

STUDIES ON THE EFFECTS OF OESTRADIOL, PROGESTERONE AND SOME STEROIDAL CONTRACEPTIVES ON THE ADRENAL CORTEX OF ALBINO RATS

By

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INTRODUCTION

Sexual dimorphism as regards the adrenal glands of some animals is well known. The adrenal glands of females are significantly bigger than that of the males.

Antistiban (1962) stated that cholesterol and ascorbic acid contents of the adrenal glands showed the above mentioned sex difference particularly in animals of middle age.

Roux et al. (1962) demonstrated in vitro a difference in adrenal metabolic activity in pregnant guinea pigs when compared with non pregnant.

Ricci et al. (1963) stated that in pregnancy an increase in nuclear volume occurred especially in zona fasciculata. This was accompanied by a rise in the soluble and insoluble protein contents which might be considered as evidence of adrenocortical hypertrophy in pregnancy.

Whitly and Stoner (1957) however, were unable to detect any increase in the weight of the adrenals when they examined the latter in pregnant women immediately after death. On the other hand by means of histochemical tests they did observe an increase in the width of the inner part of the zona fasciculata.

Progesterone also may play a role in stimulating the adrenal cortex. Larina (1963) ascertained that progesterone when administered together with ACTH caused a rise in the output of all steroids produced by slices of rabbit adrenals more than that when ACTH was administered alone.

Synthetically prepared combinations of oestrogens and progestogens are nowadays used on a large scale for contraception. Continuous administration of oestrogens in prolonged use of contraceptive pills may affect the adrenocortical activity either directly or indirectly. Increased plasma cortisol as well as its reduced metabolic clearance were reported by Pincus (1964), in long term Enovid users. Similar oral contraceptives were found to have the same effect (Besch et al. 1965 ; Sala and Castegrano, 1966).

Some of the gestogens used in the combined oral contraceptive pills have somewhat oestrogenic effects and probably small amounts are metabolised to oestrogenic compounds (Breuer et al. 1960 ; Layne et al. 1963 ; Brown, 1966).

It is therefore, apparent that oestrogenic and progestational components of oral contraceptives have certain effects on the adrenal cortex, but whether these effects are accompanied by structural changes in the adrenal is lacking.

In a previous study Zaki et al. (1964/1965), found that in the rat the hypertrophied cortical tissue, in cases of pregnancy, unilateral adrenalectomy as well as after ACTH injection, had a higher alkaline phosphatase activity than the normal adrenocortical tissue.

From these findings it was thought to study the effects of oestradiol, progesterone, and some of the combined steroid contraceptive pills on the alkaline phosphatase activity of the adrenal cortex of rats ; in an attempt to reveal to what extent these synthetic preparations affect the adrenocortical activity.

Oestrogens are known also to be the strongest reticuloendothelial system stimulants (Nicol et al. 1951, 1966). Cyclical variations in the reticuloendothelial activity are known to occur during the oestrus cycle and pregnancy in the rat and mouse (Nicol et al. 1964 and 1965). Fibrosis of the liver, lungs, spleen and coronary vessels was observed in guinea pigs after administration of ethinyl oestradiol in high doses (El-Heneidy 1966). From these probable effects of oestrogens on other

tissues than the adrenals, samples from different organs of rats given different contraceptive pills were also studied from the pathological point of view.

MATERIALS AND METHODS

Adult female albino rats, Wistar strain, of almost the same age and weight, kept on normal balanced diet were used in this investigation. These were arranged in the following groups and subgroups :

- | | |
|-----------------------------------------------------------------------------------------------------------|------------------|
| 1. Adult normal female rats | 5 rats |
| 2. Ovariectomized female rats | 6 rats |
| 3. Ovariectomized rats injected with oestradiol
dipropionate in a dose of 0.5 mg. daily for
2 weeks | 5 rats |
| 4. Ovariectomized rats injected with progesterone
in a dose of 0.5 mg. daily for 2 weeks | 6 rats |
| 5. Rats given oral contraceptive pills : | |
| (a) Lyndiol 2.5 | group of 12 rats |
| (b) Lyndiol 2 | group of 12 rats |
| (c) Gyn-anovlar | group of 12 rats |
| (d) Eugynon | group of 12 rats |
| (e) Ovulen | group of 12 rats |

Each rat of the previous subgroups was given one tablet daily in the form of even suspension using a stomach tube for a period of two weeks. In half the number of animals in each subgroup the experiment was continued for six weeks for the pathological study only. At the end of the experiment animals were sacrificed by cortical dislocation. The adrenal glands of each animals were weighed and one gland fixed in neutral buffered formaline solution for future histological studies thought to be of value in this respect. Sections, six microns in thickness, from the liver, spleen, lung and kidney were stained routinely by haematoxylin and osin. The other gland of each animal from the

previous groups was bisected and the medulla removed as far as possible, by blunt dissection using a binocular microscope. The weighed cortical tissue was homogenized for determination of alkaline phosphatase activity. The latter was expressed in terms of the increase in milligrams of hour at 37°C in Veronal buffer (pH 8.6) containing 0.5% sodium B glycerophosphate.

Adrenocortical alkaline phosphatase was done in all groups except the groups of contraceptive treated rats for six weeks, as marked atrophy was clearly observed. The pathological study was carried out in rats given contraceptive pills for two and six weeks. The six weeks duration of the experiment was chosen as animals were found to die if treatment with such a big dose was extended for a more prolonged period.

RESULTS

Data of Table 1 show that the mean alkaline phosphatase activity per gram cortical tissue of normal rats was 3 ± 0.179 .

Ovariectomy resulted in a decrease in the activity of the enzyme from 3 to 2.836. When examining the effect of oestradiol dipropionate on the alkaline phosphatase activity of the adrenal cortex of ovariectomized rats, aestradiol dipropionate was injected intramuscularly in a dose of 1/2 mg. per rat per day for a period of two weeks. Data obtained showed an increase in the activity of alkaline phosphatase to values higher than that of normal rats. A mean value of 3.896 was obtained as compared to 2.836 in ovariectomized rats and 3 in normal rats.

From the results obtained it is also clear that progesterone was without effect.

As regards the effect of different contraceptive pills (their formulae and trade names are presented in Table 2), on the adrenocortical activity of female rats, the data obtained show that the mean alkaline phosphatase activity per gram adrenocortical tissue increased from a mean value of 3 ± 0.1789 in normal rats to 4.3 ± 0.04 and 4.13 ± 0.1225 with lyndiol 2.5 and lyndiol 5 respectively. With eugynon, gyn-anovlar and ovulen the results obtained are 3.68 ± 0.447 ; 3.2 ± 0.0706 and 3.2 ± 0.1915 respectively with an increase of 0.68 ; 0.3, and 0.2 when compared with normal rats. These results show clearly that the highest increase (1.3, 1.13) was found with lyndiol 2.5, and lyndiol 5 respectively, then eugynon, gyn-anovlar and the least increase was with ovulen.

TABLE 1

Effect of Oestradiol, Progesterone and Some Steroidal Contraceptives
on the Adrenocortical Alkaline Phosphatase Activity

		No. of anim.	Mean wt. of anim. (grs)	Mean wt. of corti- cal tissue of right gland (mgm)	Mean activity per cort ical tissue of right gland	Mean activity per gm. cortical tissue	t* test
Normal	rats ♀	5	155.6	15.56	0.046	3.00	
S. E. ±				0.527	0.004	0.179	
Ovariectomized	rats	6	135.8	16.51	0.047	2.836	1.025
S. E. ±			0	0.371	0.001	0.056	P > 0.10
Ovariectomized	rats	5	174	23.24	0.088	3.896	2.723
injected with oest- radiol dipropionate							
S. E. ±				0.887	0.013	0.435	P < 0.05
Ovariectomized	rats	6	156.66	17.00	0.0475	2.868	0.431
injected with pro- gesterone							
S. E. ±				1.077	0.001	0.241	P > 0.05
<i>Rats Given :</i>							
Lyndiol	2.5	6	100	12.55	0.053	4.30	8.12
S. E. ±				0.242	0.002	0.04	P < 0.001
Lyndiol	5	6	115	14.3	0.059	4.13	5.381
S. E. ±				0.274	0.002	0.123	P > 0.001
Gynanovlar		6	95	15.36	0.050	3.30	1.685
S. E. ±				0.173	0.001	0.071	P > 0.1
Ovulen		6	130	15.85	0.050	3.2	0.769
S. E. ±				0.303	0.002	0.192	P < 0.5
Eugynon		6	109.8	15.82	0.058	3.68	3.777
S. E. ±				0.303	0.002	0.045	P < 0.01

t* test : Between the alkaline phosphatase activity per gm. cortical tissue of normal female rats and treated ones.

TABLE 2

The Synthetic Progestin-Oestrogen Preparations used in this Study

	Progestational Compound Name	Dose (mgm.)	Oestrogen Dose (mgm.)		Trade Name
			Mestranol	Ethinyl- oestradiol	
19 Nortestosterone Derivatives.	Norgestrel.				
	d 1-13-ethyl-17-ethinyl-17 B-hydroxy-4 gonene-3-one	0.5	—	0.05	Eugynon
	Ethyndiol diacetate.				
	3 acetoxy 17 alpha ethinyl 19 nortestosterone acetate	1	0.1	—	SC 11800
	Lyncoestrenol.				
	3 desoxy 17 alpha ethinyl 19 nortestosterone	2.5	0.075	—	Lyndiol 2.5
	Norethisterone acetate.				
	17 alpha ethinyl 19 nortestosterone	3	—	0.05	Gyn-anovlar
	Norethisterone acetate.				
	17 alpha ethinyl 19 nortestosterone	4	—	0.05	Anovlar
	Lyncoestrenol.				
	3 desoxy 17 alpha ethinyl nortestosterone	5	0.15	—	Lyndiol 5

PATHOLOGICAL FINDINGS

No pathological findings were detected in animals given the different contraceptive pills for a period of 2 weeks. When the rats were given the pills for 6 weeks the following pathological findings were observed.

The Liver (Fig. 1 and 2) :

It showed marked congestion, prominence of Kupffer cells as well as a marked variation in the shape and size of nuclei. Some of these nuclei showed mitotic figure. Some of the vessels were blocked with thrombi and numerous detailed bile canaliculi were also seen.

The Spleen (Fig. 3) :

It showed thickening of the capsule, prominent trabeculae extreme congestion, somewhat thickened arterioles as well as strands of fibrosis and hyperplasia of Malpighian follicles. Some giant cells were also observed.

The Lungs (Fig 4 and 5) :

Congestion was a constant finding. In some rats given lyndiol, interalveolar rupture of septa, thickening of the pleura as well as areas of pulmonary fibrosis and thickening and narrowing of the vessels were observed.

The rest of the organs showed marked congestion. These findings were generally detected in all rats given the different contraceptive preparations. However, it was marked with lyndiol 2.5.

DISCUSSION

It is becoming evident that hormones exert their regulatory effects on tissues of end organs by influencing their rate of enzymatic processes. Hertz (1954) has shown that oestrogens require the presence of folic acid, which is known to be a prothetic group of an enzyme, to produce growth of the chick's oviduct.

Changes in the concentration of certain enzymes as succinic dehydrogenase in the ovaries of rats during the different stages of the reproductive cycle have been demonstrated by Biddulph et al. (1946).



FIG. 1 : Section in the liver of rat given lyndiol 2.5 (1 pill/day) for 6 weeks ; showing liver thrombosis, marked congestion, thrombosis in one of the vessels. Stained by H and R. (X64).

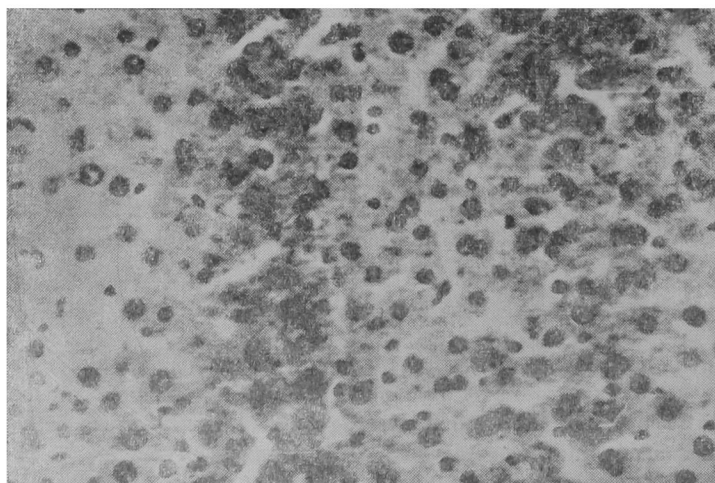


FIG. 2 : Section in the liver of rat given lyndiol 5 (1 pill/day) for 6 weeks ; showing mitosis, variation in the shape and size of nuclei. Stained by H and E. (X 128).

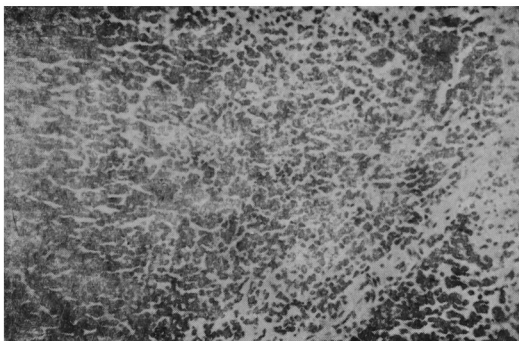


FIG. 3 : Section in the spleen of rat given lyndiol 2.5 (1 pill/day) for 6 weeks ; showing hyperplasia of Malpighia follicles, thickened tuberculae. Stained by H and E. (X 64).

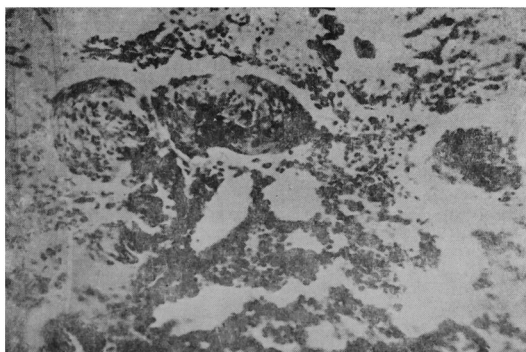


FIG. 4 : Section in the lung of rat given lyndiol 2.5 (1 pill/day) for 6 weeks ; showing thickened vessels, narrowed lumen, interstitial pulmonary fibrosis. Stained by H and E. (X 320).

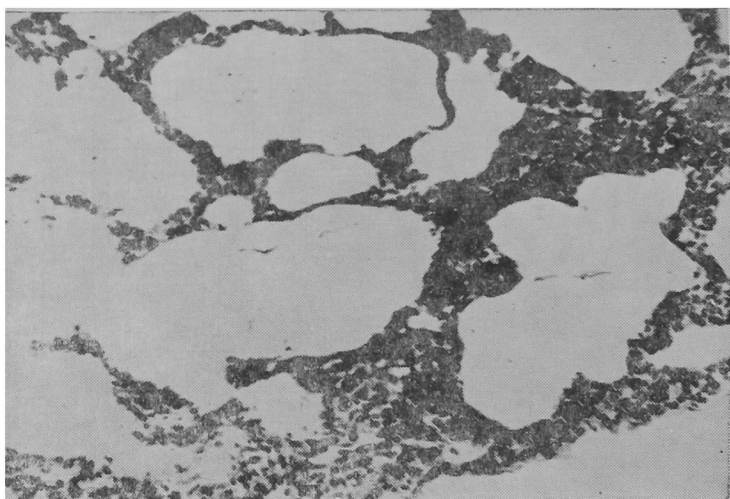


FIG. 5 : Section in the lung of rat given lyndiol 5 (1 pill/day) for 6 weeks ; showing thickened plura, interstitial pulmonary fibrosis. Stained by H and E. (X 64).

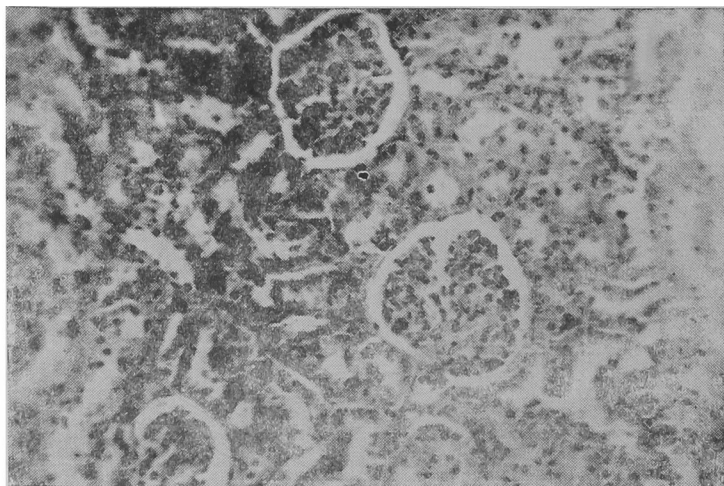


FIG. 6 : Section in the kidney of rat given gynlanovlar (1 pill/day) for 6 weeks ; showing severe congestion. Stained by H and E. (X 64).

Fluctuations in the alkaline phosphatase activity of the uterus have been correlated with the reproductive cycle in mice and in primates (Atkinson and Elftman, 1946 ; Atkinson and Engle, 1947).

In a previous study by Zaki et al. (1965), a correlation between the adrenocortical alkaline phosphatase activity and steroidogenesis was put forward. It was stated that in the rat, the hypertrophied cortical tissue in cases of pregnancy, unilateral adrenalectomy as well as after ACTH injection, showed a higher alkaline phosphatase activity. In the present investigation, when studying the effect of female sex hormones on the adrenocortical activity using the alkaline phosphatase parameter, the data obtained showed clearly a definite stimulatory effect of oestradiol dipropionate on the adrenal cortex. This was clear when using ovariectomized animals, in which the decrease in the activity of the enzyme found after ovariectomy was regained and the activity exceeded that of normal rats by the injection of oestradiol dipropionate. Kitay (1965), studying the effects of oophorectomy and various doses of oestradiol 17-beta on corticosterone production by rat adrenal tissue in vitro, found that oophorectomy after puberty resulted in a significant reduction in adrenal corticosterone production. Studies on the in-vitro effect of oestradiol on 11beta hydroxylation by homogenates of rat adrenals were carried out by Domenico and Nelson (1970). They reported an increase in 11 beta hydroxylation with the addition of oestrogen when the reaction was supported by succinate. They also added that a previous study indicated increased 11 beta hydroxylation by adrenals from animals treated in-vivo by oestrogens.

Surina (1970) found also that administration of oestradiol dipropionate to sexually mature male rats, caused hypertrophy of the adrenal glands, increased the activity of 3B- α -dehydrogenase (the enzyme responsible for the transformation of pregnenolone to progesterone), RNA content, and reduced the amounts of ascorbic acid in the cells of the fasciculata and reticularis in the same way as it occurred under the effect of ACTH. He concluded that the similarity of morphological changes occurring in the adrenal cortex of rats under the effect of oestradiol and ACTH confirms the fact that a rise in the activity of the adrenal cortex under conditions of oestrogen administration was caused by excessive discharge of endogenous ACTH into the blood. Stabler and Ungar (1970) found that 20 alpha hydroxysteroid dehydrogenase which is found in the female but not the male adrenal glands of mice, was activated by injection of oestradiol into adult mice.

In the present investigation progesterone, when injected intramuscularly in ovariectomized rats, was found to have no effect on the alkaline phosphatase activity of the adrenals, in spite of the fact that it is the precursor of corticosteroids. However, Larina (1963) found that progesterone when administered together with ACTH caused a rise in the output of all steroids produced by slices of rabbit's adrenals more than that when ACTH was administered alone. That ovarian hormones have a stimulatory effect on the adrenal glands was shown by the sexual dimorphism detected as regards the adrenals of some animals (Antistiban, 1962), or during pregnancy (Roux et al. 1962 ; Ricci et al. 1963, and Zaki et al. 1964).

Cassano et al. (1958) and Angervall and Lundi (1960) put forward that the hypertrophy during pregnancy is due to increased ACTH in all phases of gestation. Roux et al. (1962) found high metabolic activity of the adrenals of pregnant guinea pigs as compared to non-pregnants.

In spite of the fact that pregnancy constitutes a type of stress accompanied by increased ACTH as stated before, the combined effects of ACTH and the high concentration of progesterone may be other factors responsible for the increased adrenocortical activity under such conditions.

In short, during pregnancy or when oestrogen and/or progesterone are administered, the adrenocortical activity under such conditions on the combined actions of these hormones and their indirect effects on the pituitary ACTH. One can say that a new state of balance between higher centers, the pituitary gland, and the hormone secretory glands is reached during each phase of pregnancy. In this respect, it must be mentioned also that during pregnancy, besides the increased secretion of pituitary gonadotrophins and ACTH, the placental hormones, mainly chorionic gonadotrophins, circulating in the blood stream constitute additional factors and that these hormones may be responsible in part for the changes occurring in the adrenal cortex.

The synthetically prepared combinations used nowadays on a large scale for contraception, have a different role in this respect. First, the relative amounts of progestogens and oestrogens used in the different combinations are variable, and whether these synthetic preparations exert their actions as that of the natural endogenous hormones or not ? The second is that administration of these combinations is known to

affect the endocrine control of ovulation through inhibition of the pituitary gonadotrophins. The inhibition of the secretion of the endogenous oestrogens and the marked decrease in progesterone accompanying ovulation inhibition caused by some contraceptive pills, together with the high oestrogenic effects of some of these preparations constitute a new hormonal environment which, beyond doubt, has its effect on the adreno-cortical functional activity. From the present investigation, when different types of contraceptive pills were administered to rats for two weeks, a variable state of hyperactivity was found with any of the combinations used especially with lyndiol 2.5 and lyndiol 5.

Changes in adrenal size and function in rats caused by progestational compounds were mentioned by Edgren et al. (1959). They found that 6—methyl—17—acetoxy progesterone caused marked adrenal atrophy and some depression of adrenal function, while 17 alpha—ethiny1—19—nortestosterone caused no significant decrease in adrenal size.

Higashiyama (1965) studying the effect of different kinds of synthetic progestogens on the weight of endocrine organs in female rats, stated that 17—acetoxy progesterone and 6 methyl—17 acetoxy progesterone suppressed the hypothalamo—hypophysial adrenal system and inhibit ACTH production and secretion.

Baker et al. (1965) studying the histological response of the adrenal cortex to 17—ethiny1—17—hydroxy5—10—estrine—3—one in female rats, came to the conclusion that after a daily dose of 1.5 mg. /100 mg. B.W. given for 27—43 days, lipids were depleted from all adrenocortical zones, and the parenchymal cells of the zona glomerulosa became smaller. Adrenal suppression by medroxy progesterone was mentioned by Fekete and Szeberényi (1965).

David and Kovacs (1966) & 1967) stated that when female rats were given a daily injection of one mg. of oestrone acetate for ten days, the adrenal weight increased and the corticosterone concentration in the adrenal venous blood decreased. They concluded that the treatment with oestrone decreases the sensitivity of the adrenal to the stimulatory effect of ACTH and the secretion of corticosterone.

Sarkar (1969) studying the effect of some corticosteroids on intact female rats, stated that oestradiol prevented any alteration in adrenal cholesterol concentration caused by corticosteroids.

In the present investigation, a significant increase in the adrenocortical alkaline phosphatase activity was detected especially with lyndiol 2.5, and lyndiol 5. Thus the question raised was whether these variable degrees of hyperactivity of the cortical tissue as detected from alkaline phosphatase activity were accompanied by structural changes in the adrenal or not? At first it was assumed that the contraceptive pills used, exert a condition resembling that of pregnancy. But from a preliminary histological study done by the same authors, the histological pictures of all adrenals after two weeks of steroid contraceptive pill administration showed great resemblance to adrenals of ACTH stimulated rats, mainly that the glomerulosa was very much reduced or even absent. It was replaced by a very cellular zone consisting of small closely packed cells with small rounded nuclei. The fasciculata was markedly enlarged and the capillaries in the inner fasciculata were congested. There was no evidence of enlargement in the size of the cells or nuclei. The reticularis formed also a wide prominent zone in which the cells were separated by congested and dilated sinusoids. This picture is very similar to that of ACTH stimulated adrenals rather than to the histological pictures of adrenals of pregnant rats. Oral contraceptives may participate in inducing a state of stress. However, it must be noted that the dose given was high, the duration of the experiment was two weeks. Examining other tissues as liver, spleen, lungs, ... etc., no pathological changes were found. But, when the experiment was prolonged to six weeks, pathological changes were apparent, especially with lyndiol 2.5.

Oestrogens were found by Nicol et al. (1951 & 1965) to be the strongest RES stimulants. Cyclical variation in the RE activity are known to occur during the oestrous cycle and pregnancy in the rat and mouse (Nicol et al. 1964, 1965). Fibrosis of the liver, lungs, spleen and coronary vessels were observed in guinea pigs after administration of ethinyl oestradiol in high doses (El Heneidy, 1966). So the pulmonary fibrosis and the thickening and the narrowing of vessels detected in the animals treated with contraceptive pills in this study may be due to the mentioned effect of the oestrogenic component of the pill on the reticuloendothelial system.

In a preliminary study before the present investigation, it was found that using small doses for short periods were stimulatory, but higher doses for prolonged periods were suppressive to the adrenals. This may be due mainly to adrenal exhaustion. The effect of duration

and dose of each component of the pill on the adrenal histology, alkaline phosphatase activity, as well as corticosterone content are now under investigation.

ABSTRACT

The present work was carried out to study the effects of female sex hormones, oestradiol and progesterone as well as some of the different synthetically prepared steroid combinations, used on a large scale for contraception, on the adrenocortical activity in experimental animals.

In a previous study on rats the adrenocortical alkaline phosphatase activity was found to be a good and reliable parameter reflecting the cortical activity. So the present experimental study included the biochemical determination of this enzyme in the adrenal cortex of normal female rats, ovariectomized rats and ovariectomized rats injected with oestradiol or progesterone. Also adrenocortical alkaline phosphatase activity was determined in groups of rats given different contraceptive pills (lyndiol 2.5, lyndiol 5, gyn—anovlar, eugynon and ovulen) one tablet/rat/day for a period of two weeks.

A pathological study was also carried out on the rats given the contraceptive pills for two weeks. As no pathological finding were detected, the experiment was extended to six weeks. After such a period the animals started to die and definite pathological changes were detected mainly in the liver, spleen and lungs.

From the results obtained, it was clear that ovariectomy resulted in a decrease in the activity of the adrenal cortex. Injection of progesterone in these animals was without effect, while oestradiol injection caused a significant increase in the activity of alkaline phosphatase of the adrenal cortex to values exceeding that of normal females.

As regards the effect of the different contraceptive pills on the adrenocortical alkaline phosphatase activity the data obtained showed that there was an increase in the activity of the adrenal cortex with any of the pills. The increase was highest with lyndiol 2.5 and lyndiol 5 and least with ovulen. The increase with lyndiol 2.5 or lyndiol 5 was about double that of eugynon, four times that of gynanovlar and about six times that of ovulen.

A preliminary histological study revealed a striking similarity between the glands subjected to the effects of oral contraceptives and those stimulated by ACTH mainly in the narrowing or even absence of the zona glomerulosa.

The pathological study in the groups of animals given the pills for six weeks revealed marked congestion of all organs and in some animals given lyndiol 2.5 or lyndiol 5, the liver showed prominence of kupffer cells, variation in the size and shape of nuclei, dilated canaliculi and some vessels blocked with thrombi were observed.

The spleen showed thickening of the capsule, congestion, thickened arterioles as well as hyperplasia of Malpighian follicles.

The lungs showed congestion and in some of the animals given lyndiol interalveolar rupture of septa, thickening of the pleura as well as areas of pulmonary fibrosis were observed.

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