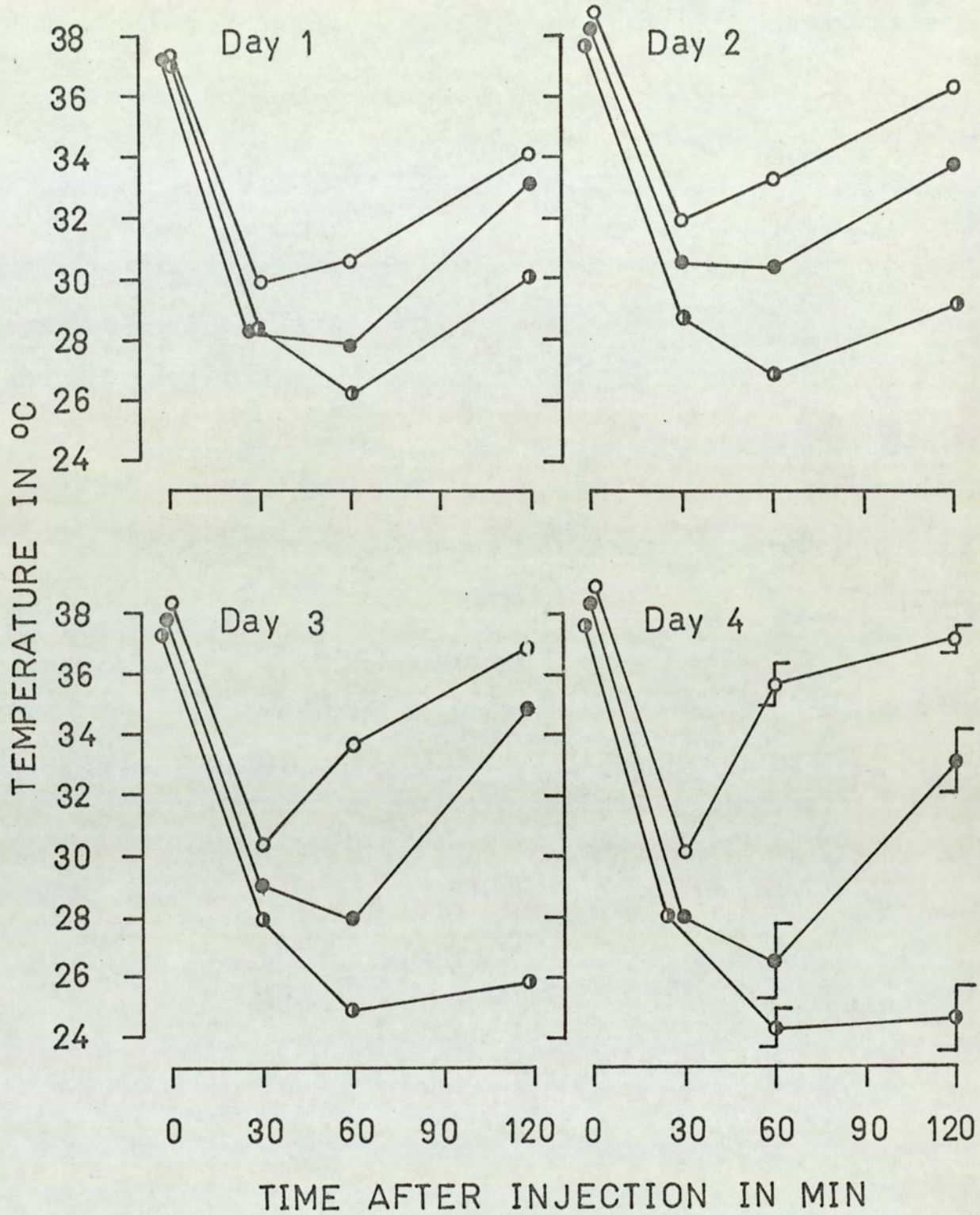


Fig. 18

Effects of pentobarbitone sodium (50 mg/kg) administered intravenously on the oesophageal temperatures of female mice pretreated with mestranol or lynestrenol. Animals were injected subcutaneously with mestranol (0.5 mg/kg) for 1, 2, 3 or 4 days (●—●); or lynestrenol (5.0 mg/kg) for 1, 2, 3, or 4 days (○—○); or the vehicle only (5.0 ml/kg, ●—●)

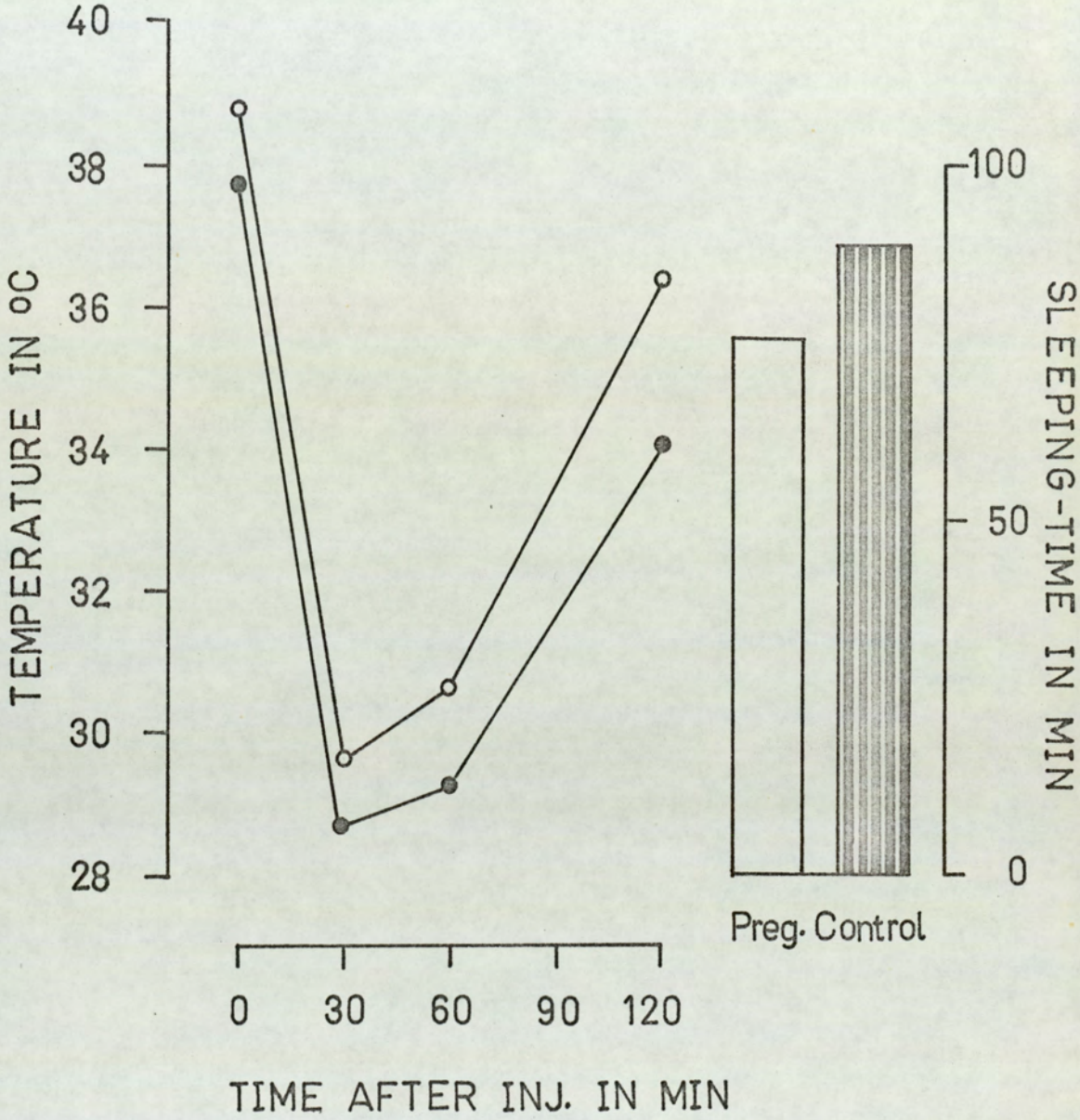


Fig. 19

Effect of pregnancy on the duration of pentobarbitone sleep and degree of pentobarbitone induced hypothermia. 16-18 day pregnant mice (O—O and open bars) and control female mice (●—● and vertical bars) received an intravenous injection of pentobarbitone sodium (50 mg/kg).

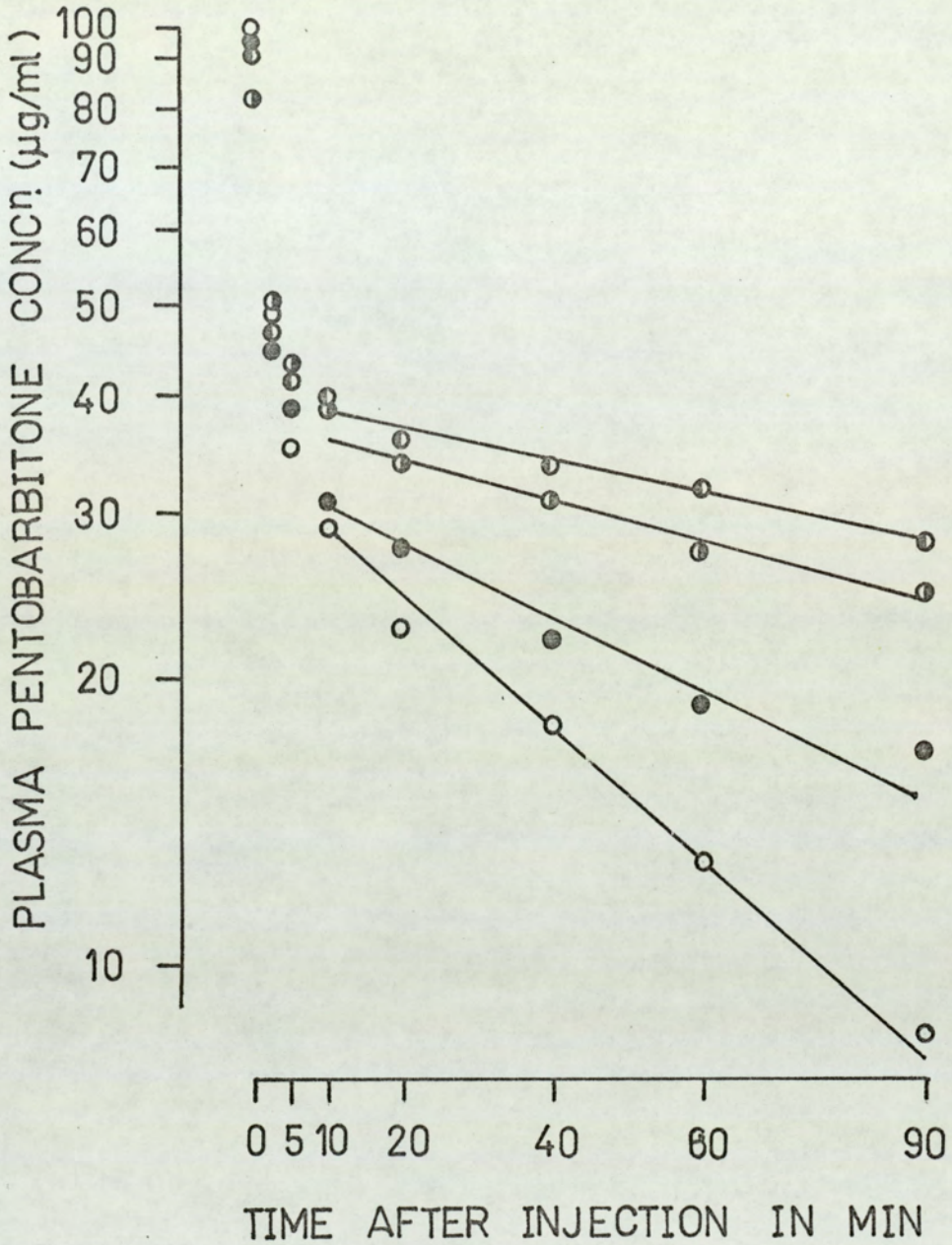


Fig. 20

Effects of pretreatment with mestranol, lynestrenol or SKF 525A on plasma pentobarbitone levels in female mice at various intervals after its injection. Animals were pretreated as follows: mestranol (0.5 mg/kg) subcutaneously for 4 days (●—●); or lynestrenol (5.0 mg/kg) subcutaneously for 4 days (○—○); or SKF 525A (50 mg/kg) intraperitoneally, once only 90 min before the barbiturate (◐—◐); or the vehicle only (5.0 ml/kg ◉—◉).

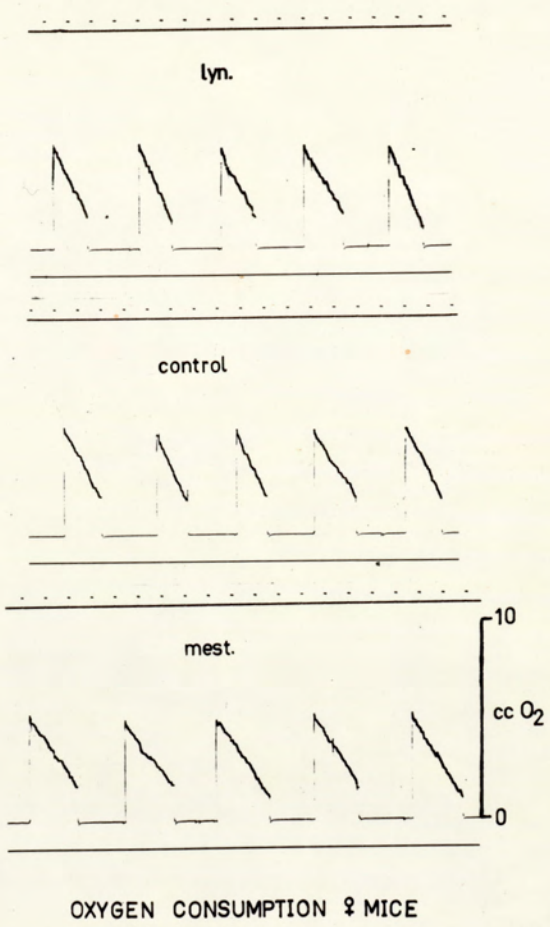


Fig. 21

Trace showing the effect of pretreatment with mestranol or lynestrenol on the rate of oxygen consumption by female mice. Animals were pretreated with mestranol (0.5 mg/kg) subcutaneously for 4 days; or lynestrenol (5.0 mg/kg) subcutaneously for 4 days; or the vehicle only (5.0 ml/kg).

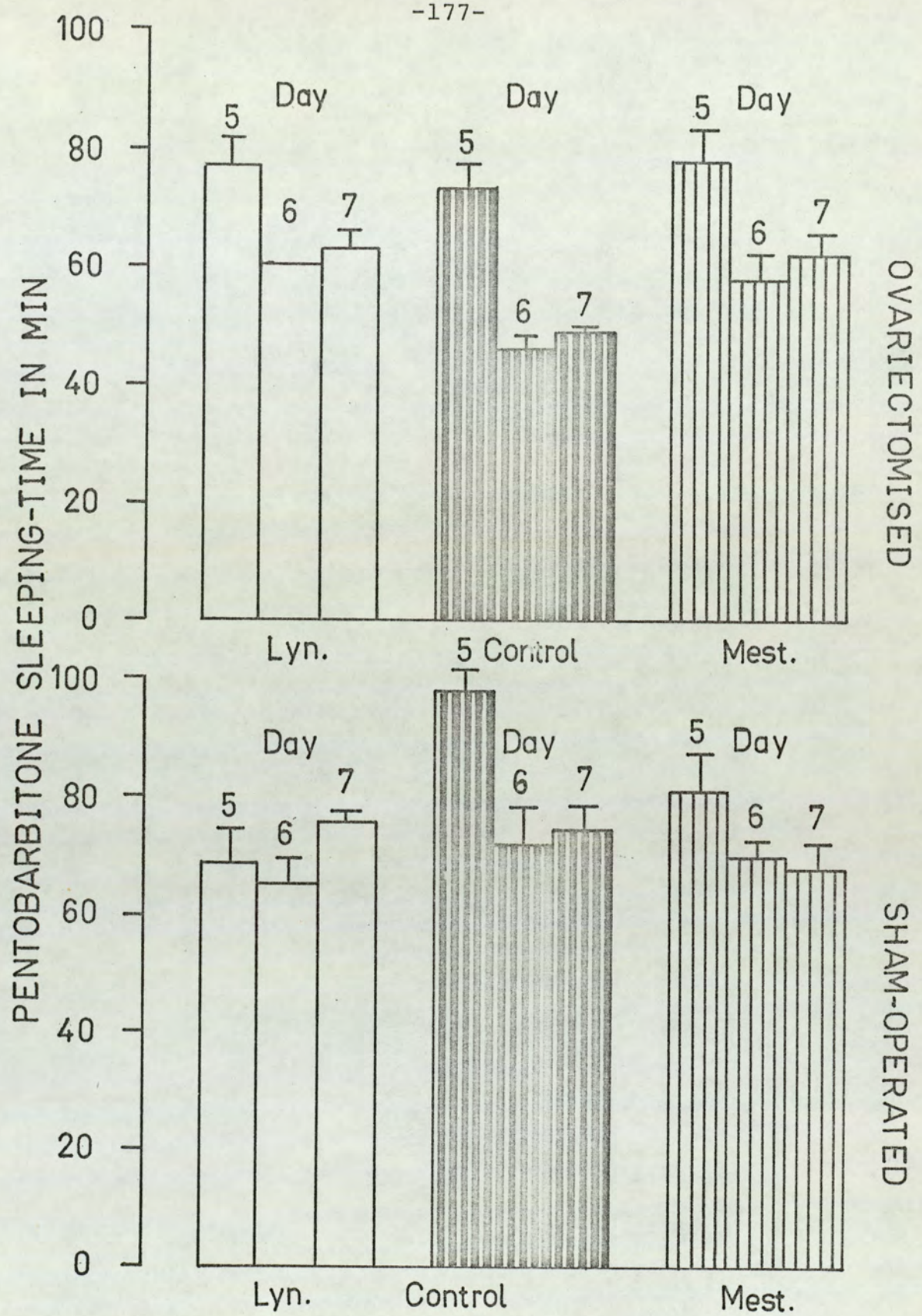


Fig. 22

Effect of mestranol or lynestrenol on the duration of pentobarbitone sleep and onset of barbiturate tolerance in ovariectomised or sham-operated immature rats. Animals were pretreated with mestranol (0.5 mg/kg, twice daily) subcutaneously for 4 days; or lynestrenol (5.0 mg/kg, twice daily) subcutaneously for 4 days; or vehicle only (2.5 ml/kg, twice daily). 18, 42 and 66 hr after the last steroid injection they received intraperitoneal injections of pentobarbitone sodium (30 mg/kg).

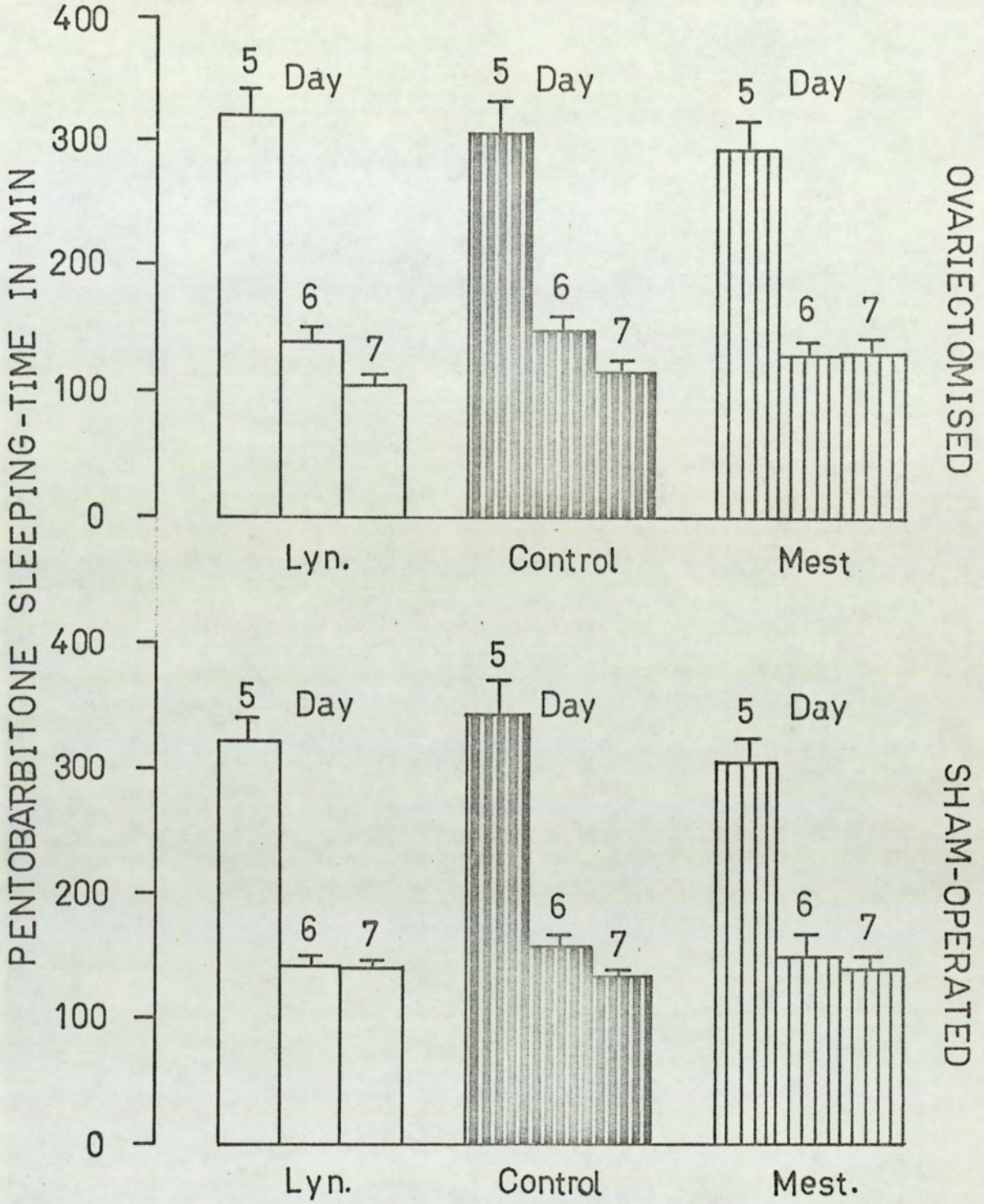


Fig. 23

Effect of mestranol or lynestrenol on th e duration of pentobarbitone sleep and onset of barbiturate tolerance in ovariectomised or sham-operated immature rats. Animals were pretreated with mestranol (0.5 mg/kg, twice daily) subcutaneously for 4 days; or lynestrenol (5.0 mg/kg, twice daily) subcutaneously for 4 days; or vehicle only (2.5 ml/kg, twice daily). 18, 42 and 66 hr after the last steroid injection they received intraperitoneal injections of pentobarbitone sodium (50 mg/kg).

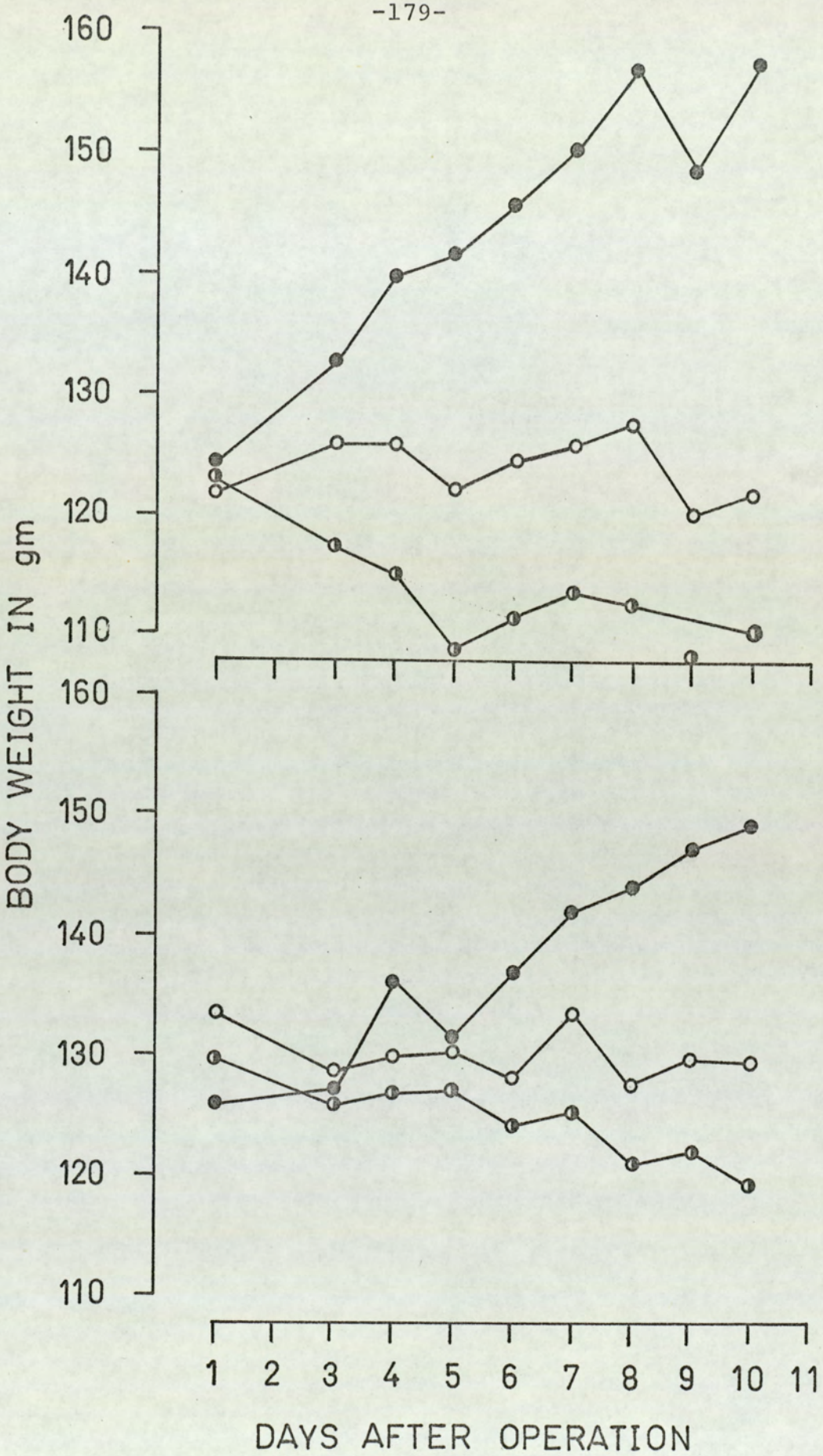


Fig. 24

Effect of mestranol or lynestrenol on the rate of growth in ovariectomised or sham-operated immature rats. Animals were pretreated with mestranol (0.5 mg/kg, twice daily) subcutaneously for 10 days (●——●), or lynestrenol (5.0 mg/kg, twice daily) subcutaneously for 10 days (o——o), or vehicle only 0.5 ml/kg, twice daily ●——●)

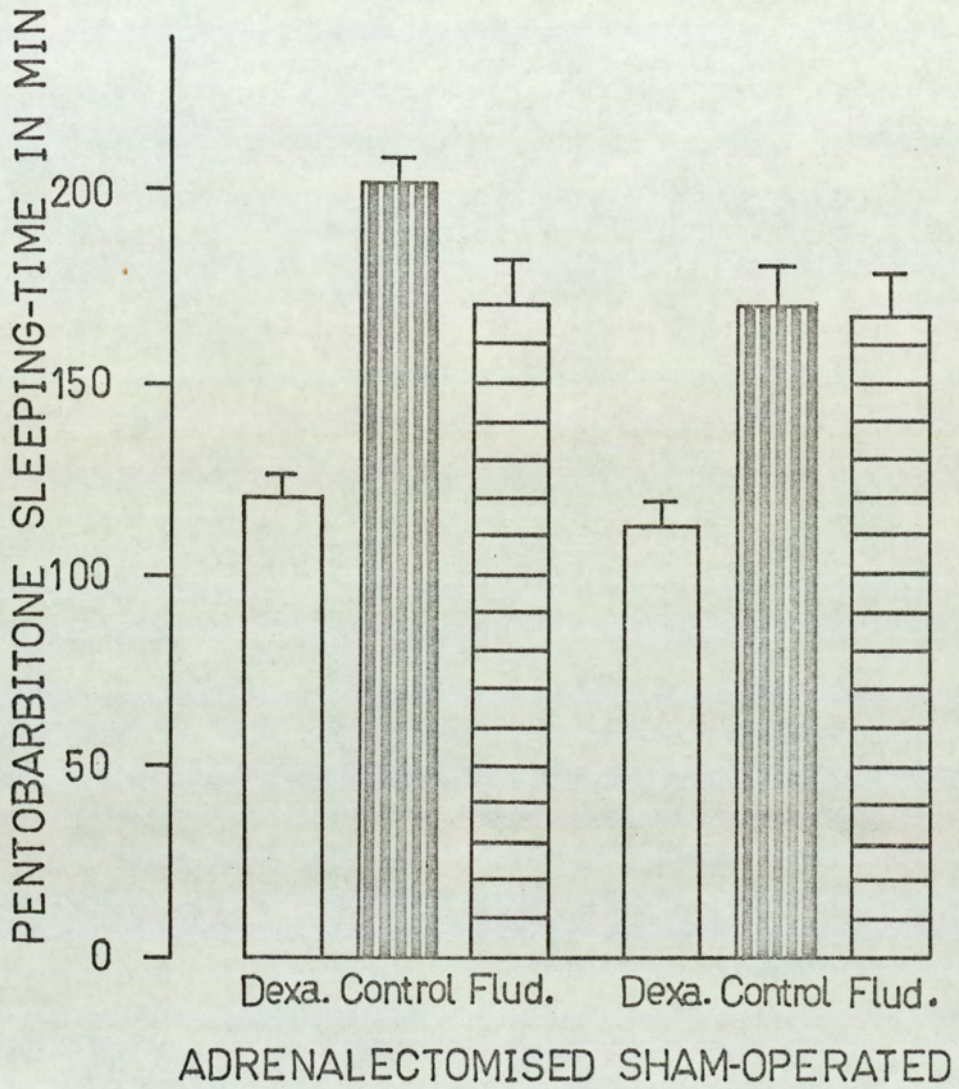


Fig. 25

Effect of dexamethasone or fludrocortisone on the duration of pentobarbitone sleep in adrenalectomised or sham-operated mature female rats. Animals were pretreated with dexamethasone (1.0 mg/kg, daily) subcutaneously for 4 days; or fludrocortisone (1.0 mg/kg, daily) subcutaneously for 4 days; or vehicle only (2.5 ml/kg, daily). 18 hr after the last injection of steroid they received an intraperitoneal injection of pentobarbitone sodium (35 mg/kg).

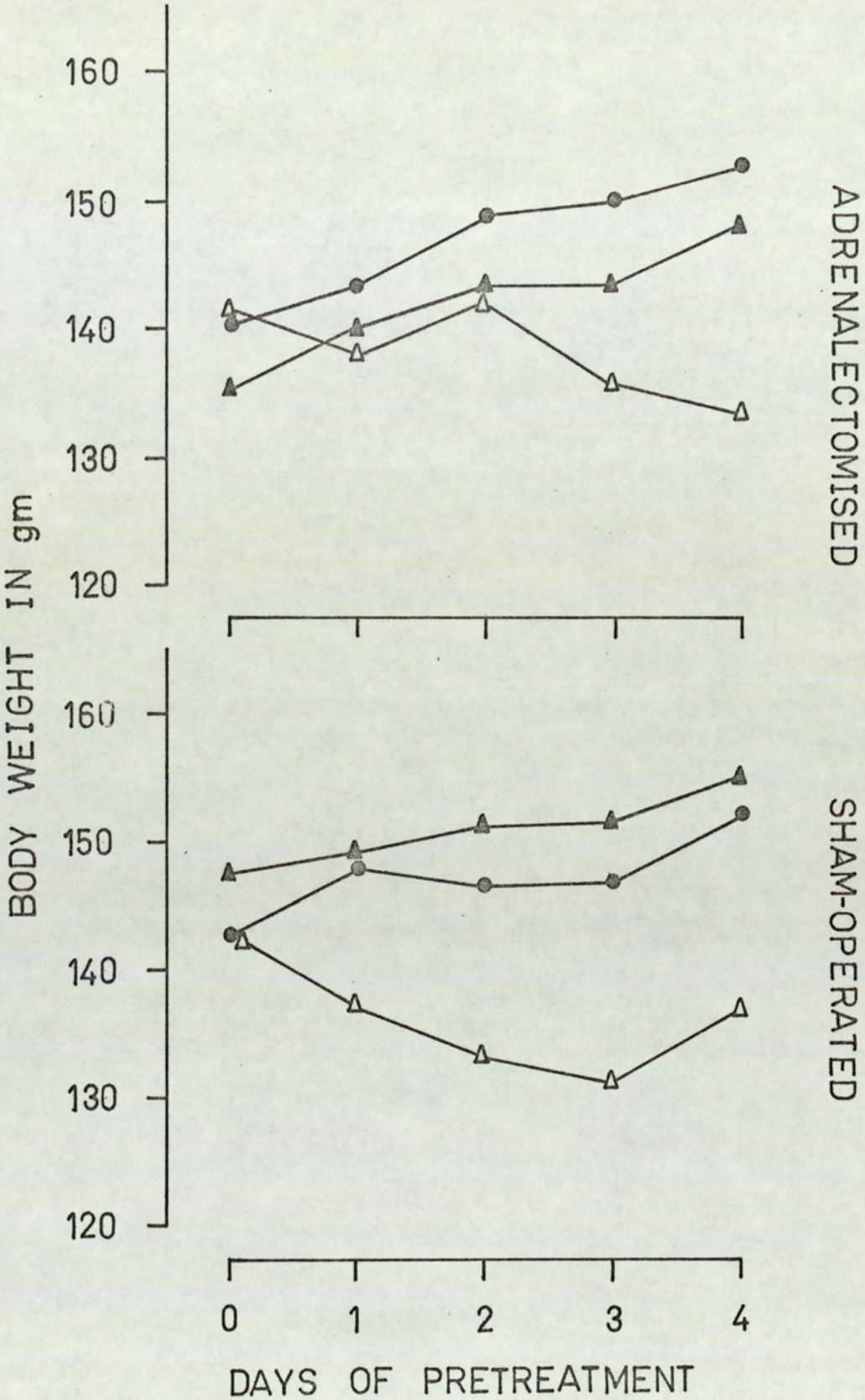


Fig. 26

Effect of dexamethasone or fludrocortisone on the body weight of adrenalectomised or sham-operated mature female rats. Animals were pretreated with dexamethasone (1.0 mg/kg, daily) subcutaneously for 4 days (Δ——Δ), or fludrocortisone (1.0 mg/kg, daily) subcutaneously for 4 days (▲——▲), or vehicle only (2.5 ml/kg, daily, ●——●)

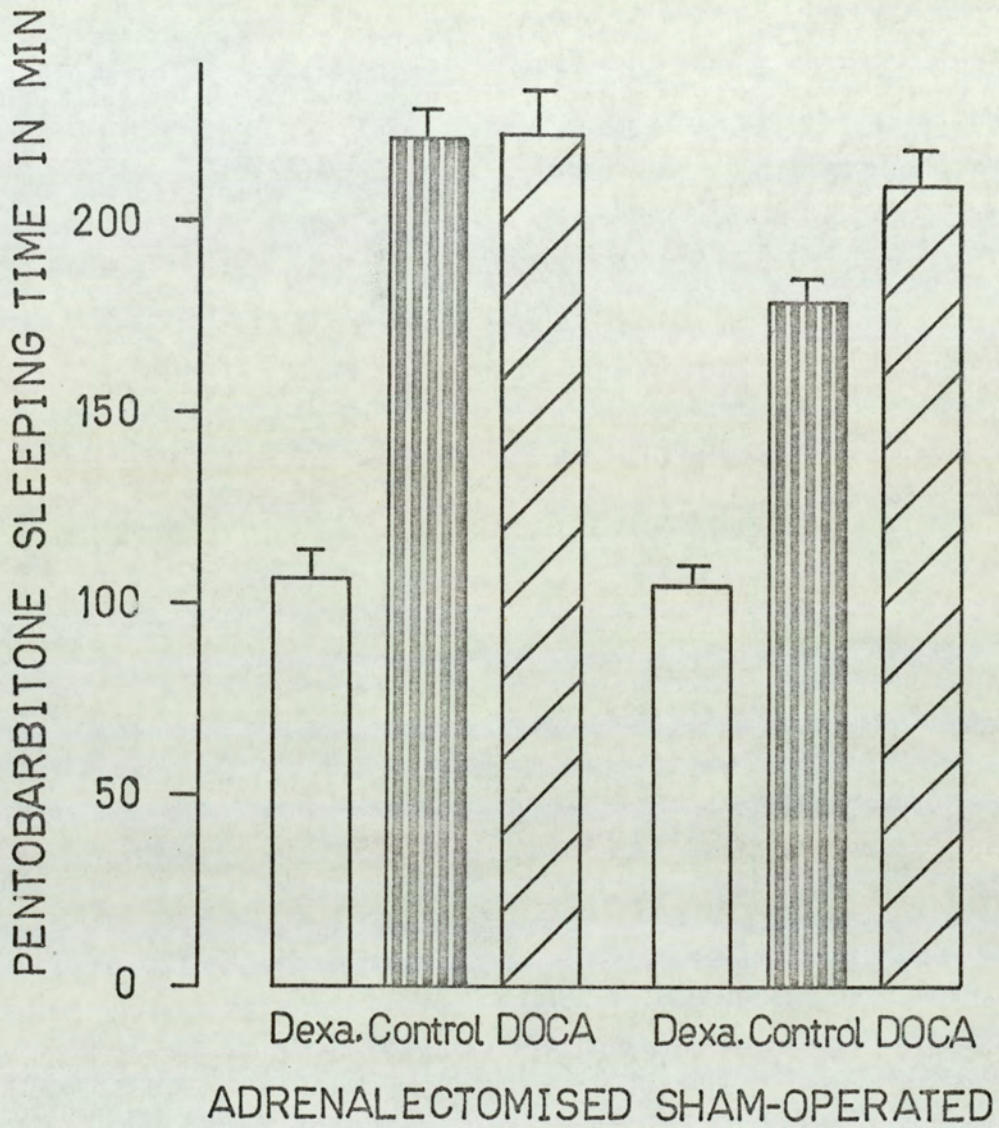


Fig. 27

Effect of dexamethasone or DOCA on the duration of pentobarbitone sleep in adrenalectomised or sham-operated mature female rats. Animals were pretreated with dexamethasone (1.0 mg/kg, daily) subcutaneously for 4 days; or DOCA (5.0 mg/kg, daily) subcutaneously for 4 days; or vehicle only (2.5 ml/kg, daily). 18 hr after the last steroid injection they received an intraperitoneal injection of pentobarbitone sodium (35. mg/kg).

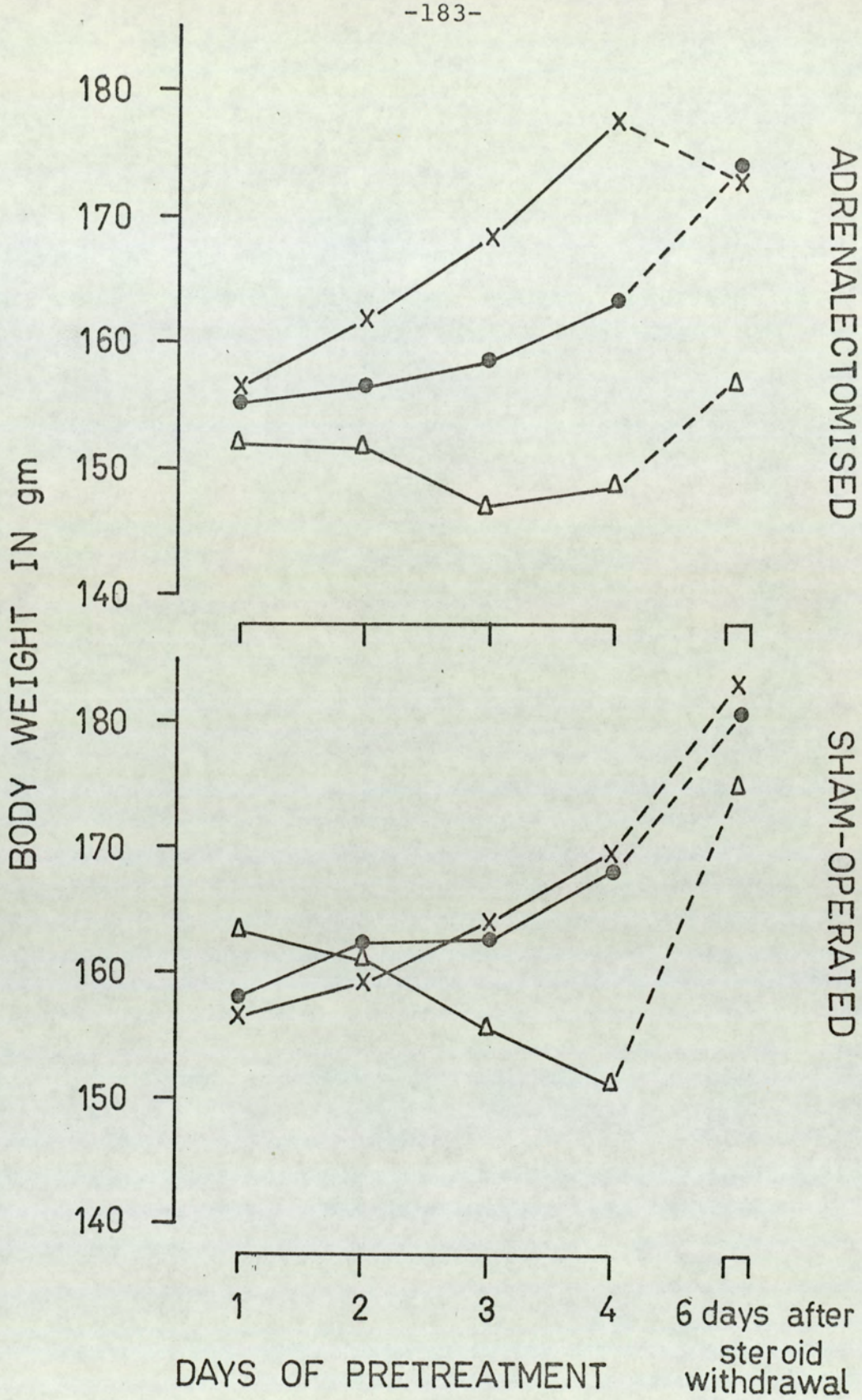


Fig. 28

Effect of dexamethasone or DOCA on the body weights of adrenalectomised or sham-operated mature female rats. Animals were pretreated daily with dexamethasone (1.0 mg/kg, daily) subcutaneously for 4 days (Δ——Δ); or DOCA (5.0 mg/kg, daily) subcutaneously for 4 days (X——X); or vehicle only (2.5 ml/kg, daily, ●——●).

CHAPTER III - SEX STEROIDS AND STIMULANTS

Introduction :-

Lichtensteiger in 1968 showed that cyclic variations occurred in the catecholamine content of the rat hypothalamus during the oestrous cycle. It is also known that there are temperature changes associated with the menstrual cycle in women. Israel and Schneller (1950) showed that increased progestational activity (progesterone) is thermogenic whilst increased oestrogenic activity is hypothermic in women. It appears, therefore, that brain amine levels, sex steroids and body temperature regulation may be inter-related in some way.

It has also been observed that increased progestational activity both in man and experimental animals is associated with increased monoamine oxidase activity (See, for example, Cohen & co-workers, 1964; Southgate & others, 1968). The occurrence of depression in a proportion of susceptible women receiving progestational agents may be due to an increased MAO activity at a central level, according to Grant and Pryse Davies (1968).

It was possible that these steroids, by inducing changes in MAO activity at both central and peripheral sites, might be associated with quantitative changes in the potency of certain drugs, for example, the sympathomimetic stimulants which may exert their action by release of the biogenic

amines.

Amphetamine is known to release endogenous biogenic amines peripherally (Burn & Rand, 1958) and there may be a similar mechanism in the brain whereby amphetamine produces its central effects (Stein, 1964; Rech, 1964; Weissman et al, 1966; Dingell et al, 1967; Wolf et al, 1969; Mennear & Rudzik, 1966 & 1968; and Frey & Magnussen, 1968). Some workers believe that amphetamine exerts some of its effect by a direct central stimulation and this is usually based upon its chemical similarity with noradrenaline (NA) and the observation that reserpine and certain other amine depletors do not antagonise amphetamine-induced locomotor hyperactivity (Rossum et al, 1962; Smith, 1963 & 1965). Nevertheless, evidence is predominantly in favour of an indirect central action for amphetamine. Moore (1963) showed that the marked increase in toxicity seen in crowded mice treated with d (+) - amphetamine compared with the l(-) isomer correlated well with the greater NA-releasing properties of d (+) amphetamine. Weissman et al (1966) demonstrated that there exists a critical level of NA at the receptor derived from a functional pool in the adrenergic neurone in the CNS which is extremely susceptible to blockade of NA synthesis at the tyrosine hydroxylase step. Thus, a methyl tyrosine (aMT), a potent tyrosine hydroxylase inhibiter blocks amphetamine induced locomotor activity. (Weissman & Koe, 1965; Weissman et al, 1966; Dingell et al, 1967) and amphetamine toxicity (Mennear & Rudzik, 1966). Reserpine and a methyl meta tyrosine (aMmT) although blocking toxicity to some extent (Mennear & Rudzik, 1966) by amine depletion do not disrupt amine synthesis and, therefore, do

not prevent amphetamine-induced locomotor hyperactivity. Recently Carr and Moore (1969) have developed a cerebro-ventricular perfusion technique in live cats to demonstrate that NA is released from endogenous stores, by dexamphetamine.

The role of central amines in the control of body temperature and their importance in amphetamine-induced hyperthermia has been the subject of some controversy. Nor-adrenaline (NA) and 5 hydroxytryptamine (5HT) are present in relatively high concentrations in the hypothalamus, which is known to be the seat of body temperature control (Review of Cooper, 1966). Injections of these monoamines into the third ventricle (the hypothalamus forms part of the wall of this), produce varying effects upon the body temperature. Feldburg and Myers (1964) showed that 5HT injected into the anterior hypothalamus or lateral ventricle of the cat caused the animal's temperature to rise and NA injected into these sites caused it to fall. However, injection of NA and 5HT into the cerebral ventricles of the conscious mouse produced opposite effects upon body temperature (Brittain & Handley, 1967). More direct evidence for monoamines acting as the mediators of amphetamine hyperthermia was provided by Carr and Moore (1969) when they showed the release of tritiated NA from the hypothalamus of conscious cats by dexamphetamine perfusion. Other workers believe that amphetamine exerts its hyperthermia by a calorigenic effect due to the NA released from peripheral sympathetic nerve endings in adipose tissue. The hyperthermia is thought to be a consequence of the increased metabolic rate resulting from the increased availability and utilization of free fatty acids (Gessa, Clay & Brodie, 1969).

In contrast with amphetamine, fencamfamin (2-ethylamino-3-phenyl norcamphane) has two remarkable properties, central stimulation with only slight peripheral circulatory effects. Its pharmacological profile may be summarized as follows: (for details, see Hotory et al, 1961): it has a sympathomimetic action causing central excitation together with a slight pressor activity, but has no effect upon the spleen and nictitating membrane. At least peripherally, it is thought to have an indirect action, since cocaine abolished its pressor activity while NA and A enhanced it. In contrast with amphetamine, it is only partially metabolised in the liver (51% excreted unchanged in the urine of rats).

This Chapter reports our findings with dexamphetamine, dl-amphetamine and fencamfamin in mice pretreated with the sex steroids. The effects of these three sympathomimetics have been studied on body temperature and locomotor activity, together with their acute toxicities in mice pretreated with lynestrenol or mestranol. Dexamphetamine is approximately twice as potent as fencamfamin and dl-amphetamine on a weight for weight basis and, therefore, doses of the drugs were chosen to give approximately equal activity. The doses of stimulant drugs to produce marked hyperactivity was found to be half that necessary to produce hyperthermia (except fencamfamin which is not hyperthermic). The effects of amine depletors, reserpine and tetrabenazine, and the monoamine oxidase inhibiter nialamide on certain of the effects of these stimulants were also studied.

SECTION I

Effects of lynestrenol or mestranol pretreatment in female mice, upon hyperthermia induced by stimulant drugs

dl-amphetamine :-

Groups of 15 female mice were pretreated for 4 days with mestranol (0.5 mg/kg) or lynestrenol (5.0 mg/kg) or with their oily vehicle (controls). On the 5th day they all received dl-amphetamine (20 mg/kg; intraperitoneally and their oesophageal temperatures measured at intervals for the next 4 hr. The peak hyperthermias (average temperature above original) for control and lynestrenol-pretreated mice of 2.4°c and 1.1°c respectively occurred at 1 hr; the peak hyperthermia for mestranol-pretreated animals of 3.1°c occurred at 2 hr. After 4 hr the temperature of lynestrenol-pretreated animals returned to normal whereas the average temperature of control animals was still 1.3°c above their original. The average temperature of mestranol-pretreated animals was still 2.77°C above their original. Thus lynestrenol pretreatment shortened the duration and intensity of dl-amphetamine hyperthermia whereas mestranol prolonged the duration and increased the intensity of hyperthermia (See Fig. 29).

Dexamphetamine :-

Groups of 10 female mice were pretreated with lynestrenol (10 mg/kg) or mestranol (1.0 mg/kg) as above. On the 5th

day their received dexamphetamine (10 mg/kg) intraperitoneally. Their body temperatures were measured periodically over the next 4 hr.

The results were similar to those obtained with dl-amphetamine but not so marked (See Fig. 30). It was, therefore, decided that this experiment and further experiments should be repeated using a pretreatment time of 6 days. The results were now very similar to those obtained with dl-amphetamine (See Fig. 31).

Fencamfamin :-

Groups of 10 female mice were pretreated for 6 days with lynestrenol or mestranol and on the 7th day they received fencamfamin (20 mg/kg) intraperitoneally and their temperatures measured at intervals for the next 4 hr.

Mestranol pretreatment induced fencamfamin to become hyperthermic (a rise of 1.85^{o}C was noted at 2 hr), an effect not produced at this dose level in untreated animals. Lynestrenol was without effect. (See Fig. 32). It was also noted that 1 hr after fencamfamin injection the mestranol-pretreated animals were far more excited than the control animals. This took the form of fighting and vocalisation.

The observed mestranol potentiation of hyperthermia with dl-amphetamine and dexamphetamine, and the induced hyperthermia with fencamfamin may be due to blockade of drug metabolism in the liver and/or monoamine oxidase inhibition affecting biogenic amines.

If the potentiating effect of mestranol was due to an

inhibition of MAO activity, whether of central and/or peripheral origin, a similar effect should be produced by nialamide. Nialamide (60 mg/kg) was administered to groups of 10 female mice intraperitoneally 4 hr prior to the stimulant drug; control groups received saline (5.0 ml/kg) prior to receiving the stimulant drug. The hyperthermic effect of dexamphetamine (10 mg/kg) was potentiated whilst fencamfamin (20 mg/kg) was induced to become hyperthermic (See fig. 33).

The effect of pretreatment with SKF 525A (50 mg/kg) intraperitoneally 90 min before receiving the stimulant drug, was tested in groups of 10 female mice; control mice received saline (5.0 ml/kg) prior to receiving the stimulant drug. SKF 525A, a known liver microsomal enzyme inhibiter (See Chapter II, page 125), failed to reproduce the potentiation of hyperthermia seen in animals pretreated with mestranol and nialamide. (See Fig. 34).

Pretreatment with the central amine depletor tetrabenazine, which was found to be most effective in producing hypothermia when injected as a pH 1.5 solution (See Fig. 35), completely blocked dexamphetamine (10 mg/kg) hyperthermia. Dexamphetamine was administered intraperitoneally 90 min after tetrabenazine (30 mg/kg) * also administered intraperitoneally. Reserpine, which depletes both central and peripheral biogenic amines is known to reduce dexamphetamine toxicity possibly by reducing hyperthermia (Mennear & Rudzik, 1966). Thus, administration of amine depletors had mimicked lynestrenol pretreatment by

*Tetrabenazine probably has peripheral amine depleting properties at this high dose level. Thus, the hypothermia and amphetamine hyperthermia blockade may be peripherally mediated.

blocking dexamphetamine hyperthermia. This is in accordance with the postulate that lynestrenol increases MAO activity.

SECTION II

Effects of lynestrenol or mestranol pretreatment in female mice on the increased locomotor activity induced by stimulant drugs

Dexamphetamine :-

Groups of 5 female mice were pretreated with lynestrenol (10 mg/kg) or mestranol (1.0 mg/kg) or their oily vehicle (controls) for 6 days. On the 7th day they received dexamphetamine (5.0 mg/kg). The spontaneous locomotor activity was measured during the duration of activity of the drug: approximately 6 hr for dexamphetamine and 4 hr for fencamfamin. Activity counts were recorded by a pen recorder. The average total counts from 3 experiments are shown in Table 32 and half-hourly counts for one experiment are shown in Fig. 36.

Lynestrenol pretreatment reduced the duration of action of the drug while mestranol increased it. The average total count of lynestrenol-pretreated mice in 3 experiments was less than those recorded from 3 control experiments. The average total count of mestranol-pretreated mice in 3 experiments was not dissimilar to the controls.

Fencamfamin:-

Sex steroid-pretreated mice received fencamfamin (10 mg/kg)

and the spontaneous locomotor activity was measured for the duration of activity of the drug. Again, the duration of action of fencamfamin was shortened by pretreatment with lynestrenol and also the total average count from 3 experiments was less than controls. In the mestranol-pretreated mice, both the duration of action of the drug and the total average count from 3 experiments were increased (See Table 33 and Fig. 37).

Pretreatment with tetrabenazine (30 mg/kg), intraperitoneally, in pH 1.5 solution 3o min prior to injection of the stimulant, totally abolished activity to fencamfamin (10 mg/kg) and dexamphetamine 5.0 mg/kg). (See Table 34).

Since a solution at this pH caused the animals a large degree of stress a different solvent for tetrabenazine was tried. Tetrabenazine (30 mg/kg) was dissolved in 10% dimethyl sulphoxide and injected intraperitoneally 30 min before dexamphetamine (5.0 mg/kg). (See Table 34). The initial locomotor activity of dexamphetamine was abolished but the effects lasted for less than 2 hr. Therefore, the pH of the tetrabenazine solution seemed to play an important part in the activity spectrum of tetrabenazine. Tetrabenazine has a high affinity for fat depots (Pfieffer et al, 1962) and pH changes may alter fat solubility.

Pretreatment with reserpine (2 mg/kg) for 1 hr, 4hr or 24 hr failed to block dexamphetamine (5.0 mg/kg) hyperactivity to any extent (See Table 35), during the 3 hr of observation. However, 4 hr pretreatment with reserpine did reduce the duration of action of dexamphetamine activity.

SECTION III

Effects of lynestrenol or mestranol pretreatment upon the acute toxicities of the stimulant drugs under crowded and uncrowded conditions

Acute toxicity under uncrowded conditions :-

Five groups of 8 mice were pretreated with lynestrenol, mestranol or their oily vehicle subcutaneously and used to determine the LD50 of dl-amphetamine, dexamphetamine and fencamfamin.

4 days pretreatment with the sex steroids failed to influence the LD50 dose of dl-amphetamine in groups of uncrowded mice.

Likewise 6 days pretreatment with the sex steroids failed to change the LD50 of either dexamphetamine or fencamfamin. It is of interest to note that the LD50 of dl-amphetamine and dexamphetamine are fairly similar by this route. (See Table 36).

Acute toxicity under crowded conditions :-

Five groups of 8 mice pretreated with lynestrenol, mestranol or their oily vehicle were used to determine the LD50 of dl-amphetamine, dexamphetamine and fencamfamin.

Six days pretreatment with mestranol (1.0 mg/kg) produced a significant increase in the toxicity of dexamphetamine compared with controls. The LD50 dose is calculated from the pooled results of 2 experiments. A similar

pretreatment with 10 mg/kg of lynestrenol failed to produce any significant differences in the LD50 of dexamphetamine. The LD50 dose is calculated from the pooled results of 2 experiments. (See Table 36).

Six days pretreatment with lynestrenol (10 mg/kg) or mestranol (1.0 mg/kg) did not alter the LD50 dose of fencamfamin in crowded conditions, when compared with the controls. In fact, crowding itself failed to produce any increase in the toxicity of fencamfamin. This is in marked contrast to dexamphetamine where a sevenfold increase in toxicity was observed. (See Table 36 and Fig. 38).

Monoamine oxidase inhibitors are known to potentiate amphetamine toxicity to a greater degree than fencamfamin (Brittain, Jack & Spencer, 1964) which agrees with these results assuming mestranol to have monoamine oxidase inhibitory activity. The absence of any protection with lynestrenol pretreatment, however, remains unexplained.

SECTION IV

Effects of lynestrenol or mestranol on mouse brain noradrenaline, dopamine and 5-hydroxytryptamine

The pooled brains of 6 groups of 8 mice pretreated with lynestrenol (10 mg/kg), mestranol (1.0 mg/kg) or vehicle (controls) for 6 days, were extracted and assayed for NA, DA and 5HT, by the method of Spencer and Turner (1969). The results are expressed as nanograms per gram of brain (See Table 37).

Lynestrenol slightly decreased the levels of nor-

adrenaline, dopamine and 5HT (between 10 and 25%, P = 0.1 - 0.05), whereas mestranol elevated the levels of 5HT only (by 35%, P = 0.1 - 0.05).

SECTION V

(1) Pressor responses due to fencamfamin, dexamphetamine and nor-adrenaline in anaesthetised female mice

Female TO mice, weighing approximately 25 g, were anaesthetised with a fluothane/O_2 gas mixture. The pressor drugs were administered intravenously in the tail vein. Pulse pressure changes were measured in the carotid artery via an arterial cannula (See Methods, page 58).

50 and 150 ng NA produced fairly consistent, but small, increases in arterial pressure in 6 experiments, (See Table 38). Both fencamfamin and dexamphetamine were pressor in large doses ranging from 15 - 100 ug. In one experiment the pressor responses were equivalent whilst in another the pressor response to fencamfamin (30 ug) was approximately equal to that of half the dose of dexamphetamine (15 ug), (See Table 38).

(2) Tachyphylaxis to fencamfamin and cross-tachyphylaxis to dexamphetamine in anaesthetised female mice

Tachyphylaxis to both high and low doses of fencamfamin was demonstrated. In the same experiment a slight cross tachyphylaxis to a low dose of dexamphetamine was demonstrated (See Fig. 39).

During the course of these experiments it was observed

that dexamphetamine was far more liable to produce heart block than fencamfamin.

Fencamfamin was found to be half to one times as pressor as dexamphetamine in mice in contrast to the minimal pressor effects found in rats (Hotovy et al, 1961). Tachyphylaxis to fencamfamin plus a slight cross tachyphylaxis to dexamphetamine suggest that fencamfamin pressor effects are mediated through peripheral catecholamines. This is in agreement with the results of Hotovy and co-workers, (1961).

DISCUSSION

The results in this Chapter demonstrate that both mestranol and lynestrenol alter quantitatively the response of female mice to the activity of certain stimulant drugs. Again, as in Chapter II, Part 1, a quantitative difference between the mestranol- and lynestrenol-pretreated animals was observed since the direction of their effects upon the activity of the stimulant drugs was opposite. The fact that these steroids are physiologically antagonistic may have a bearing on these results. It was found necessary to double the steroid dosage and extend the pretreatment time from 4 to 6 days in order to obtain significant and reproducible effects. Possibly this was necessary because the steroids were affecting the mediators (biogenic amines) of the stimulant drug activity rather than a direct action upon the drug itself, a phenomenon which might require a longer period of time.

Mestranol increased whilst lynestrenol reduced the

hyperthermia induced by dl-amphetamine (20 mg/kg) and dexamphetamine (10 mg/kg); fencamfamin (20 mg/kg) failed to induce hyperthermia in control and lynestrenol pretreated mice, but did induce hyperthermia after mestranol pretreatment. The duration of locomotor hyperactivity induced by dexamphetamine (5 mg/kg) and fencamfamin (10 mg/kg) was potentiated by mestranol and reduced by lynestrenol. The intensity of hyperactivity was similarly affected but not so marked.

In uncrowded conditions the acute toxicities of dl-amphetamine, dexamphetamine and fencamfamin remained unaltered by steroid pretreatment. Under crowded conditions the usual increases (Chance, 1946) in dl-amphetamine and dexamphetamine toxicities were still further increased by mestranol but remained unchanged after lynestrenol (in one experiment lynestrenol conferred some protection against crowding toxicity). Crowding was shown to increase the toxicity of fencamfamin only marginally; steroid pretreatment was without effect on its toxicity in crowded conditions. Whole brain levels of DA, NA and 5HT were determined by the method of Spencer and Turner (1969) after pretreatment with steroid for 6 days. The levels of each amine were slightly reduced with lynestrenol ($P = 0.1 - 0.05$) while 5HT was increased with mestranol ($P = 0.1 - 0.05$).

There are several possible explanations of these steroid-induced changes of stimulant activity of these drugs. They may alter the activity of the drug metabolising microsomal enzymes of the liver as occurred with the barbiturates (Chapter II, Part 1). However, pretreatment with SKF 525A, which inhibits the amine oxidases which metabolize

amphetamine in the liver failed to mimic the potentiating effect of mestranol upon hyperthermia. It is unlikely that fencamfamin metabolism would be markedly affected since in rodents 50% of it is excreted unchanged in the urine (Hotovy, et al, 1961). If the actions of dl-amphetamine, dexamphetamine and fencamfamin are due predominantly to the release of endogenous amines (peripherally and/or centrally), as the wealth of evidence suggests, then an increase (with lynestrenol) or a decrease (with mestranol) of tissue MAO activity should change the potency of these drugs. Evidence from the literature (Kuwabara et al, 1967; Southgate et al, 1968; Grant & Pryse Davies, 1968) supports the contention that lynestrenol (progestin) increases MAO activity. Indirect experimental evidence points to mestranol having MAO inhibitory activity since its potentiating effects upon hyperthermia were mimicked by pretreatment with the MAO inhibitor, nialamide.

Hotovy and co-workers, (1961) showed that the peripheral pressor effects of fencamfamin are weaker than those of amphetamine in cats. The results obtained in blood pressure experiments in mice are in agreement with their work. The toxic effects of dexamphetamine upon the mouse heart appeared to be greater than those of fencamfamin. Also fencamfamin induces hyperactivity (thought to be centrally mediated, Weissman & Koe, 1965; Weissman et al, 1966; Dingell et al, 1967) without hyperthermia (thought to be peripherally mediated, Gessa, Clay & Brodie, 1969). Also the hyperactivity of both dexamphetamine- and fencamfamin-treated mice was blocked by the central amine depletor tetrabenazine, but not by the general amine depletor,

reserpine. A possible explanation is that guanethidine (and prenylamine), like tetrabenazine, is able to deplete central amine stores controlling locomotor activity which are resistant to reserpine (Pfeiffer et al, 1968).

Thus, the peripheral effects of dl-amphetamine and dexamphetamine would be most affected and those of fencamfamin least affected by steroid pretreatment. The data reported in the results supports this. Mestranol potentiated dl-amphetamine and dexamphetamine induced hyperthermia and aggregation toxicity (enhanced by hyperthermia; Askew, 1962; Clark et al, 1967) to a greater degree than those induced by fencamfamin. However, the increased locomotor activity induced by all three stimulants was affected similarly by steroid pretreatment.

The effects of lynestrenol and mestranol upon brain amine levels may be a reflection of their effects upon MAO activity. However, it is possible that steroid-induced changes occur in the amine content of aminergic neurons which control the anterior pituitary, and which are sensitive to hormonal levels (See Introduction, page 28). Goldberg and Salama (1969) showed that under certain stressful conditions, there is a 13 - 15% rise in rat brain 5HT levels which is accompanied by an increase in amphetamine toxicity. Since there is also a rise in mouse brain 5HT when pretreated with mestranol, this might provide an alternative explanation of the increased amphetamine toxicity in these animals.

The changes in the activity spectrum of "three" stimulant drugs induced by lynestrenol and mestranol may be due to changes in tissue MAO activity and/or brain amine content. This further supports the theory that stimulant

drugs exert their activities through amine release rather than through a direct mechanism.

30 min LOCOMOTOR ACTIVITY COUNTS FOR THE DURATION OF ACTION OF DEXAMPHETAMINE

Time	Lyn	Lyn	Lyn	Cont	Cont	Cont	Mest	Mest	Mest
0.5	2500	2500	2600	2900	2800	2700	2700	2900	2500
1	2600	2300	2200	2700	2500	2600	2900	2500	2700
1.5	2500	2500	2300	2700	2200	2500	2700	2400	2900
2	2800	2500	2200	2600	2000	2300	2500	2700	2000
2.5	2300	2400	2100	2800	2200	2100	2600	2600	2900
3	1900	1900	1900	2300	2200	1800	2400	2400	2200
3.5	1300	1600	2200	1500	2200	1800	2400	1500	2600
4	400	1500	2000	900	2400	1600	1900	1600	1400
4.5	100	600	800	500	1900	1400	1800	1400	1300
5	100	300	400	200	1900	1100	1700	1500	400
5.5	-	-	-	100	1100	900	1300	1300	600
6				100	800	600	1000	1100	300
6.5				-	300	700	700	1000	600
7					-	100	400	900	700
7.5						-	200	600	300
8							100	900	100
8.5							-	300	-
Totals	16500	18100	18700	19700	24500	22200	27800	27600	23600

TABLE 32

Dexamphetamine (5 mg/kg) was administered intraperitoneally on the seventh day of experiment, 18 hr after the sixth injection of steroid. The results are from 3 separate experiments.

30 min LOCOMOTOR ACTIVITY COUNTS FOR THE DURACTION OF ACTION OF FENCAMFAMIN

Lyn	Lyn	Lyn	Cont	Cont	Cont	Mest	Mest	Mest
2,200	2,700	2,900	2,500	3,300	2,300	2,800	1,800	2,000
3,300	2,900	1,700	2,600	3,500	2,500	2,900	2,100	1,800
1,000	900	400	2,000	2,100	1,800	2,300	2,000	1,900
600	700	400	1,500	1,300	1,400	2,000	2,000	2,000
100	300	400	1,400	800	1,400	1,800	1,400	1,800
100	400	-	200	300	800	1,700	2,100	2,000
100	300	-	200	200	600	1,000	2,300	1,500
-	100	-	100	-	300	1,200	2,300	1,200
	-		100		-	300	1,900	500
			-			-	1,500	200
							1,300	100
							700	100
7,400	8,300	5,800	9,700	11,500	11,100	16,000	21,900	15,100

TABLE 33

Fencamfamin (10 mg/kg) was administered intraperitoneally on the seventh day of experiment, 18 hr after the sixth injection of steroid. The results are from 3 separate experiments.

30 min LOCOMOTOR ACTIVITY COUNTS						
Time	Tbz in pH 1.5 HCl & Dex	pH 1.5 HCl & Dex	Tbz in pH 1. 5 HCl & Fencam.	pH 1.5 HCl & Fencam.	Tbz in 10% DMSO & Dex	10% DMSO & Dex.
0.5	600	2,600	200	2,300	200	2,500
1	400	2,900	200	2,500	800	2,900
1.5	300	2,500	200	1,800	1,100	2,600
2	400	2,100	300	1,400	1,100	2,700
2.5	300	2,500	200	1,400	1,400	2,300
3	100	2,500	200	800	1,900	2,300
3.5	300	1,500	-	600	2,300	2,400
4	400	1,000	100	300	2,000	1,500
4.5	300	700	-	-	1,800	1,000
5	100	300	-	-	700	700
5.5	-	100	-	-	300	200
Total	3,200	19,100	1,400	11,100	13,600	21,100

TABLE 34

Dexamphetamine (5 mg/kg) or fencamfamin (10 mg/kg) was administered 0.5 hr after tetrabenazine (30 mg/kg) had been injected intraperitoneally; controls received the vehicle (10 ml/kg) intraperitoneally 0.5 hr before the stimulant.

30 min LOCOMOTOR ACTIVITY COUNTS						
	Reserpine 1 hr pre-treatment	Control Saline	Reserpine 4 hr pre-treatment	Control Saline	Reserpine 24hr pre-treatment	Control Saline
0.5	3,600	2,600	2,100	3,400	2,200	2,900
1	1,600	2,500	2,300	2,100	2,800	2,700
1.5	1,400	2,500	2,200	1,800	2,600	2,200
2	1,100	3,000	1,400	2,400	2,100	2,300
2.5	1,200	3,000	700	2,700	1,500	1,900
3	1,200	3,000	200	1,800	1,000	1,700

TABLE 35

Dexamphetamine (5 mg/kg) was administered at various times after reserpine (2 mg/kg) had been injected intraperitoneally; controls received saline (10 ml/kg) intraperitoneally at similar times before the stimulant.

ACUTE TOXICITIES mg/kg

(Confidence limits for 95% probability)

Stimulant	Crowded			Uncrowded		
	Lyn	Control	Mest	Lyn	Control	Mest
Fencamfamin	48 (41.7-55.2)	52 (46.4-58.2)	50 (43.1-58.0)	56 (49.1-63.8)	53 (46.9-59.9)	55 (49.0-63.3)
Dexamphetamine	14 (10.5-18.6)	14 (11.2-17.5)	*7.4 (5.7-9.6)	94 (82.5-107.2)	95 (89.2-114)	100 (83.3-120)
dl-amphetamine	21 (14.5-30.4)	19.5 (13.5-28.3)	17 (11.3-25.5)	145 (120.8-174)	139 (111.3-173.8)	137 (114.2-164.4)

TABLE 36

The stimulant drug was administered intraperitoneally on the seventh day of experiment (except dl-amphetamine: fifth day), 18 hr after the last injection of steroid. The results are calculated from 5 groups of 8 mice for each pretreatment.

* Significantly different from relevant controls ($P = < 0.05$)

Drug	Dose	Pressor responses mm. Hg.	Av. mm. Hg. (± S.E.)
NA	50 ng	14, 14, 16, 11, 12, 16	13.8 ± 0.84
NA	150 ng	16, 18, 19, 19, 16, 20	18.0 ± 0.68
Dexamphetamine	30 ug	6 mm)	same experiment
Fencamfamin	30 ug	5 mm)	
Dexamphetamine	15 ug	17 mm)	same experiment
Fencamfamin	30 ug	14 mm)	

TABLE 38

Pressor responses of anaesthetised female mice due to fencamfamin, dexamphetamine or nor-adrenaline.

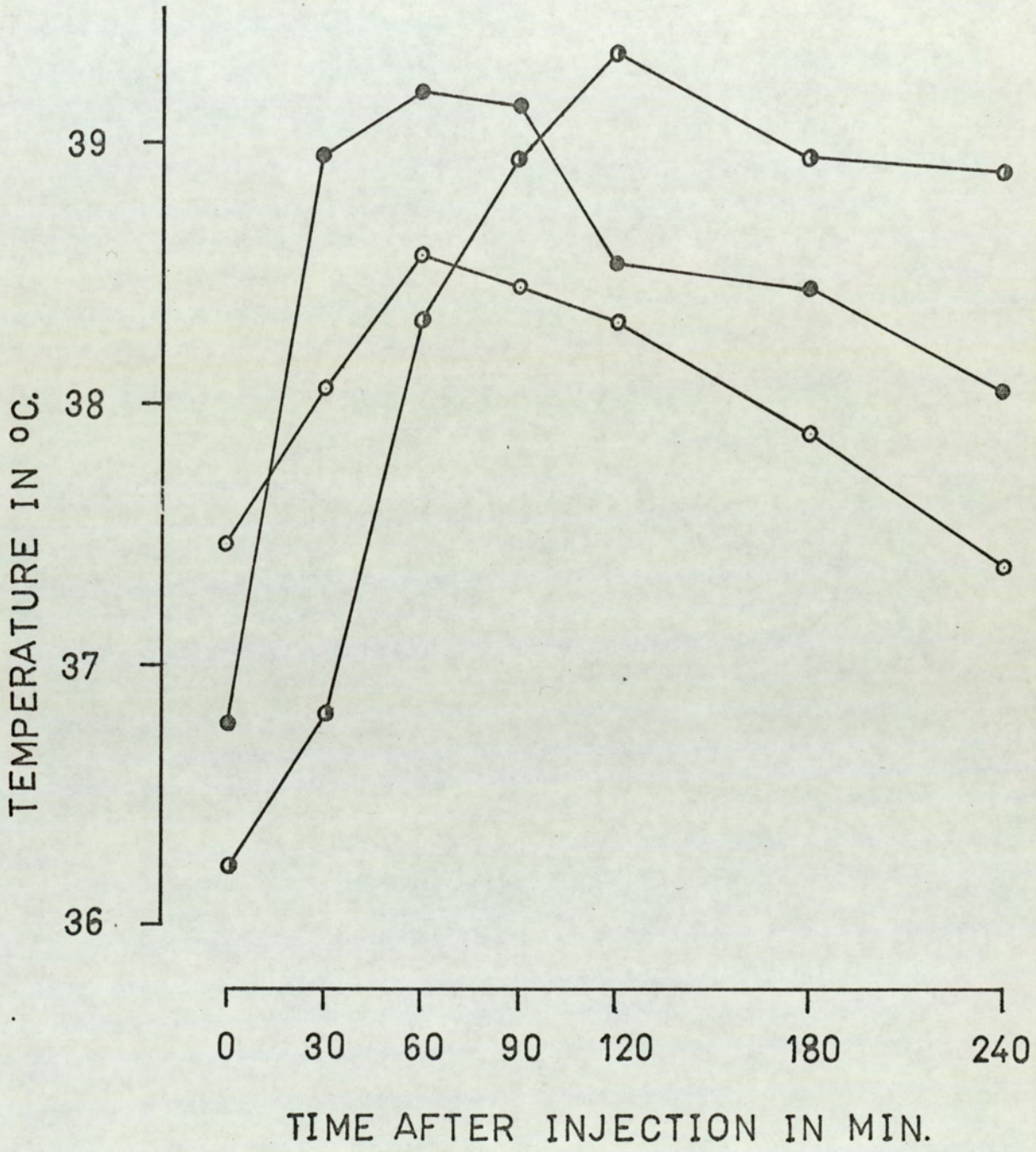


Fig. 29

Effect of mestranol or lynestrenol on dl-amphetamine-induced hyperthermia in female mice. Animals were pretreated with mestranol (0.5 mg/kg, daily) subcutaneously for 4 days (●——●); or lynestrenol (5.0 mg/kg, daily) subcutaneously for 4 days (○——○); or vehicle only (5.0 ml/kg, daily ●——●). On the fifth day they received an intraperitoneal injection of dl-amphetamine (20 mg/kg) and their oesophageal temperatures measured over the next 4 hr.

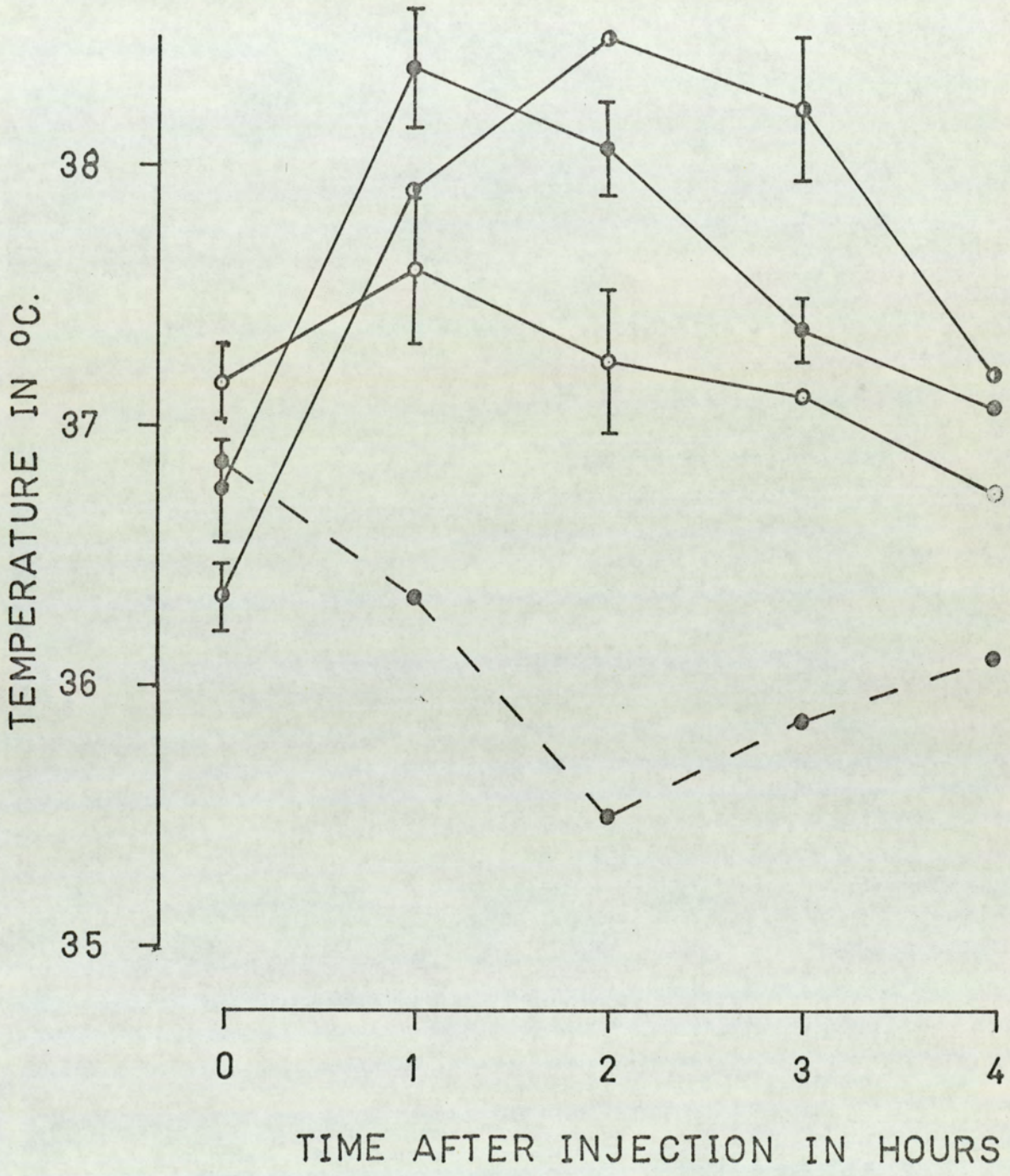


Fig. 30

Effect of mestranol or lynestrenol on dexamphetamine-induced hyperthermia in female mice. Animals were pretreated with mestranol (1.0 mg/kg, daily) subcutaneously for 4 days (●——●); or lynestrenol (10 mg/kg, daily) subcutaneously for 4 days (○——○); or vehicle only (5.0 ml/kg, daily ●——●). On the fifth day they received an intraperitoneal injection of dexamphetamine (10 mg/kg) and their oesophageal temperatures measured over the next 4 hr.

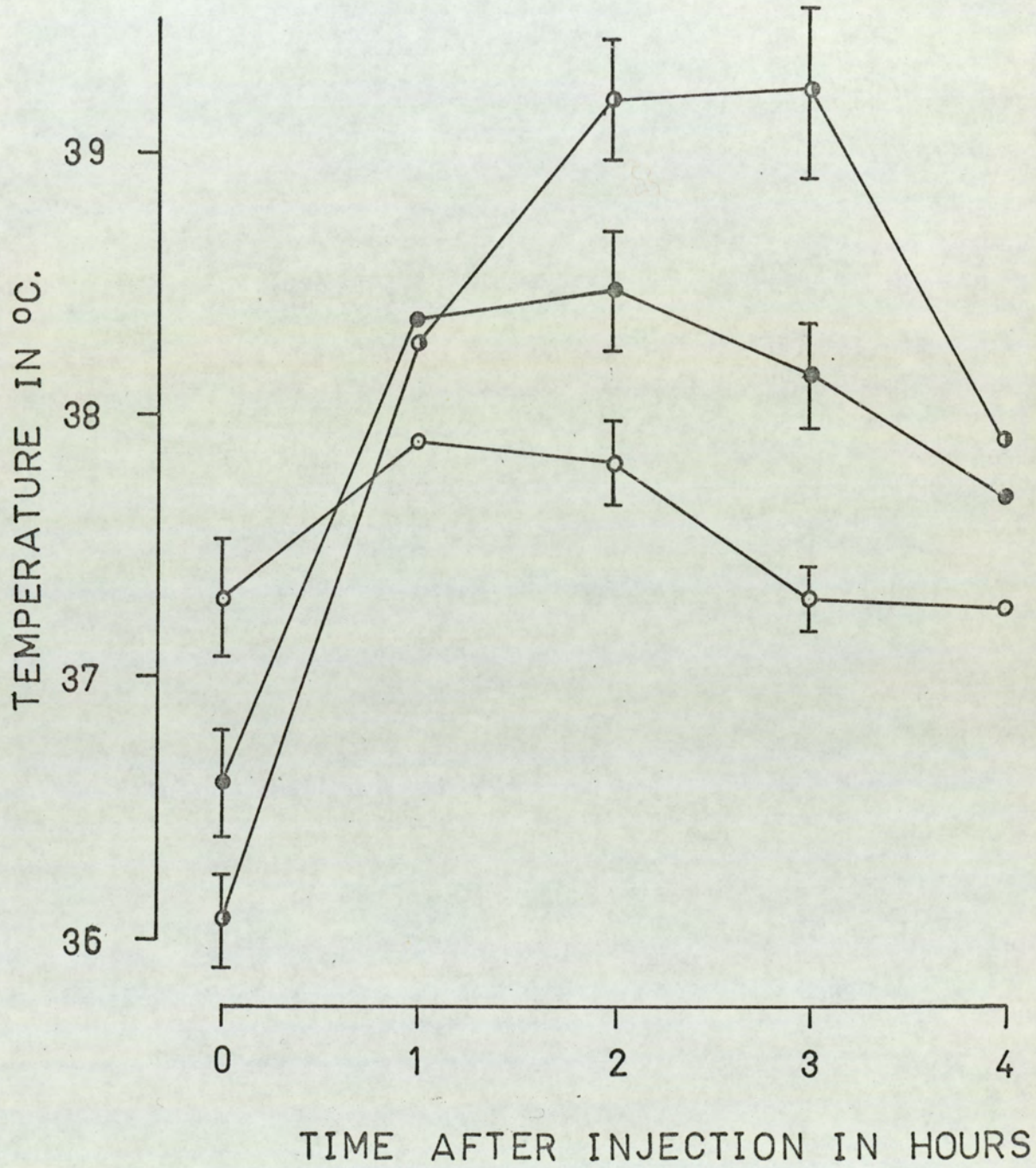


Fig. 31

Effect of mestranol or lynestrenol on dexamphetamine-induced hyperthermia in female mice. Animals were pretreated with mestranol (1.0 mg/kg, daily) subcutaneously for 6 days (●——●); or lynestrenol (10 mg/kg, daily) subcutaneously for 6 days (○——○); or vehicle only (5.0 ml/kg, ●——● daily). On the seventh day they received an intraperitoneal injection of dexamphetamine (10 mg/kg) and their oesophageal temperatures measured over the next 4 hr.

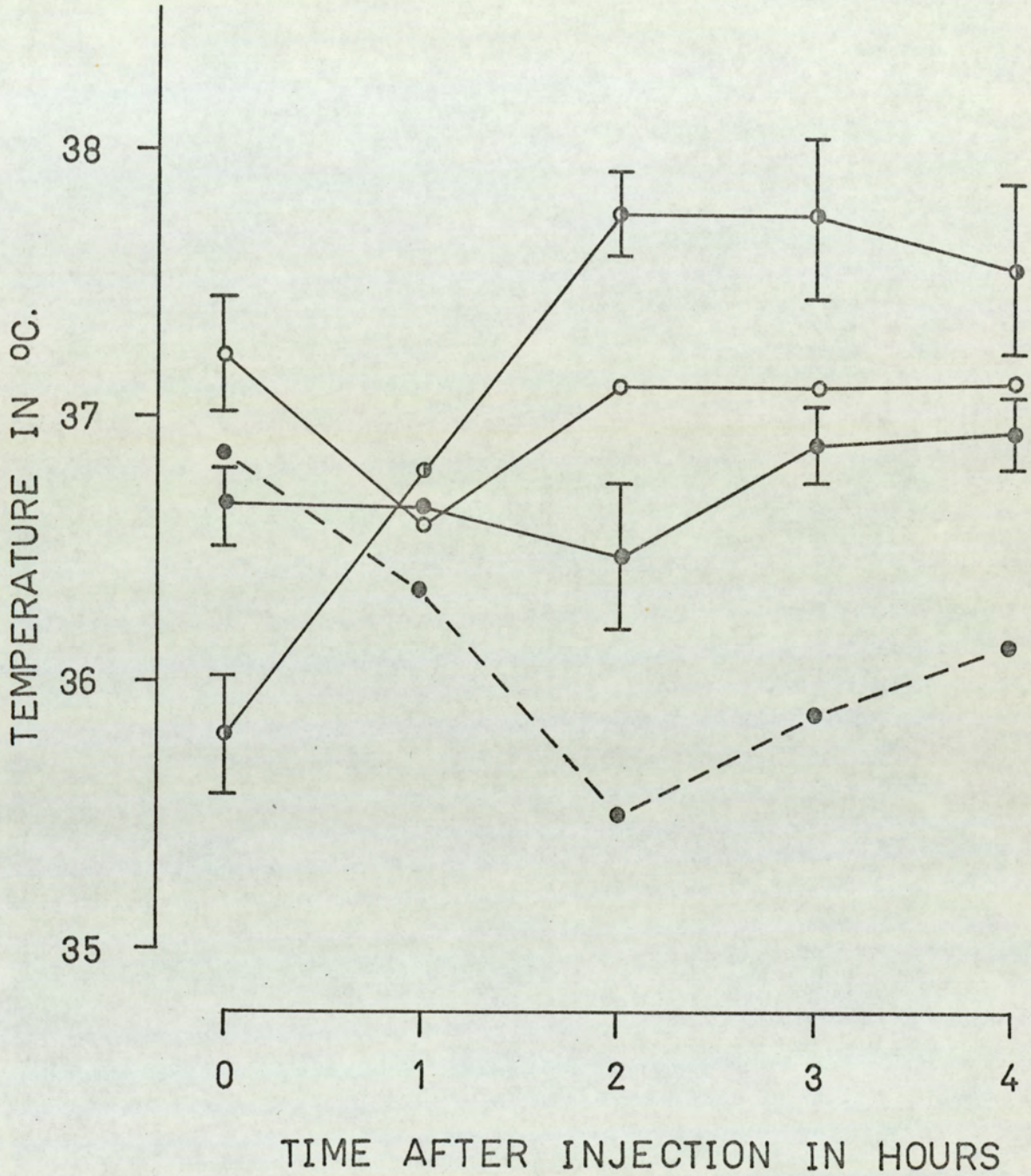


Fig. 32

Effect of mestranol or lynestrenol on body temperature after fencamfamin in female mice. Animals were pre-treated with mestranol (1.0 mg/kg, daily) subcutaneously for 6 days (◐——◐); or lynestrenol (10 mg/kg, daily) sub-cutaneously for 6 days (○——○); or vehicle only (5.0 ml/kg, daily ●——●). On the seventh day they received an intra-peritoneal injection of fencamfamin (20 mg/kg) and their oesophageal temperatures measured over the next 4 hr.

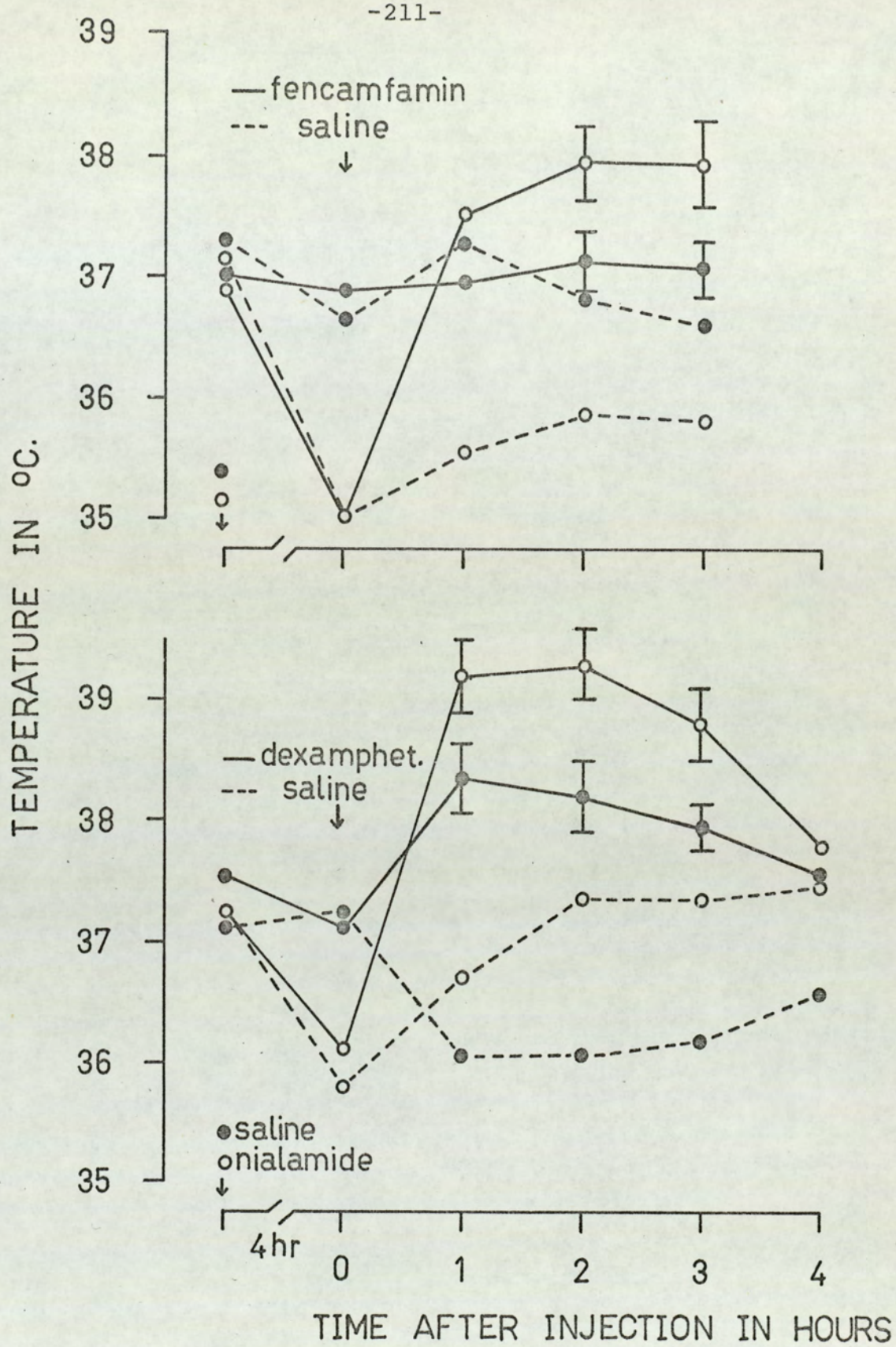


Fig. 33

Effect of pretreatment with nialamide on the hyperthermic response of female mice to dexamphetamine or fencamfamin (if any). Animals were pretreated with nialamide (60 mg/kg) intraperitoneally 4 hr before receiving an intraperitoneal injection of dexamphetamine (10 mg/kg) or fencamfamin (20 mg/kg). Their oesophageal temperatures were measured over the next 4 hr.

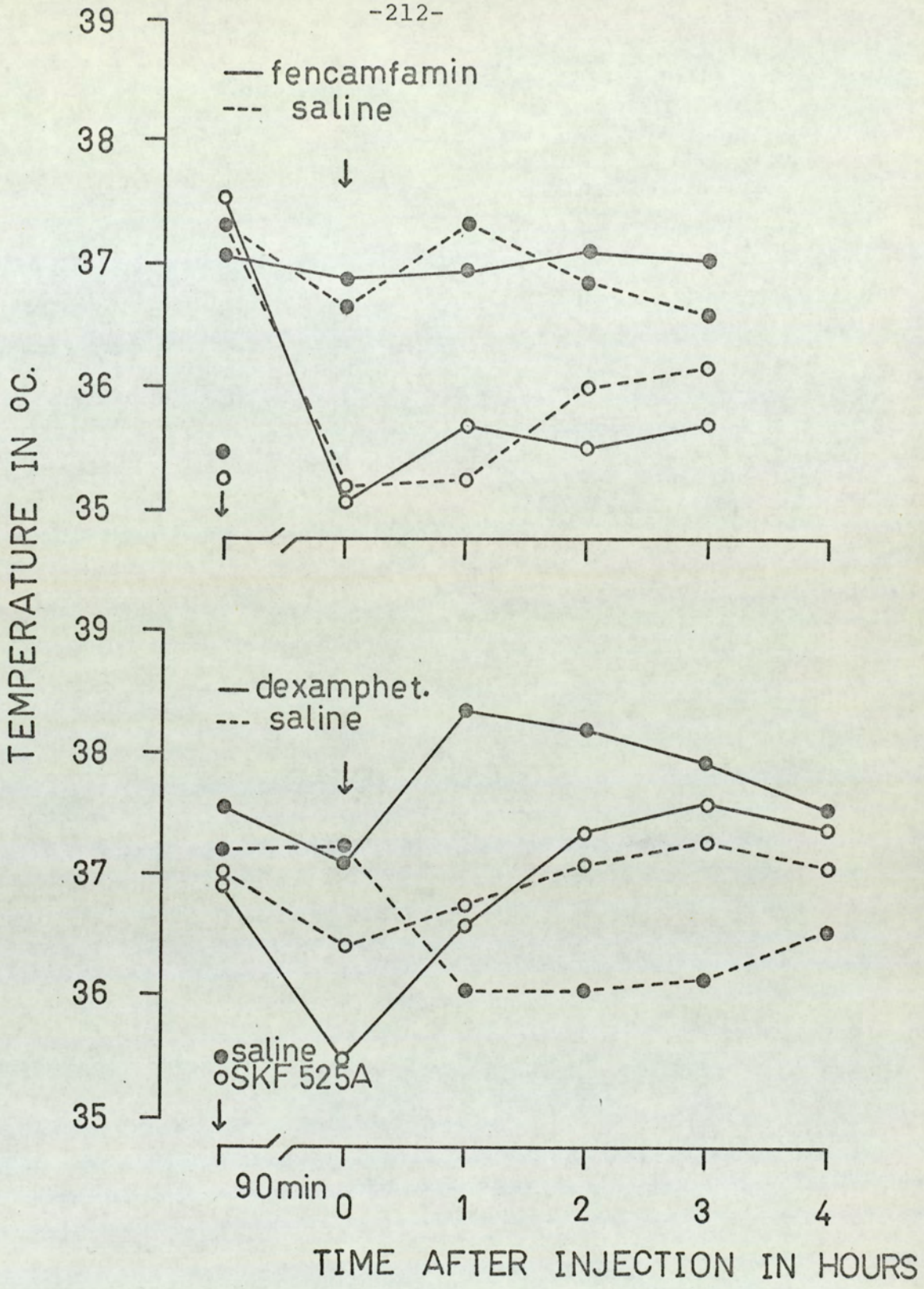


Fig. 34

Effect of pretreatment with SKF 525A on the hyperthermic response of female mice to dexamphetamine or fencamfamin (if any). Animals were pretreated with SKF 525A (50 mg/kg) intraperitoneally 90 min before receiving an intraperitoneal injection of dexamphetamine (10 mg/kg) or fencamfamin (20 mg/kg). Their oesophageal temperatures were measured over the next 4 hr.

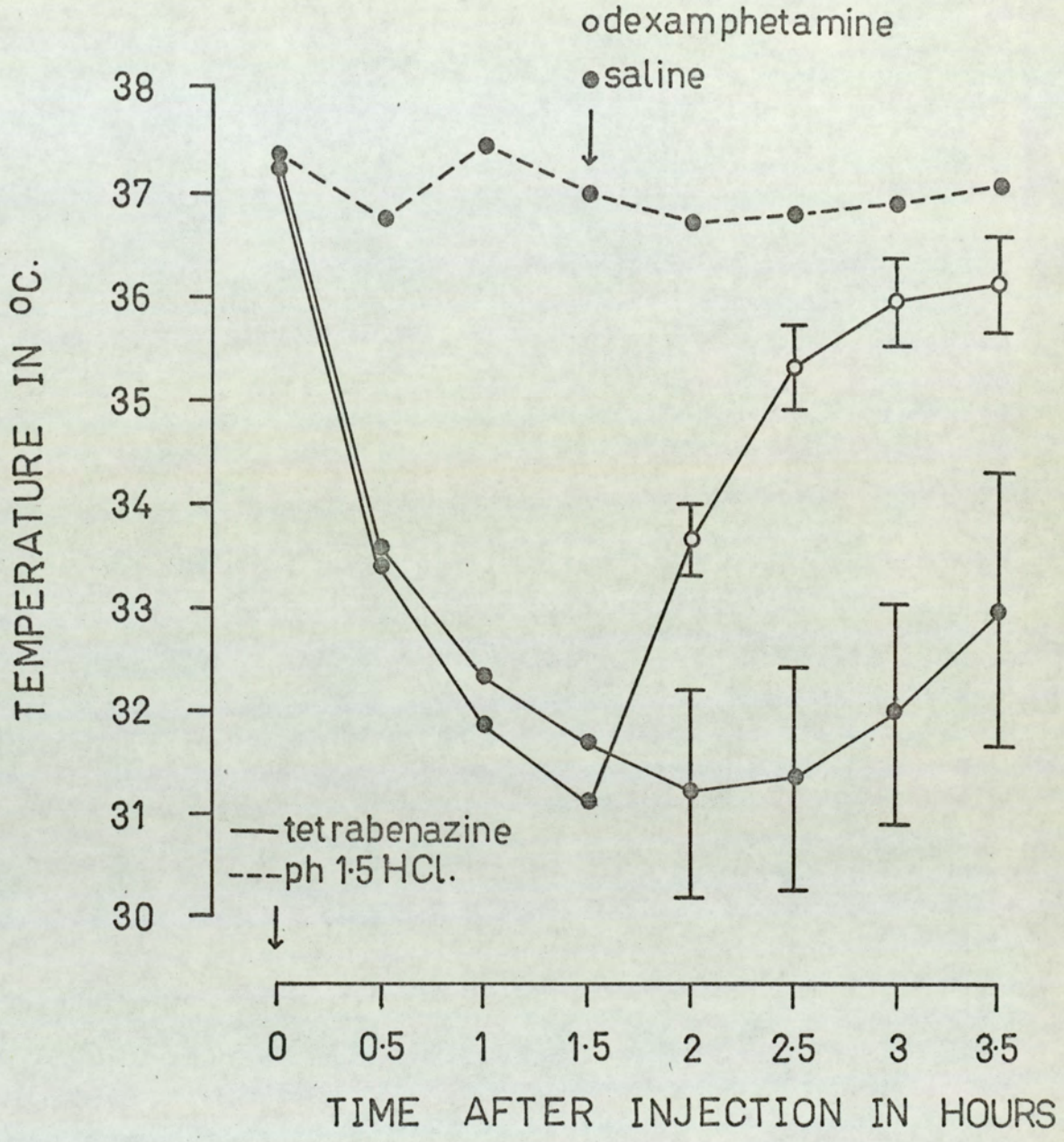


Fig. 35

Antagonism of dexamphetamine hyperthermia by tetrabenazine in female mice. Dexamphetamine (10 mg/kg) was administered 90 min after tetrabenazine (30 mg/kg); both drugs were injected intraperitoneally.

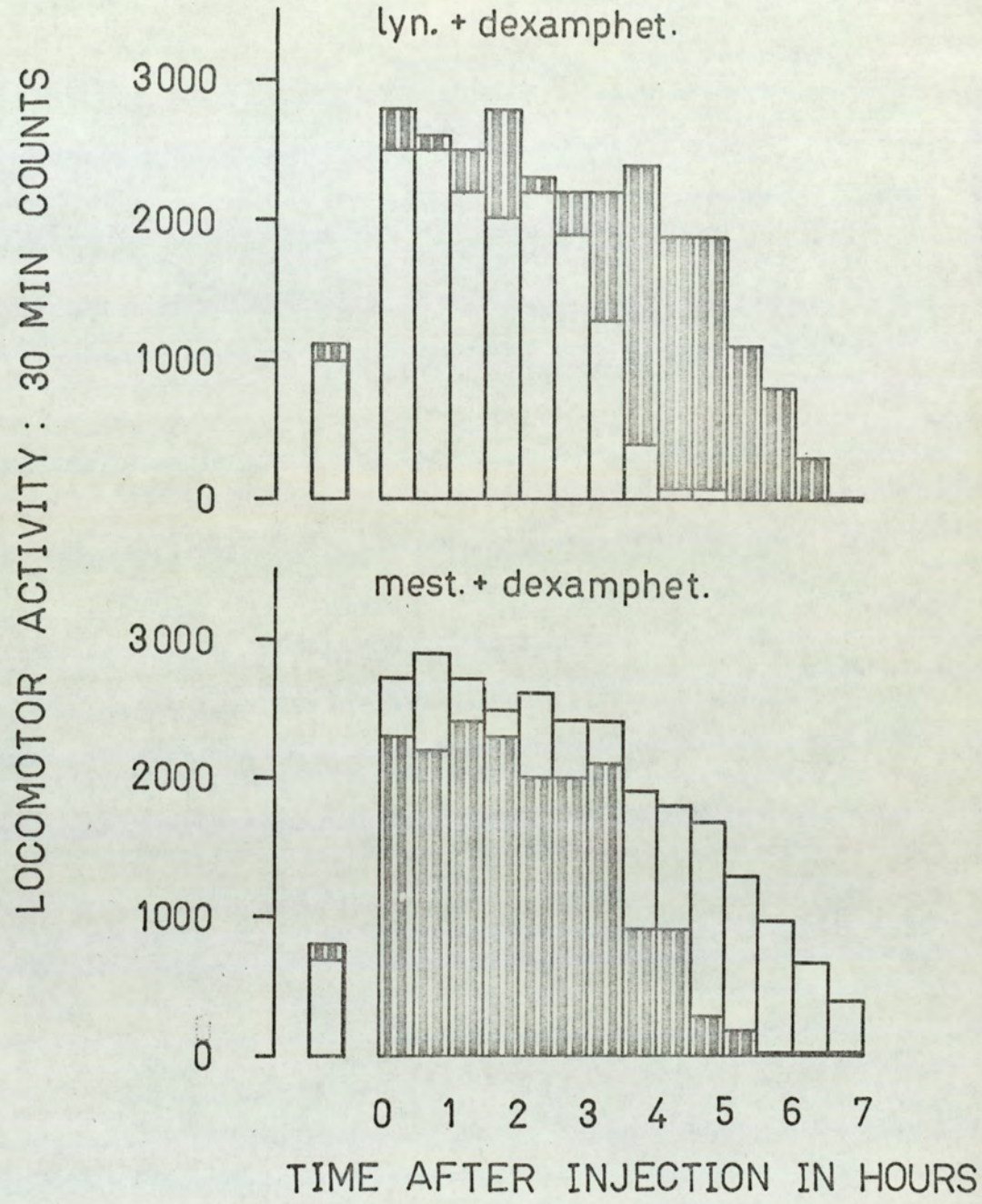


Fig. 36

Effect of mestranol or lynestrenol on dexamphetamine-induced locomotor activity in female mice. Animals were pretreated with mestranol (1.0 mg/kg, daily) subcutaneously for 6 days (open bars); or lynestrenol (10 mg/kg, daily) subcutaneously for 6 days (open bars); or vehicle only (5.0 ml/kg, daily, closed bars). On the seventh day they received an intraperitoneal injection of dexamphetamine (5.0 mg/kg) and ½ hr activity counts then taken for the duration of drug activity.

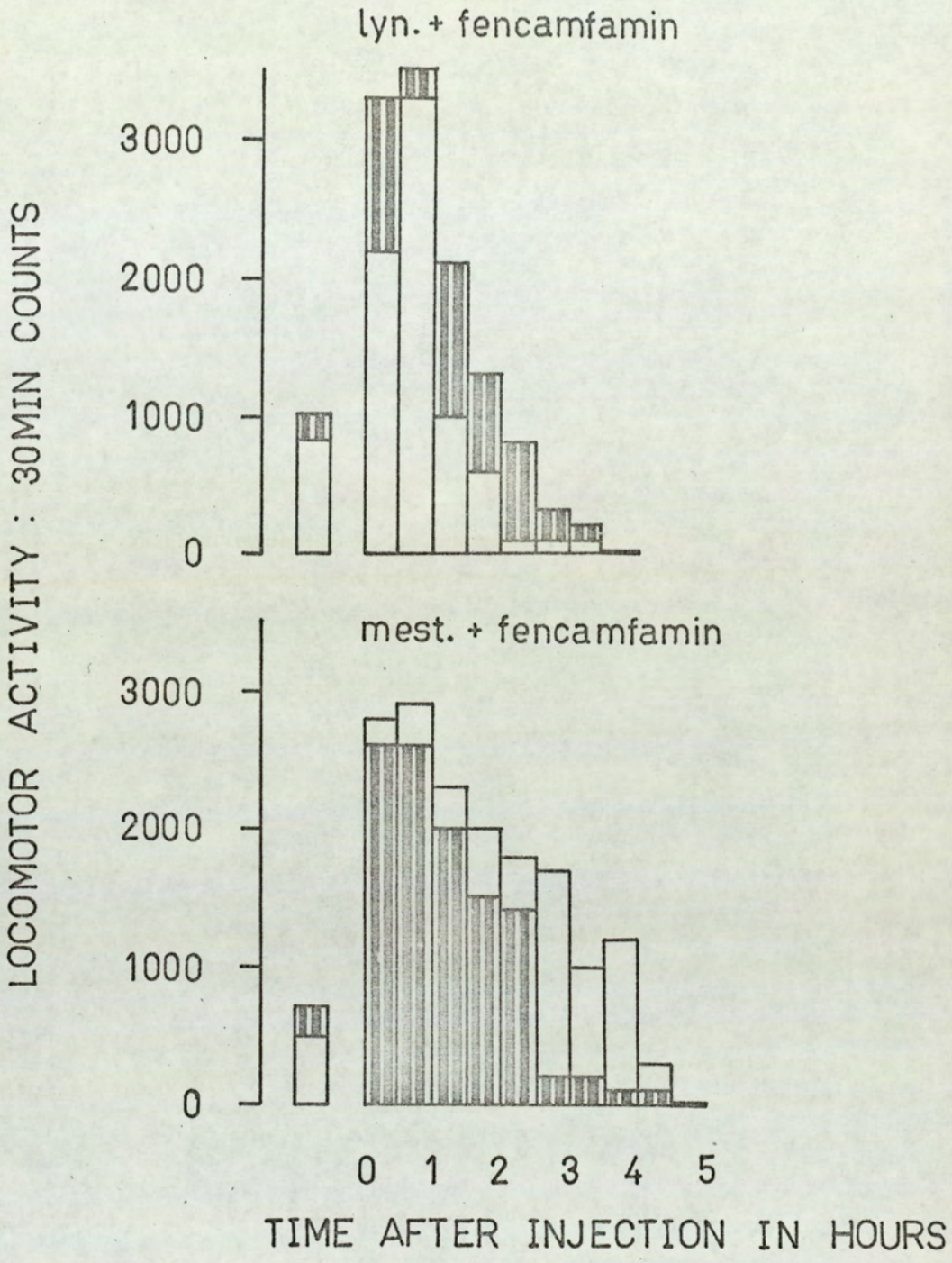


Fig. 37

Effect of mestranol or lynestrenol on fencamfamin-induced locomotor activity in female mice. Animals were pretreated with mestranol (1.0 mg/kg, daily) subcutaneously for 6 days (open bars); or lynestrenol (10 mg/kg, daily) subcutaneously for 6 days (open bars); or vehicle only (5.0 ml/kg, daily, closed bars). On the seventh day they received an intraperitoneal injection of fencamfamin (10mg/kg) and ½ hr activity counts taken for the duration of drug activity.

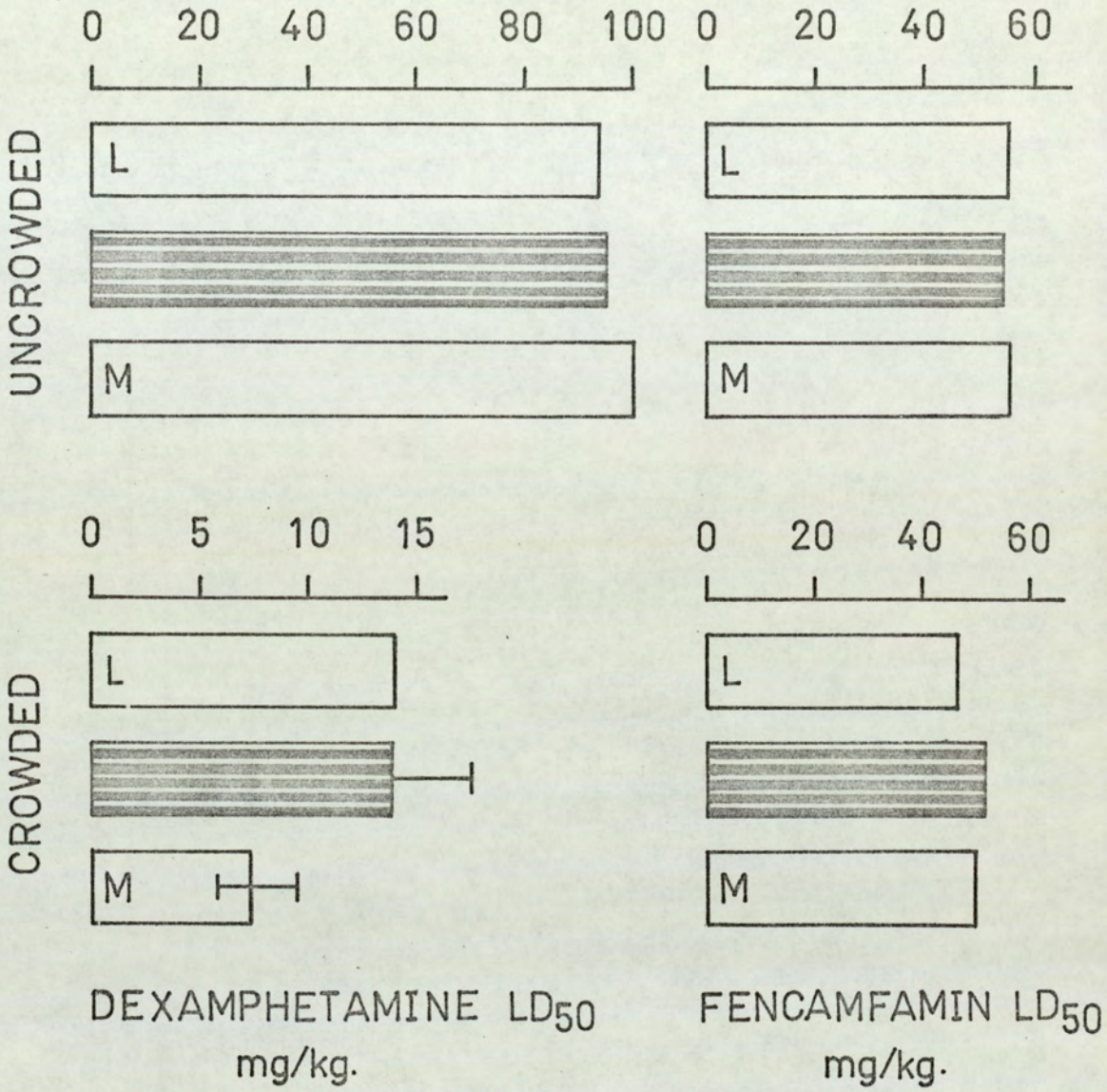


Fig. 38

Effect of mestranol (M) or lynestrenol (L) on crowded and uncrowded toxicity in mice after dexamphetamine or fencamfamin. Animals were pretreated with mestranol (1.0 mg/kg, daily) subcutaneously for 6 days; or lynestrenol (10 mg/kg, daily) subcutaneously for 6 days; or vehicle only (5.0 ml/kg, daily, closed bars). On the seventh day they received various doses of dexamphetamine or fencamfamin.

	Mestranol	Control	Lynestrenol
NA	314ng ± 7·8	329ng ± 14·7	287ng ± 9·6
DA	348ng ± 21·2	396ng ± 17·5	304ng ± 9·4
5 HT	396ng ± 24·5	295ng ± 9·2	245ng ± 9·5

Table 37

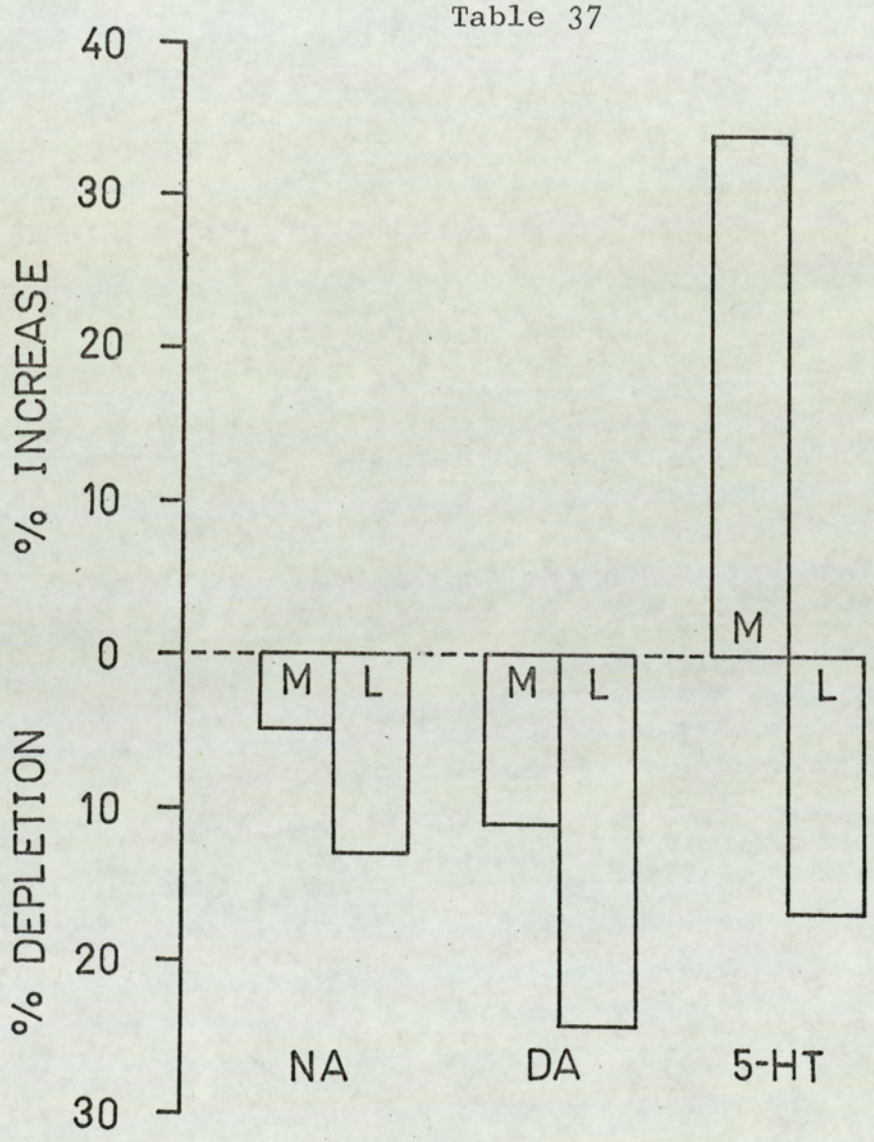


Fig. 39

Effect of mestranol (M) or lynestrenol (L) pretreatment on brain amine levels in female mice. Animals were pretreated with mestranol (1.0 mg/kg, daily) subcutaneously for 6 days; or lynestrenol (10 mg/kg, daily) subcutaneously for 6 days; or vehicle only (5.0 ml/kg, daily,) On the seventh day they were sacrificed.

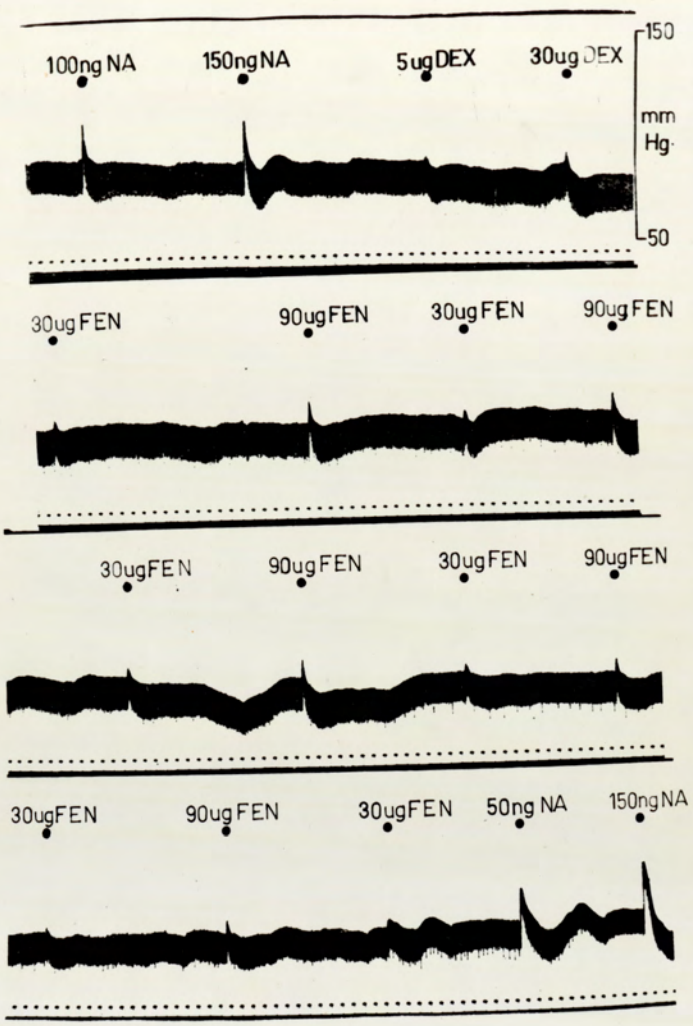


Fig. 40

Mouse blood pressure recording showing pressor responses to both fencamfamin and dexamphetamine. Tachyphylaxis to fencamfamin and cross-tachyphylaxis to dexamphetamine was demonstrated.

CHAPTER IV - SEX STEROIDS, CONVULSIONS AND ANTI-CONVULSANTS

Introduction :-

The site of discharge of an epileptic convulsion was assumed to be in the brain stem, from which may be initiated not only the motor but also the autonomic disturbances (Davis & Pollock, 1928). By occluding the basilar artery in the brain of the cat, these authors were able to perfuse the pons and medulla with picrotoxin, while the cerebrum received a normal blood supply. When the brain stem alone was stimulated by picrotoxin, Davis and Pollock (1928) produced a complete convulsion pattern including tonic and clonic seizures (the latter previously thought to be of cerebral origin).

However, when a similar technique was used to determine the convulsive site for leptazol (Jolly, Steinhaus et al, 1956), the higher centres were found to be much more sensitive to leptazol than the centres located in the pons and medulla oblongata.

Selye (1941) showed that DOCA and progesterone protected rats against leptazol convulsions and death. Since then other workers (Dashputra, Sharma & Rajapurkar, 1964; Hewett, Sarage, Lewis & Sugrue, 1964; Craig, 1966) have shown that natural and synthetic steroids possess anti-convulsant activity. They explained these effects

variously from being barbiturate-like to a direct effect upon neurons (See Introduction, page 20). Also Woolley and Timiras (1962 & 1962) demonstrated the importance of sex and sex steroids on brain excitability by measuring changes in EST in rats and mice of different sex or receiving different hormones. There are, therefore, theoretical grounds for suspecting that administration of oral contraceptive steroids to women with a history of epilepsy might need careful reconsideration (World Health Organization, 1966). This stimulated Espir, Walker and Lawson (1969) to do a controlled cross-over trial in 20 epileptic women receiving Norinyl 1. Their results showed that a low oestrogen/progestin combination pill had no significant effect on the frequency of fits compared with placebos. However, one of their patients showed increased susceptibility to the effects of the anti-convulsant drug, Primidone, which she was receiving. It was decided that the activity of oestrogen and progestin upon seizure susceptibility and the duration and intensity of anti-convulsant drugs needed to be further investigated.

The most commonly used anti-convulsant drugs in use today include phenobarbitone sodium, 5-5 diphenylhydantoin sodium (phenytoin sodium) and 'Tridione'. Although the mode of action of phenobarbitone sodium is not clear (thought to be widespread depression of the C.N.S.), the work of Knoeffel and Lehmann (1942) and Everett and Richards (1944) points to the nervous mechanism higher than the mesencephalon as the locus of anti-convulsant action for phenytoin sodium. The best method for testing anti-convulsant drugs is abolition of the tonic extensor component

of MES (according to Toman, Swinyard & Goodman, 1946) since all the clinically recognized anti-epileptic agents are effective in this test; whereas in the EST and leptazol tests phenytoin sodium and its derivatives are ineffective.

A probable explanation for the division of the anti-convulsant drugs into these two classes is that hydantoin derivatives excel in the inhibition of seizure spread but are relatively inactive in elevating EST; in contrast other drugs possess both mechanisms of anti-convulsant action (Goodman, Grewal, Brown & Swinyard, 1953).

It was shown by various workers (Rosenstein, 1960; Hernandez-Peon, 1964; & Banziger, 1965), that the benzodiazepine tranquilizers, chlordiazepoxide and diazepam have anti-convulsant activity. Hernandez-Peon (1964) showed that diazepam depressed after - discharges from the amygdala and hippocampus and this, therefore, might be the site of anti-convulsant activity.

Because of the clinical implications of female epileptics receiving steroid hormones together with anti-convulsant therapy it was decided to study the duration and intensity and duration of action of four anti-convulsant drugs mentioned above in sex steroid pretreated mice. The anti-convulsant activity of the drugs was measured using the MES test devised by Cashin and Jackson (1962) (See Methods, page 57) for the reasons stated above.

Before studying the effects of sex hormones on the anti-convulsant properties of certain centrally acting drugs it was necessary to examine the effects of sex and sex hormones on the response of female mice to electrical and

chemical seizures.

SECTION I

(1) Sensitivity of control mice to MES and leptazol

Tonic seizure threshold:

Groups of 10 female and male mice were subjected to increasing voltages until all 10 animals in both groups showed MES. The table (Table 40) shows that at 60 volts 10/10 females exhibit tonic extensor seizure but not until 70 volts do 10/10 males exhibit tonic extensor seizure.

Leptazol threshold:

Groups of 10 male and female mice of approximately equal weight received an intravenous infusion of 0.5% $^{w}/_{v}$ leptazol solution and the volume of solution necessary to produce each phase of the seizure described by Orloff et al (1949) (See Methods, page 56) recorded. There was no significant difference between male and female mice in the doses of 0.5% $^{w}/_{v}$ leptazol to produce each phase of the seizure (See Table 41).

(2) Effect of sex steroid pretreatment on MES and leptazol

Tonic seizure threshold:

Groups of 50 female mice were pretreated with lynestrenol (10 mg/kg) or mestranol (1.0 mg/kg) or their oily vehicle, for 4 days. On the 5th day they were divided into groups of 10 animals and subjected to electro-

shocks of increasing voltage. Mestranol afforded protection against tonic seizure up to 60 volts (of 30%) when compared with the other two groups in which the results were approximately equivalent (See Table 42).

Leptazol threshold:

Groups of 10 female mice were pretreated with similar doses of lynestrenol or mestranol or vehicle as above. The volume of intravenous 0.5% leptazol solution to induce each phase of the seizure was recorded for each mouse in the three groups.

Pretreatment with mestranol significantly protected the mice against the third phase of the leptazol seizure compared with controls. Lynestrenol and control results were not significantly different (See Table 43).

SECTION II

(1) ED50 of four anti-convulsant drugs v MES in control mice

Groups of 10 female mice received logarithmically spaced doses of the anti-convulsant drugs. The % protection provided by the anti-convulsant drugs v MES was plotted on log probit paper and the ED50 dose read directly (See Table 44). The treated mice were subjected to MES at 70 volts at the time of peak effect of the drugs (determined in preliminary experiments) which are :-

Sodium phenobarbitone - 2 hr after
Sodium phenytoin - 1 hr after
Chlordiazepoxide hydrochloride - 1 hr after

Diazepam - ½ hr after.

(2) ED50 of sodium phenytoin and sodium phenobarbitone in sex steroid pretreated mice

Groups of 10 female mice pretreated with lynestrenol or mestranol or vehicle as above received the anti-convulsant drug intraperitoneally at four dose levels spaced logarithmically. Controls received 0.9% saline. At the time of peak effect of the drug each group was subjected to MES at 70 volts (MES, see Methods, page 57). The % protection offered by the various doses of the anti-convulsant drug for each group was plotted on log probit paper and ED50s for both anti-convulsants in each of the groups was calculated. Compared with controls, mestranol increased the ED50 dose of both sodium phenytoin and sodium phenobarbitone whilst lynestrenol reduced them. (See Table 45 and Figs. 41 and 42).

(3) Duration of anti-convulsant action of sodium phenytoin, sodium phenobarbitone, chlordiazepoxide hydrochloride and diazepam in sex steroid pretreated female mice v MES

Female mice were pretreated for four days with lynestrenol (10 mg/kg) or mestranol (1.0 mg/kg). On the 5th day each mouse was further treated with one of the four anti-convulsants; the dose chosen was that which gave 70 - 90% protection at the drug's peak of activity (which entailed the anti-convulsant activity being determined at a variety of times after the drug's administration).

Sodium phenytoin - 20 mg/kg @ 1 hr

Sodium phenobarbitone - 40 mg/kg @ 2 hr

Chlordiazepoxide hydrochloride - 80 mg/kg @ 1 hr I.P.

Diazepam - 20 mg/kg @ ½ hr

The results were plotted and results compared. The duration of all four anti-convulsant drugs was prolonged in mice pretreated with mestranol and reduced in mice pretreated with lynestrenol. (See Figs. 43, 44, 45 & 46).

(4) Duration of anti-convulsant action of sodium phenytoin, chlordiazepoxide hydrochloride and diazepam in SKF 525A pretreated female mice v MES

Female mice pretreated with SKF 525A (50 mg/kg) (See page 125) intraperitoneally or 0.9% saline intraperitoneally received one of the anti-convulsant drugs at the dose levels above and groups of 10 were examined for protection against MES at various times afterwards. Mice receiving SKF 525A showed prolonged protection v MES with all three anti-convulsant drugs used. These effects mimicked those obtained with mestranol (See Figs. 47, 48 & 49).

DISCUSSION

The results show that mature female mice require a lower voltage to produce a tonic seizure than males of the same age. In contrast, the susceptibility of male and female mice to leptazol convulsions appear to be identical. These results partly confirm the work of Woolley and Timiras (1962) who showed that mature female rats and mice display a greater convulsive reactivity than males, an observation they attributed to the levels of circulating oestradiol

which in the females lower certain brain thresholds.

Although pretreatment of female mice with lynestrenol (progestin) produced little or no effect on their susceptibility to tonic seizures produced by electroshock or leptazol infusion, pretreatment with mestranol (oestrogen) decreased the susceptibility of the mice to both electroshock and leptazol seizures. Opposing these results, Selye (1941) showed that progesterone protected rats against leptazol convulsions and death; Woolley and Timiras (1962) showed that oestradiol lowered EST in female rats and progesterone raised it. However, brain levels of NE and 5HT have been shown to be implicated in the seizure pattern due to electroshock and leptazol; increased levels decreasing susceptibility and decreased levels increasing susceptibility (Schlesinger, Boggan & Freedman, 1963). Since mestranol increases mouse brain 5HT levels and lynestrenol decreases mouse brain levels of NE and 5HT (See Chapter III, page, 194) these changes may account for the differing susceptibility of the pretreated mice to electrical and chemical seizures. This is borne out by the fact that injection of 5HTP produces a decrease in the susceptibility of female mice to tonic seizures due to electroshock (Schlesinger, Boggan & Griek, 1968).

The duration and intensity of activity of diazepam, chlordiazepoxide hydrochloride, phenytoin sodium and phenobarbitone sodium is increased by pretreatment with mestranol or SKF 525A and decreased by pretreatment with lynestrenol in the MES test. It is suggested that the observed changes in the activity of the above anti-convulsant drugs are due to alterations in their rate of metabolism.

Confirmatory evidence for this suggestion is provided by Rümke and Noordhoek (1969) who have shown that pretreatment of mice with large doses of lynestrenol (20 and 200 mg/kg) 48 hr prior to receiving either phenytoin or phenobarbitone resulted in decreased protection against bemegride convulsions and increased metabolism of the drugs. Also both lynestrenol and mestranol have marked and opposite effects upon the metabolism of certain barbiturates (See Chapter II, page 128), and chlordiazepoxide is rapidly and extensively metabolised in mice (Coutinho Cheripko & Carbone, 1969). Finally, the potent microsomal enzyme inhibitor SKF 525A mimics the effects of mestranol on all four drugs.

Even though MES was used throughout and produced 100% tonic convulsion in all pretreated animals, the steroid induced changes in mouse brain monoamine levels and their effects on susceptibility to convulsions, may be additive to the metabolic effects of the steroids upon the anti-convulsants.

PULSE RATE - 100 per sec

PULSE WIDTH - 2 msec

SHOCK DURATION - 0.3 sec

Voltage	No. of mice showing tonic seizures	
	Male	Female
50 v	1/10	3/10
55 v	0/10	4/10
60 v	6/10	10/10
65 v	8/10	10/10
70 v	10/10	10/10

TABLE 40

A comparison in control male and female mice of susceptibility to electroshock tonic seizures with increasing voltage.

Volume of 0.5% solution of leptazol injected intravenously					
SEIZURE PATTERN					
MALE			FEMALE		
1st phase	2nd phase	3rd phase	1st phase	2nd phase	3rd phase
0.15	0.15	0.20	0.15	0.15	0.15
0.20	0.20	0.25	0.15	0.20	0.45
0.10	0.15	0.35	0.15	0.20	0.25
0.20	0.25	0.25	0.10	0.20	0.25
0.10	0.15	0.15	0.15	0.15	0.20
0.15	0.15	0.20	0.15	0.20	0.35
0.15	0.15	0.45	0.10	0.20	0.35
0.15	0.20	0.20	0.15	0.20	0.40
0.15	0.15	0.35	0.10	0.10	0.15
0.15	0.15	0.15	0.10	0.15	0.15

TABLE 41

A comparison in control male and female mice of the volumes of 0.5% leptazol solution required to produce the three typical convulsive phases of leptazol convulsions.

PULSE RATE - 100 per sec

PULSE WIDTH - 2 msec

STIMULUS TIME - 0.3 sec

Voltage	No. of mice showing tonic seizures		
	Lynestrenol	Controls	Mestranol
45 V	5/10	5/10	0/10
50 v	8/10	6/10	3/10
55 v	8/10	8/10	4/10
60 v	10/10	10/10	7/10
70 v	10/10	10/10	10/10

TABLE 42

The effect of sex steroids: lynestrenol (10 mg/kg) for four days, mestranol (1.0 mg/kg) for four days, or vehicle (controls) for four days upon seizure pattern in groups of 10 female TO mice subjected to increased voltage electroshock.

Volume of 0.5% solution of leptazol injected intravenously (± S.E.)								
SEIZURE PATTERN								
lynestrenol			controls			mestranol		
1st phase	2nd phase	3rd phase	1st phase	2nd phase	3rd phase	1st phase	2nd phase	3rd phase
0.11 ± 0.01	0.14 ± 0.01	0.26 ± 0.03	0.12 ± 0.01	0.16 ± 0.01	0.26 ± 0.02	0.16 ± 0.01	0.19 ± 0.01	0.38* ± 0.04

TABLE 43

The effect of sex steroids: lynestrenol (10 mg/kg) for four days, mestranol (1.0 mg/kg) for four days or vehicle (controls) for four days upon the volume of 0.5% leptazol to produce the three convulsive phases in groups of 10 female TO mice.

* Significant difference compared with controls ($P=<0.05$)

	Intraperitoneal ED 50 doses
Phenytoin sodium	6 mg/kg
Phenobarbitone sodium	17 mg/kg
Chlordiazepoxide hydrochloride	52 mg/kg
Diazepam	11 mg/kg

TABLE 44

The ED50 dose of four anti-convulsant drugs in female TO mice in the MES test.

Phenobarbitone sodium	Lynestrenol	Controls	Mestranol
ED50 dose mg/kg I.P.	25 *	15.5	4.2 *
Range P = 0.05	16.7 - 37.5	9.1 - 26.4	1.8 - 10.1
Phenytoin sodium	**Lynestrenol**	**Controls**	**Mestranol**
ED50 dose mg/kg I.P.	2.7 *	6.6	11.0 *
Range P = 0.05	1.5 - 4.9	4.7 - 9.2	7.3 - 16.5

TABLE 45

The effect of sex steroids: lynestrenol (10 mg/kg) for four days, mestranol (1.0 mg/kg) for four days, or vehicle (controls), upon the ED50 dose of two anti-convulsant drugs.

* Significant difference compared with controls ($P \leq 0.05$)

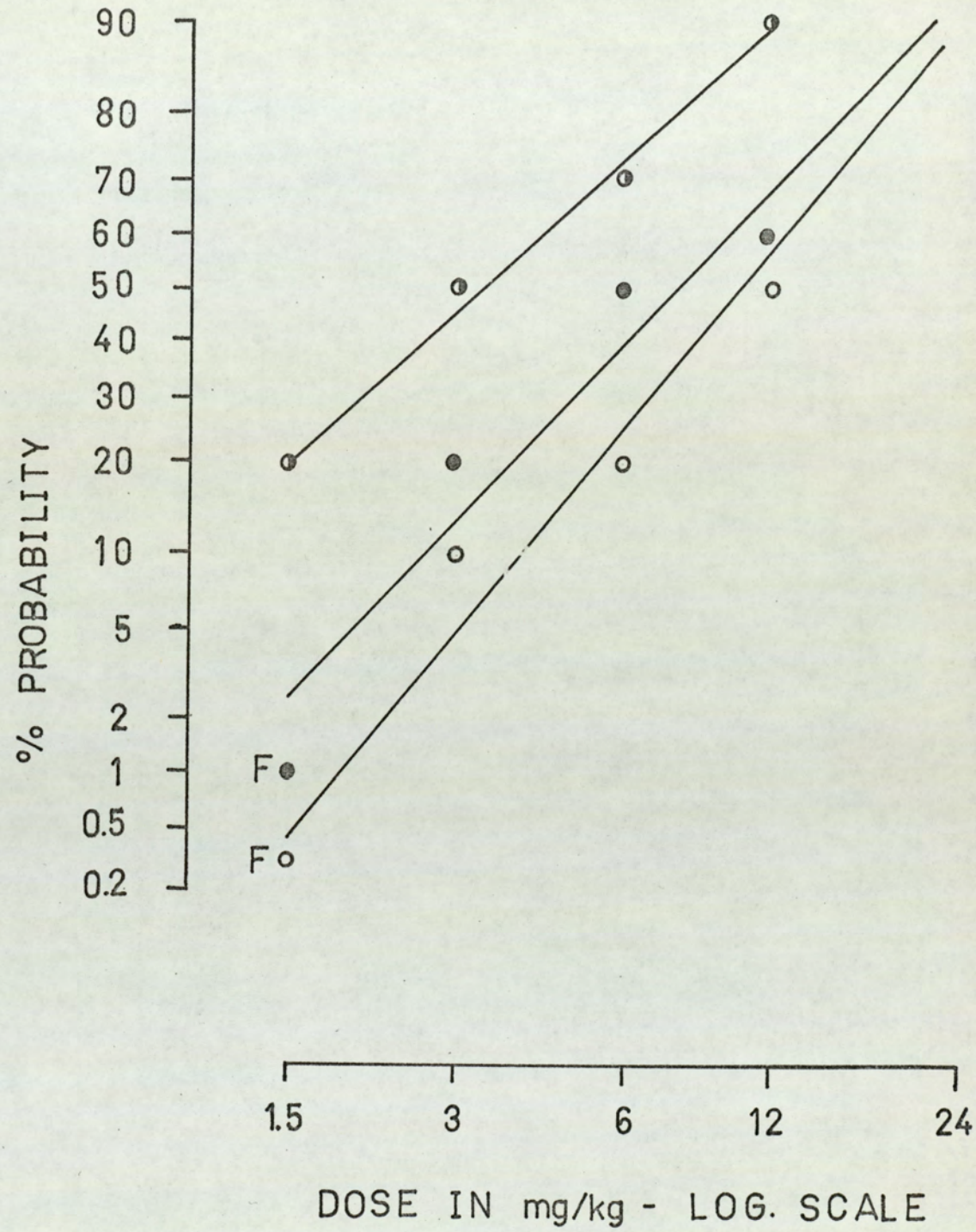


Fig. 41

Graph of % protection against MES vs log dose of phenytoin sodium in sex steroid pretreated female mice. Animals received mestranol (1.0 mg/kg, daily) subcutaneously for 4 days (●——●); or lynestrenol (10 mg/kg, daily) subcutaneously for 4 days (○——○); or vehicle only (5.0 ml/kg, daily, ●——●).

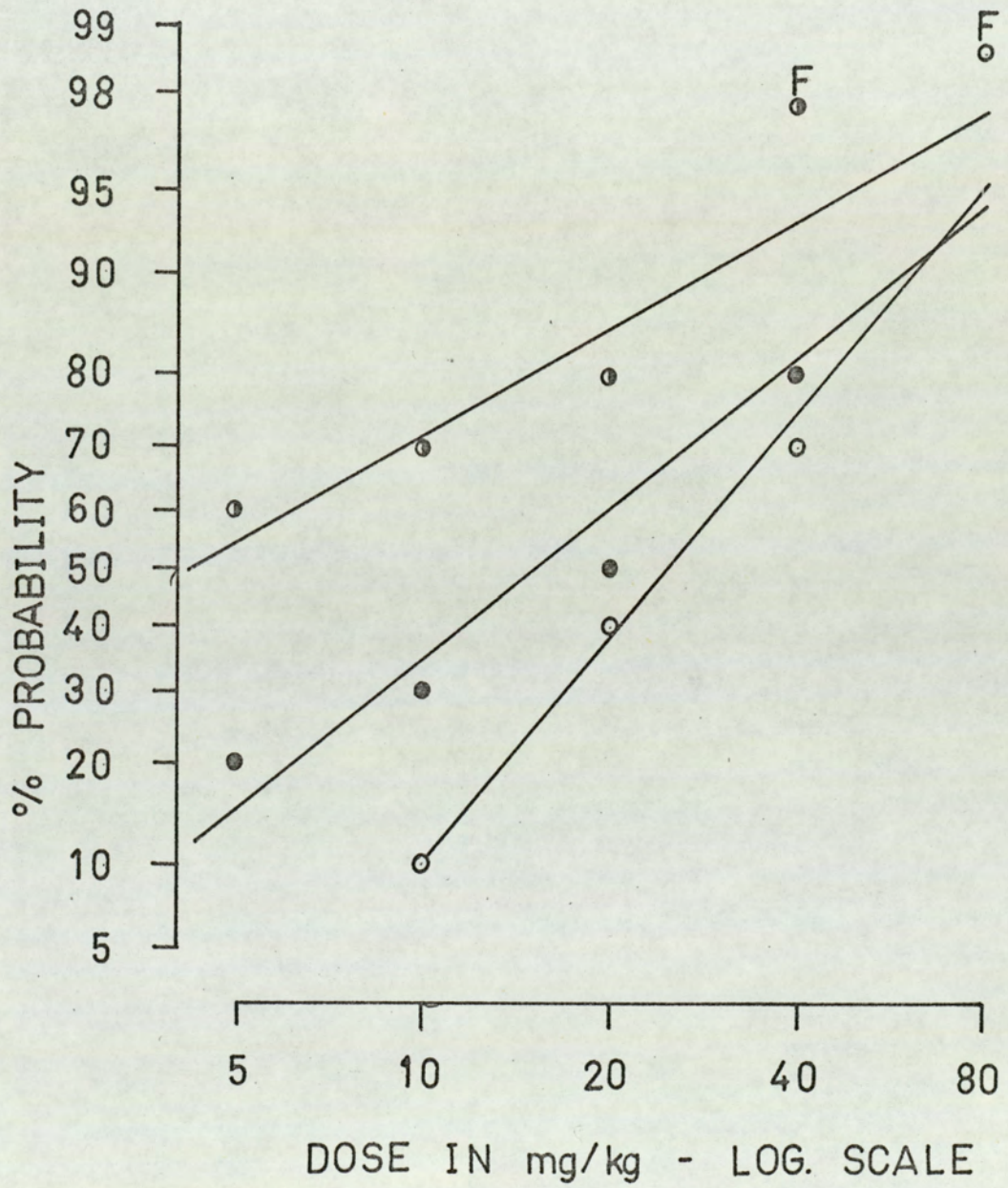


Fig. 42

Graph of % protection against MES vs log dose of phenobarbitone sodium in sex steroid pretreated female mice. Animals received mestranol (1.0 mg/kg, daily) subcutaneously for 4 days (●——●); or lynestrenol (10 mg/kg daily) subcutaneously for 4 days (○——○); or vehicle only (5.0 ml/kg, daily, ●——●).

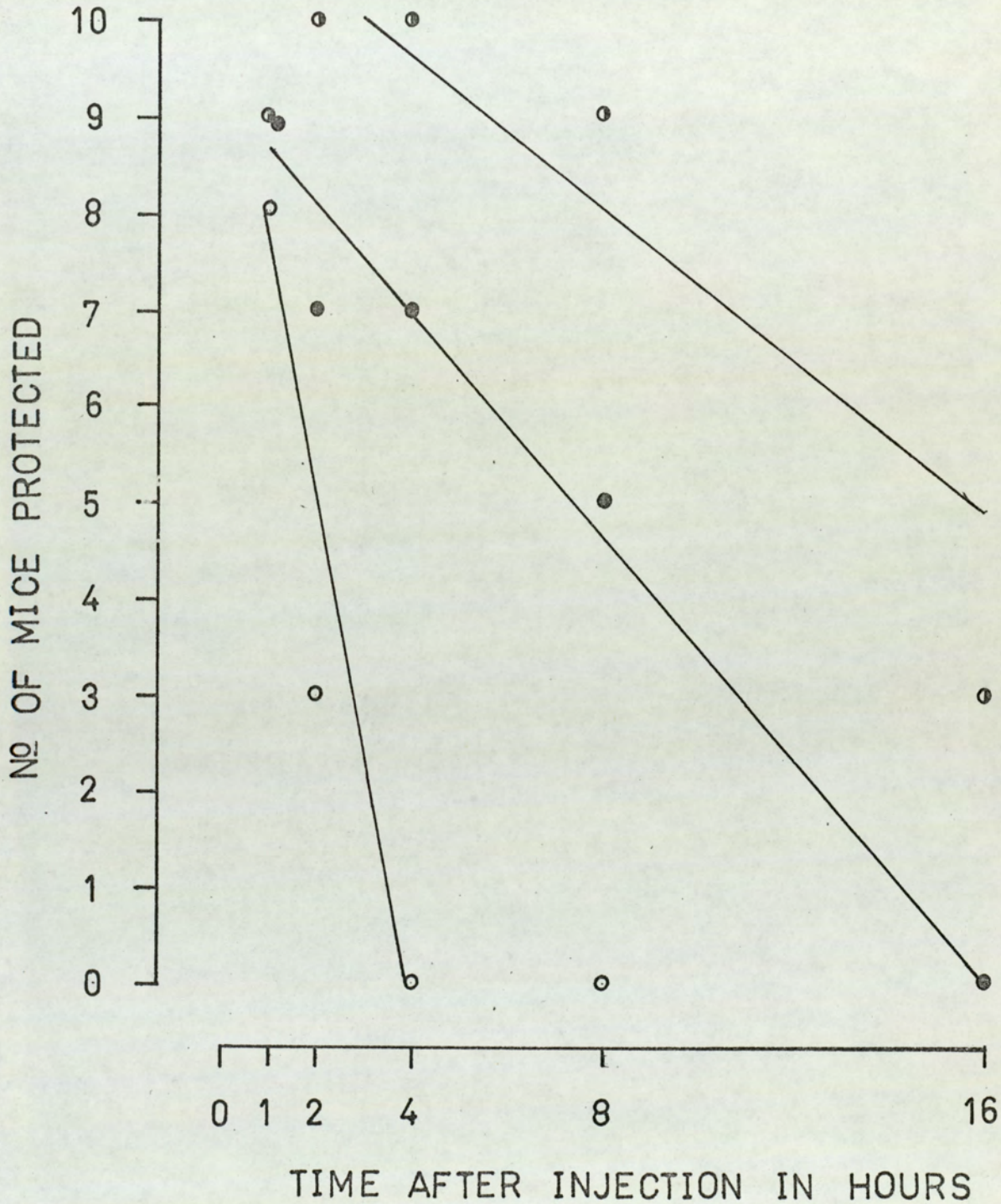


Fig. 43

Duration of action of phenytoin in female mice pretreated with mestranol or lynestrenol. Animals were pretreated with mestranol (1.0 mg/kg, daily) subcutaneously for 4 days (●——●); or lynestrenol (10 mg/kg, daily) subcutaneously for 4 days (○——○); or vehicle only (5.0 ml/kg, daily, ●——●). On the fifth day they were subjected to MES at 1 hr and various times after an intraperitoneal injection of phenytoin sodium (20 mg/kg).

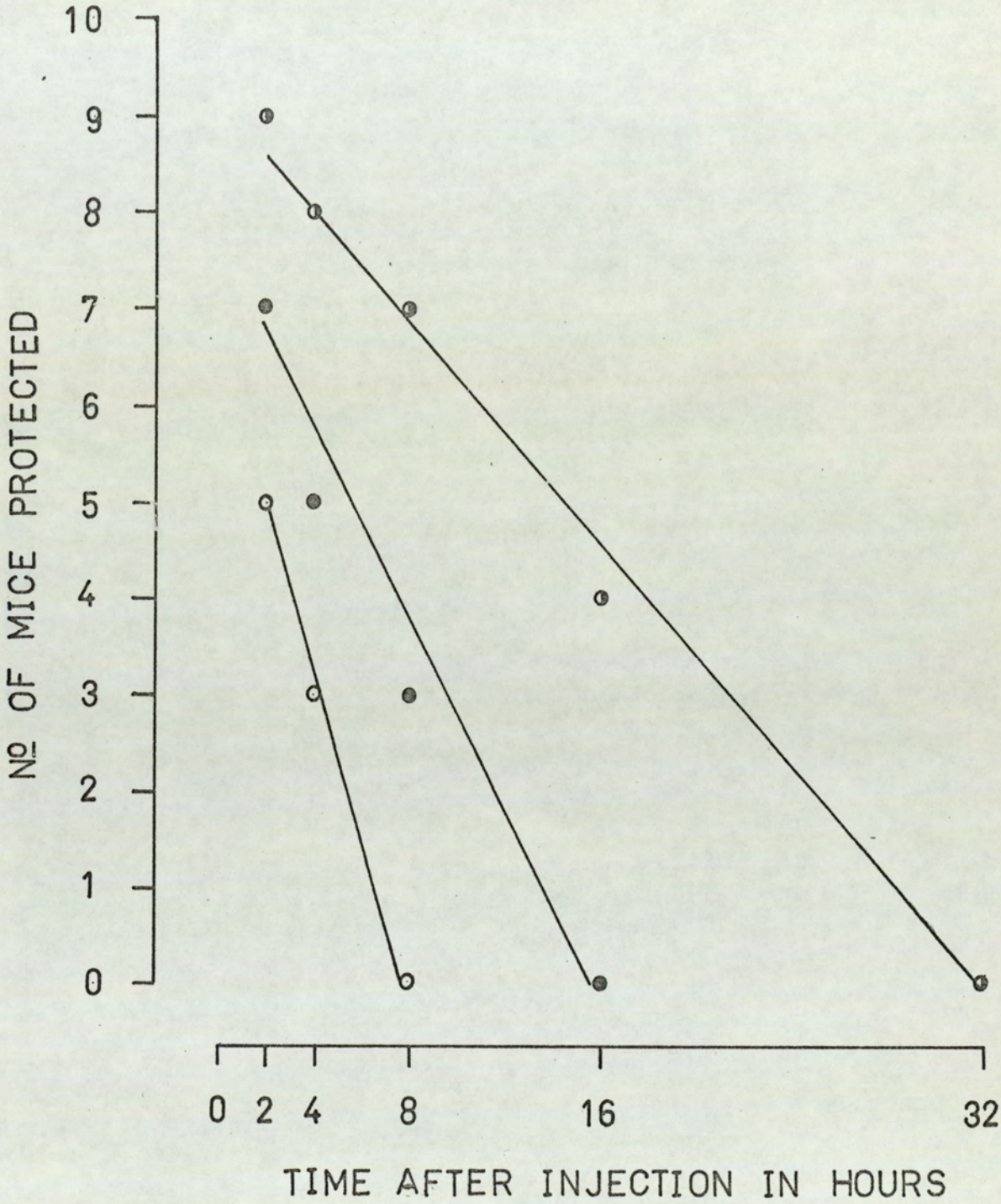


Fig. 44

Duration of action of phenobarbitone in female mice pretreated with mestranol or lynestrenol. Animals were pretreated with mestranol (1.0 mg/kg, daily) subcutaneously for 4 days (●——●); or lynestrenol (10 mg/kg, daily) subcutaneously for 4 days (○——○); or vehicle only (5.0 ml/kg daily ●——●). On the fifth day they were subjected to MES at 2 hr and various times after an intraperitoneal injection of phenobarbitone sodium (40 mg/kg)

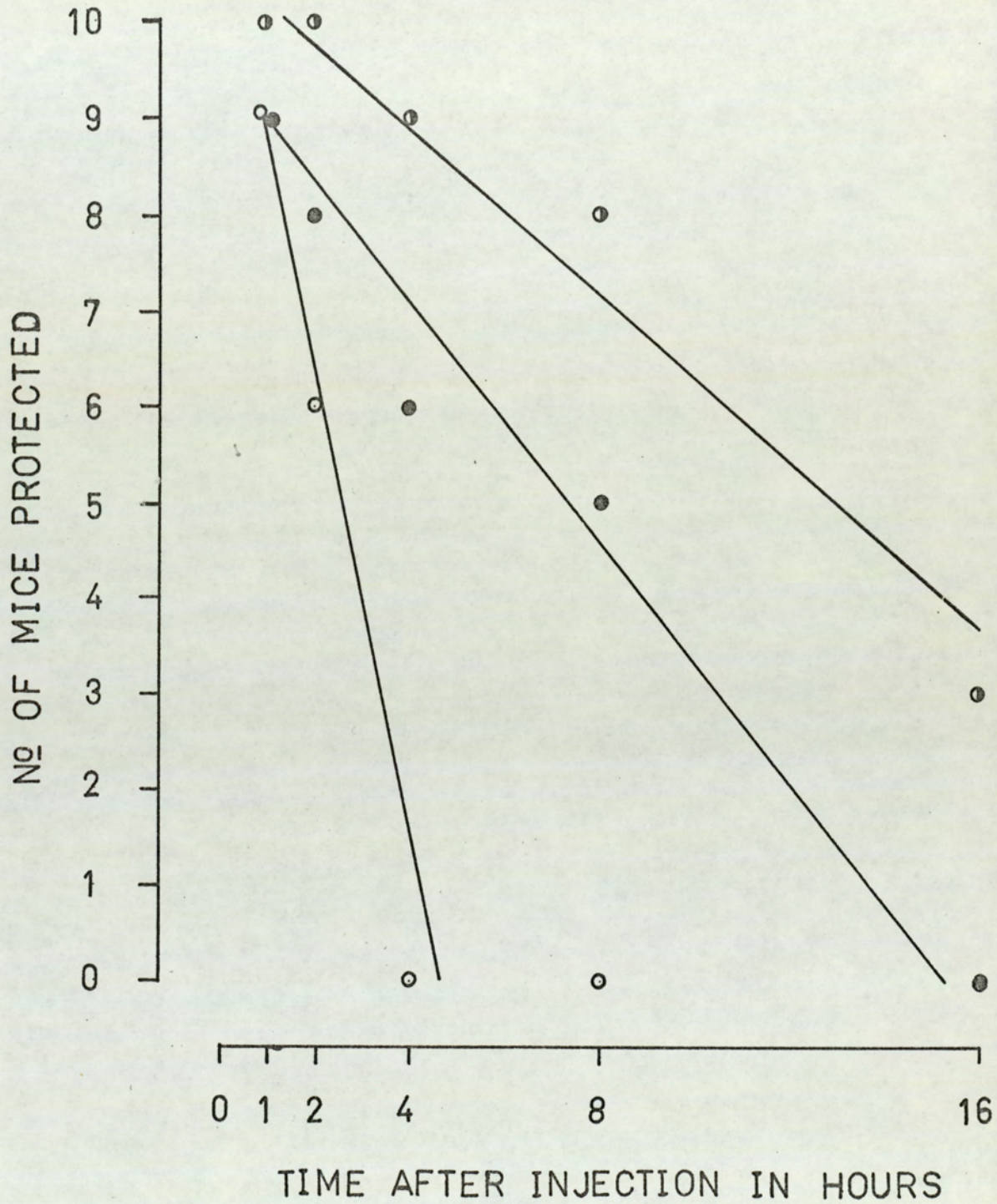


Fig. 45

Duration of action of chlordiazepoxide in female mice pretreated with mestranol or lynestrenol. Animals were pretreated with mestranol (1.0 mg/kg, daily) subcutaneously for 4 days (◑——◑); or lynestrenol (10 mg/kg, daily) subcutaneously for 4 days (○—○); or vehicle only (5.0 ml/kg daily ●—●). On the fifth day they were subjected to MES at 1 hr and various times after an intraperitoneal injection of chlordiazepoxide hydrochloride (80 mg/kg).

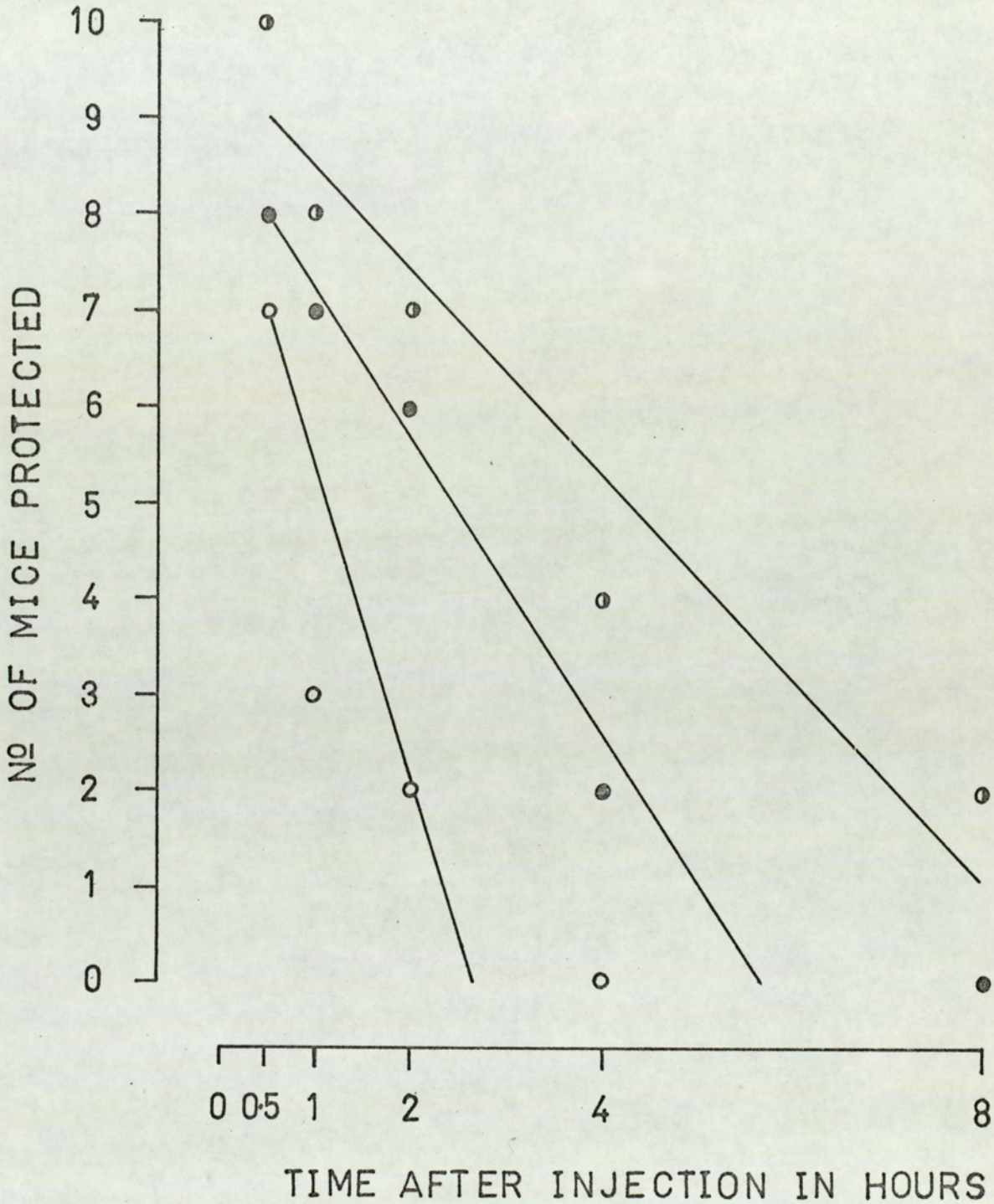


Fig. 46

Duration of action of diazepam in female mice pretreated with mestranol or lynestrenol. Animals were pretreated with mestranol (1.0 mg/kg, daily) subcutaneously for 4 days (◐——◐); or lynestrenol (10 mg/kg, daily) subcutaneously for 4 days (○——○); or vehicle only (5.0 ml/kg, daily, ●——●). On the fifth day they were subjected to MES at ½ hr and various times after an intraperitoneal injection of diazepam (20 mg/kg).

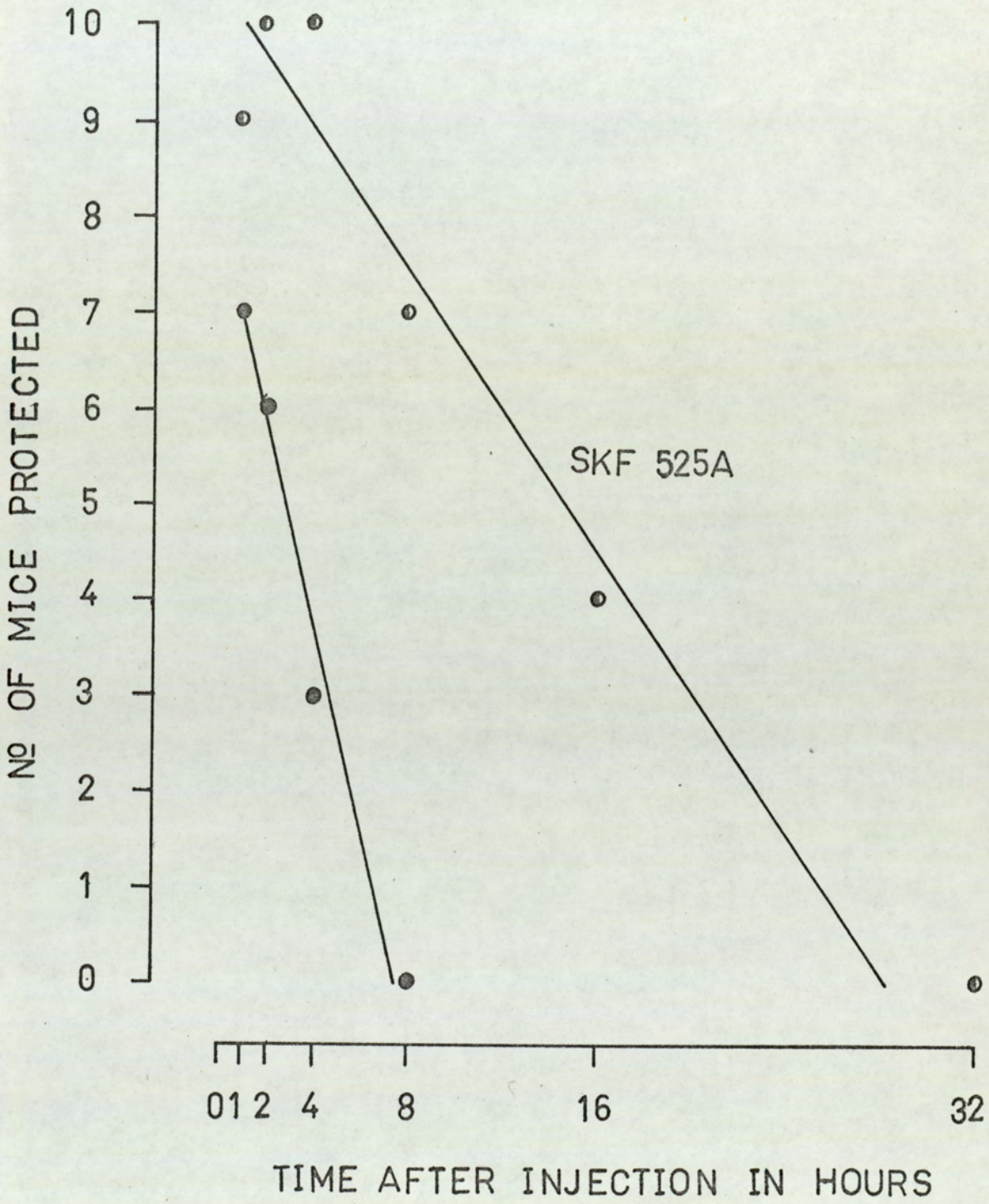


Fig. 47

Duration of action of phenytoin in female mice pretreated with SKF 525A. Animals received an intraperitoneal injection of SKF 525A (50 mg/kg) 90 min before receiving phenytoin sodium (20 mg/kg) also administered intraperitoneally. They were subjected to MES at 1 hr and various times after the anti-convulsant drug.

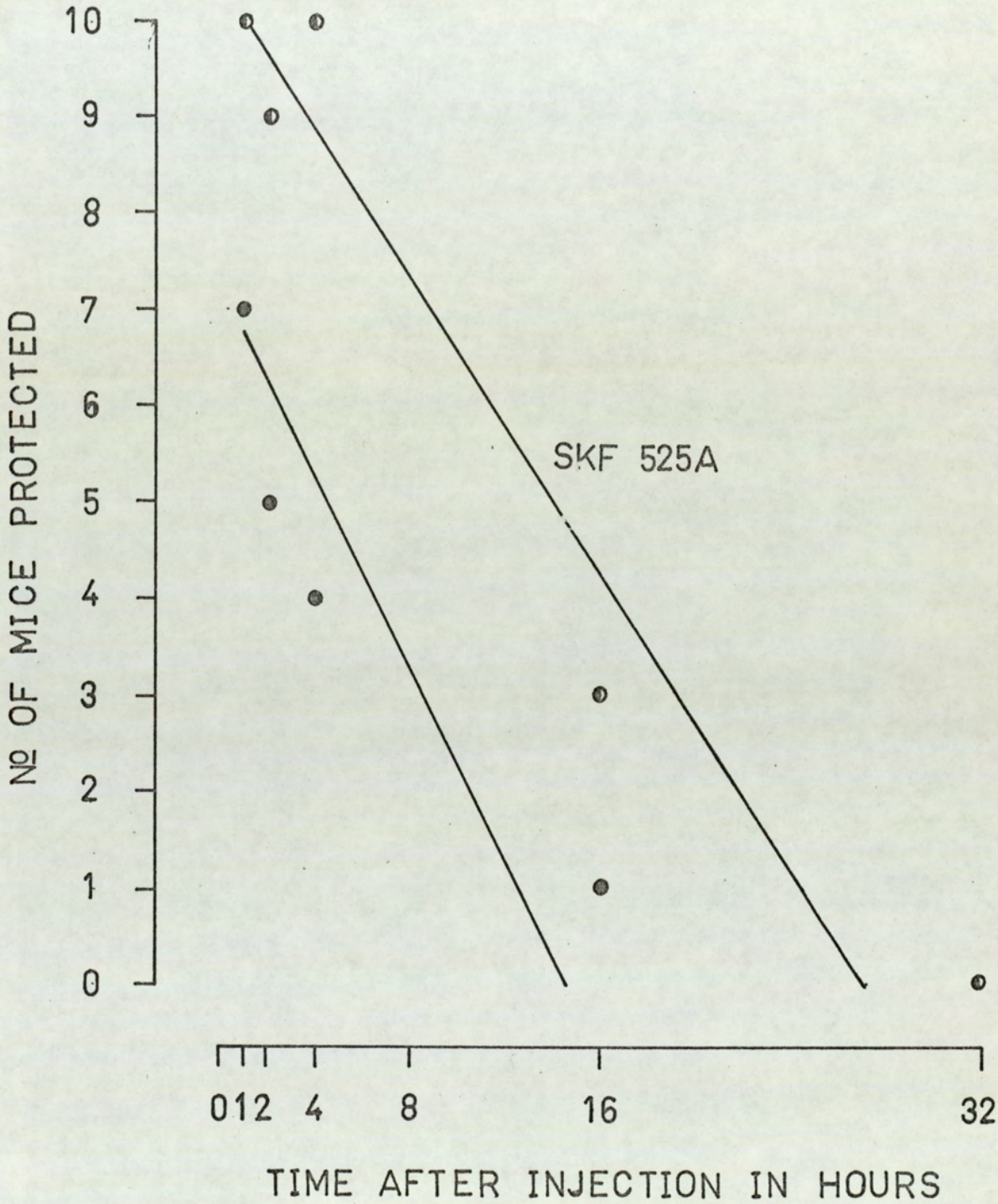


Fig. 48

Duration of action of chlordiazepoxide in female mice pretreated with SKF 525A. Animals received an intraperitoneal injection of SKF 525A (50 mg/kg) 90 min before receiving chlordiazepoxide hydrochloride (80 mg/kg) also administered intraperitoneally. They were subjected to MES at 1 hr and various times after the anti-convulsant drug.

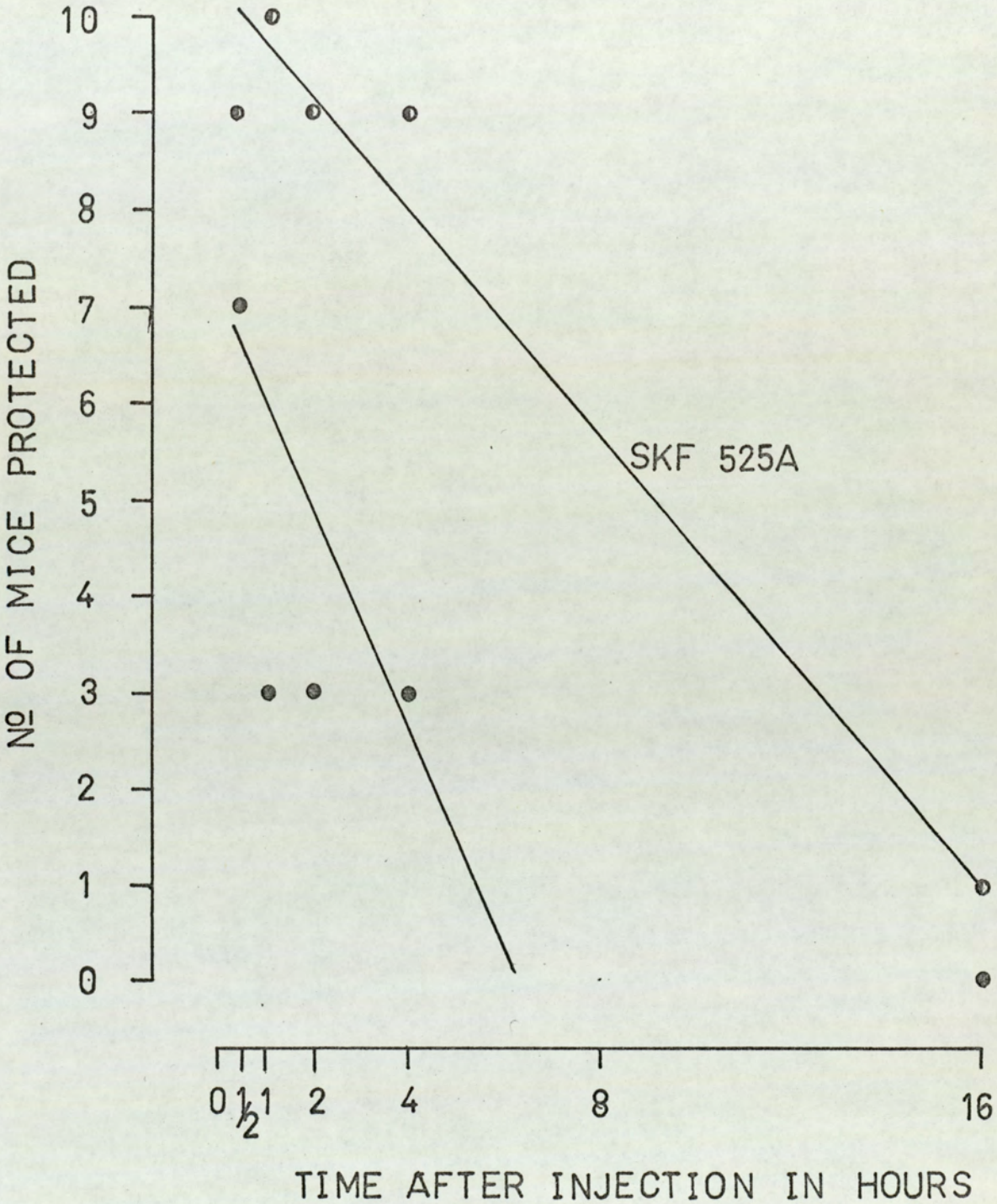


Fig. 49

Duration of action of diazepam in female mice pretreated with SKF 525A. Animals received an intraperitoneal injection of SKF 525A (50 mg/kg) 90 min before receiving diazepam (20 mg/kg) also administered intraperitoneally. They were subjected to MES at ½ hr and various times after the anti-convulsant drug.

GENERAL DISCUSSION

(a) Principal conclusions

In this thesis, a study has been made of the effects of pretreatment with a number of synthetic adrenocortical and sex steroids on the responses of mice and rats to various centrally acting drugs. Evidence was presented in the Introduction describing the many ways in which steroid hormones are able to modify the responses of different species of animals to these drugs. These modifications have been variously ascribed to changes in the animal's body temperature, central sensitivity, levels of chemical transmitters and hormonal milieu brought about by steroid pretreatment.

The majority of available information, including some very recent work, suggests that the drug metabolic pathways in the liver or other enzyme systems present in the brain and plasma are the most likely targets for steroid modification of drug response. In their normal hormonal roles, steroids are known to be enzyme inducers and their exact locus of action appears to be at the level of the nuclear proteins, i.e. DNA (Thompson et al, 1966; Beato et al, 1968; Barker & Warner, 1967; O'Malley & McGuire, 1969). Whether steroids which reduce the activity of certain centrally active drugs do so by inducing enzyme systems in the liver, or elsewhere, which metabolise these drugs remains to be established. However, it has been

suggested that steroid hormones are naturally occurring substrates for drug-metabolising enzymes in liver microsomes (Kuntzman, 1964) and further that drugs and steroids are alternate substrates for a common microsomal mixed-function oxidase system, (Tephly & Mannering, 1968). Thus, prior administration of either steroid or drug promotes the metabolic degradation of the other. Other workers believe that steroids act as regulators of metabolic pathways and their presence or absence determines how quickly drugs are degraded. The terminal oxidase (involving the haemoprotein, cytochrome P_{450}), which is the rate determining step of the liver enzyme system hydroxylating a number of drugs and steroids, has been implicated as the locus of action of certain steroids (Davies et al, 1967; Kato et al, 1968).

Because it is well known that the age, sex, species, and strain of the animal may be implicated in the response of the animal to centrally acting drugs, these factors were considered throughout.

With the introduction of potent synthetic anti-fertility steroids which are used clinically over long periods, the possibility arose of centrally acting drugs being administered concomitantly. Consequently we have examined the effects of certain of these synthetic steroids (for example, dexamethasone, fludrocortisone, lynestrenol and mestranol) upon the response of mice and rats to analgesic, hypnotic, stimulant and anti-convulsant drugs.

The most significant and consistent observation in this thesis has been that in both male and female mice, pretreatment with female sex steroids can affect markedly

the intensity and duration of the pharmacological action of certain barbiturates and related drugs. Thus, oestrogen (e.g. mestranol) prolonged whilst progestin (e.g. lynestrenol) reduced the hypnotic effect of pentobarbitone and hexobarbitone and the anti-convulsant activity of phenobarbitone phenytoin , chlordiazepoxide and diazepam. Without exception these drugs are metabolised almost entirely in the liver (Quinn, Axelrod & Brodie, 1958; Noordhoek, 1968; Coutinho, Cheripko & Carbone, 1968). It appeared to us, therefore, that this was the most likely target for steroid/drug interaction. When prior administration of SKF 525A (a potent microsomal enzyme inhibitor), to control mice before administration of these drugs (above) mimicked the effect of oestrogen pretreatment, our suggestion was largely confirmed. It was further supported when a study of the rate of degradation of pentobarbitone sodium in the blood and plasma of sex steroid pretreated mice was made; the rate of metabolism of the barbiturate was enhanced in progestin pretreated mice and delayed in oestrogen pretreated animals; blood levels in both groups were similar to controls (therefore, central sensitivity to the drug must be similar, Brodie, 1956).

However, the further prolongation of barbiturate hypnosis in oestrogen pretreated mice by SKF 525A was much greater than might have been expected from a mere summation of the two effects. An increase in central sensitivity of oestrogen pretreated mice to barbiturates was suspected. By raising the environmental temperature by $10^{o}C$ thus preventing barbiturate-induced hypothermia, and thereby maintaining the rate of drug metabolism (Fuhrman & Fuhrman,

1961; Morris, 1963) it was hoped to abolish any steroid influence upon hypnosis. This largely succeeded in progestin pretreated mice (i.e. no further shortening of sleeping-time was observed) when their sleeping-times were similar to controls. Oestrogen pretreated animals, however, still slept longer. This was considered by us to be a manifestation of increased central sensitivity to barbiturates.

Since pentobarbitone and hexobarbitone are metabolised in the liver by enzyme systems dependent upon TPNH + O_2 (by oxidation of the terminal or penultimate C atoms of the sidechain, Cooper & Brodie, 1955), the effects of sex steroid pretreatment upon O_2 consumption in mice were studied. Oestrogen decreased whilst progestin increased the rate of O_2 consumption in mice (10%). This possibly has an influence on the response of these animals to barbiturates.

We have shown that mestranol slightly antagonises whilst lynestrenol slightly potentiates morphine 'analgesia'. An opposite effect would have been expected if the steroids were exerting their 'usual' effect upon liver microsomes. But if the theory of Beckett et al (1956) is accepted then antagonism by mestranol and potentiation by lynestrenol of the production of the active metabolite, normorphine, would account for these differences. In our hands pentazocine possessed no 'analgesic' properties in mice. Its potency as a morphine antagonist was potentiated by sex steroid pretreatment; some tentative explanations were put forward in the Discussion (Chapter I, page 100).

The potentiation of the duration of locomotor activity

hyperthermia, and crowded toxicity after dexamphetamine by oestrogen (mestranol) in female mice, and conversely the antagonism of two of these three parameters by progestin (lynestrenol) was thought to be again a manifestation of the metabolic effect of these steroids at the liver microsomal level. However, two observations tended to disprove this theory. Firstly, SKF 525A did not mimic the oestrogen effect but the monoamine oxidase inhibitor (MAOI) nialamide did. Also another central stimulant fencamfamin, which is known to be poorly metabolised in rodents (Hotovy et al, 1961), was similarly affected by sex steroid pretreatment (that is, mestranol induced hyperthermia and increased locomotor activity whilst lynestrenol decreased locomotor activity). Since progestins are known to increase MAO levels in the brain and plasma (Kuwabara et al, 1967; Southgate et al, 1968; Grant & Pryse Davies, 1968), the effects of the oestrogen are mimicked by nialamide, it seemed that these observations might offer an alternative explanation of the observed differences in sympathomimetic drug activity. Present evidence points to a predominantly indirect action of these sympathomimetic drugs upon the CNS via a release of biogenic amines such as dopamine and noradrenaline. These in turn rely to some extent upon presence of MAO present in the brain for their inactivation. Therefore, steroid-induced changes in MAO levels might account for the observed alterations in sympathomimetic activity. A brief study of brain amines in sex steroid pretreated mice revealed an increase in 5HT levels after oestrogen and a decrease in levels of dopamine,noradrenaline and 5HT after lynestrenol. Further experiments were thought to be necessary before

drawing too many conclusions from these results.

In our hands the potent glucocorticoid (dexamethasone) and mineralocorticoid (fludrocortisone) produced little or no effect upon morphine analgesia or barbiturate anaesthesia (fludrocortisone did potentiate morphine analgesia slightly). These results are in marked contrast with those obtained by Winter and Flataker (1951 and 1962) who maintain that cortisone antagonises whilst DOCA potentiates morphine analgesia and barbiturate hypnosis. They suggest that these effects are due to the direct action of the steroids upon the brain stem, the former producing 'arousal' and the latter 'depression' of neuronal mechanisms.

A detailed study of the effects of the four types of steroid upon pentobarbitone anaesthesia in female rats revealed a species difference when compared with results obtained in female mice. Although both the oestrogen and progestin produced slight changes in pentobarbitone anaesthesia, they were always in a similar direction and depended upon the age of the animal rather than any other factor - a point previously noted by Fujii et al (1965).

Ovariectomy slightly reduced pentobarbitone hypnosis in immature rats but this was not so in mature rats. Neither ovariectomy nor sex steroid pretreatment altered the degree or time of onset of the rapidly developed barbiturate tolerance in young rats. It was noted that in immature rats, where both sex steroids slightly reduced pentobarbitone sleeping-time, their adrenal glands were significantly enlarged. This had been noted by other workers (Selye & Collip, 1936; Gemzell, 1952; Gibson et al, 1967) and was offered by Robillard et al (1954) as an

explanation of the increased sleeping-times to pentobarbitone in mature rats receiving natural female sex steroids, i.e. increased corticosteroid levels inducing decreased metabolism of the barbiturate. Compared with mice, female rats appear to be insensitive to the effect of mestranol and lynestrenol upon barbiturate hypnosis but it seems relevant at this juncture to point out that the rat is the only species known which demonstrates a general sex difference in response to drugs (the female metabolises certain drugs, e.g. hexobarbitone, antipyrine and aniline, slower than the male, Quinn et al, 1958). This sex difference can be nullified by administration of the opposite sex steroid. Kato et al (1962, 1968) believe that in the rat it is the level of androgen which controls the activity of drug oxidation in liver microsomes. This would account for the observed sex difference to barbiturates and might account for the lack of significant effects of female sex steroids in female rats upon barbiturate hypnosis.

In female rats, we have shown that adrenalectomy and DOCA administration markedly increased the response of those animals to pentobarbitone. This confirms the work of Tureman et al (1952) Robillard et al (1954) and Bousquet et al (1965). Dexamethasone,on the other hand, reduced pentobarbitone sleeping-time in intact and adrenalectomised female rats. Fludrocortisone appeared to act as a replacement hormone in that the pentobarbitone sleeping-times of adrenalectomised animals returned to normal whilst the times in intact animals remained unchanged. Since glucocorticoids are potent enzyme inducers in rat liver, we have

postulated that this is the mechanism whereby dexamethasone produces its effect. The effects of adrenalectomy and DOCA have been variously ascribed to changes in brain sensitivity to the barbiturate (Winter et al, 1952) or decreased liver metabolism (Remmer, 1958), but in our opinion the latter seems more likely.

Thus, in general mice appear to be most sensitive to the effects of female sex steroid-induced changes in barbiturate hypnosis and insensitive to the effects of adrenocortical steroids - and vice versa in female rats. The significance of this hormonal species difference on the response of these animals to drugs in other pharmacological situations remains to be assessed.

Throughout this work it was observed that the animals' sensitivity to various stimuli and drug action was modified by steroid pretreatment. For instance, the reaction times of mice to various nociceptive stimuli were increased with all four types of steroid. An increased sensitivity to barbiturates and both chemical and electro-shock procedures was noticed in mice after mestranol (oestrogen) pretreatment. A direct effect upon neuronal mechanism by the steroid would account for these effects (See Review of Woodbury, 1958) but the observed differences in amine levels after steroid pretreatment previously discussed are thought to be implicated.

(b) Clinical implications

Early clinical observations during hyper or hypo-hormonal states (e.g. during pregnancy, menstruation, Addison's and Cushing's diseases) have shown an exacerbation

of both clinical epileptic seizures and EEG abnormalities (Gowers, 1885; Logethetis & co-workers, 1959; Quarton et al, 1955). Animal experimentation has since shown that steroid pretreatment is capable of altering brain excitability, EEG patterns,central amine and drug metabolism. Under these circumstances changes in the activity of centrally acting drugs might also be expected.

With the growing use of prophylactic steroid therapy the clinical implications of the observations above are vast. Crawford and Rudofsky (1966) have shown in women that pregnancy, administration of oral contraceptives, or stilboestrol, leads to a reduction in the urinary levels of the major metabolites of both pethidine and promozine - an indication of reduced drug metabolism. Similarly, Espir and co-workers (1969) reported that one of their female epileptic patients, receiving a low-dose oestrogen/ progestin combination pill, showed an increased susceptibility to the effects of the anti-convulsant drug "Primidone'.

We have shown that in mice sex steroid administration, albeit in large doses (50 x human dose) produces changes in drug metabolism. However, it should be emphasised that mice metabolise most drugs far more rapidly than man (e.g. biological half life of hexobarbitone is 19 min in mouse and 360 min in man, Quinn et al, 1958) and because of their surface area/volume ratio are far more vulnerable to body temperature changes. A direct comparison between effects of steroids in mouse and man is difficult because the direction of oestrogen and progestin effects on drug metabolism in man, albeit from only a few clinical

observations, are similar but in mouse appear to be opposite.

Finally, the occurrence of depression in a number of susceptible women receiving contraceptive preparations has suggested that these steroids induce changes in both central monoamine oxidase activity (Grant & Pryse Davies, 1968) and tryptophan metabolism (Winston, 1969; Price & Toseland, 1969; and Rose, 1969). Progestins are thought to raise brain MAO activity and thereby induce depression. Other workers believe that these steroids inhibit the decarboxylation of tryptophan to yield tyramine and 5HT and induce depression by this means. In support of both theories, Shaw (1969), has shown a significant decrease in amines of the brain stem of suicide cases (successful). The unfortunate consequences of reductions in MAO activity on the intensity of effects of central stimulant drugs is well known (Mason, 1962; Dally, 1962; Hay, 1962). That we have produced changes in the activity of central sympathomimetic drugs and brain amines in mice pretreated with these steroids emphasises the possibility of abnormal clinical effects when amphetamine-like drugs are administered to patients receiving this type of steroid therapy.

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