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Oral Effectiveness of the Dimethyl Ether of Diethylstilbestrol and of Various Steroid Hormones on the Mammary Glands of Mice and Rabbits

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INTRODUCTION

In 1938 Dodds and associates culminated several years of investigation on the estrogenic activity of various synthetic compounds with the introduction of 4, 4′-dihydroxystilbene or "stilbestrol" (Dodds, Goldberg, Lawson and Robinson). This was quickly followed by the more potent derivative 4, 4′-dihydroxy-α:β-diethylstilbene or "diethylstilbestrol" (Dodds, Lawson and Noble, 1938). The former compound, being the mother substance of a series of estrogenic agents, was named stilbestrol by Dodds and the terminology was adopted by the Council on Pharmacy and Chemistry of the American Medical Association (1941). However, the more potent diethyl derivative displaced the use of the original compound, but the term stilbestrol persisted. As a result the terms stilbestrol and diethylstilbestrol are used interchangeably at present to refer to the diethyl derivative.

Compounds of this series possess several remarkable features. They are prepared from common laboratory reagents, hence are relatively inexpensive. When administered parenterally they are generally as potent or more potent than the natural estrogens, weight for weight (Sondern and Sealey, 1940; Sealey and Sondern, 1941; Morrell, 1941). In addition, when administered orally, they undergo very little loss of potency, apparently as a result of their ability to resist inactivation by the liver (Allen, 1941).

The importance of the stilbestrol series of compounds to clinical medicine is obvious. In addition, the introduction of these compounds placed the estrogenic treatment of domestic animals on a practical and economical basis for the first time.

In ruminants, the relative effectiveness of the stilbestrol compounds administered orally may be considerably less than parenterally, possibly because of inactivation in the rumen. As measured by the lactation inhibiting effect, diethylstilbestrol orally administered to goats was only about 1 per cent as effective as when administered subcutaneously (Mixner, Meites and Turner, 1944). In the cow, oral administration of synthetic estrogens in the drinking water or feed resulted in the utilization of less than 10 per cent of the dose given (Folley and Malpress, 1944). Oral administration of synthetic estro-

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gens may nevertheless be feasible in ruminants also, by virtue of the relative inexpensiveness of such compounds.

In view of known stimulating effects of estrogens on growth of the mammary glands, oral use of diethylstilbestrol and its derivatives as a mammary stimulant in laboratory and domestic animals is of considerable interest.

In 1939, Bishop et al. demonstrated the effectiveness of orally administered diethylstilbestrol in the induction of mammary proliferation by studying biopsy specimens taken before and after oral administration of 280 milligrams of diethylstilbestrol to a castrate woman.

Shimkin and Grady (1940) administered diethylstilbestrol in oil twice weekly by stomach tube to male mice of the C_3H strain and induced breast tumors. This tumor development following estrogen treatment is known to be associated with development of the otherwise rudimentary mammary glands.

In considering oral administration of hormonal substances to experimental animals, use of a stomach tube has obvious drawbacks. Inclusion of the substance into the everyday diet of the animal is not only easier, but permits a more continuous and sustained intake throughout the day.

Noble (1939a) was first to administer diethylstilbestrol in drinking water to rats. Unfortunately, mammary growth was not studied. Lewis and Turner (1941a), however, administered diethylstilbestrol to male mice in drinking water and observed extensive mammary duct proliferation similar to that produced by injection. This required approximately six times as much diethylstilbestrol as by injection.

EXPERIMENTAL

The present work was undertaken to establish some standard of dosage for the development of the mammary glands of experimental animals by administration of estrogen in the feed. Dimethyl ether of diethylstilbestrol was used. This compound has been shown to be one of the most potent estrogens for oral administration to the fowl (Japp, 1945).

In both mice (Emmens, 1939) and rats (Sondern, Sealey and Kartsonis, 1941) the dimethyl ether appears to be required in five times the dosage of diethylstilbestrol to elicit unit assay response by oral administration. However, although the diethers of diethylstilbestrol have higher threshold values for unit response, the action of a single administration is more prolonged than in the case of diethylstilbestrol.

Sondern, Sealey, and Kartsonis (1941) have reported that the oral estrogenic potency of the diethers of diethylstilbestrol, as measured by vaginal cornification in the rat, decreases rapidly as the length

of the chain in the alkoxy group increases (Table 1). The material was administered in water or alcohol, and the authors suggested that the results may have been due in part to a decrease in the absorption from the intestinal tract as a result of the lower water solubility of the higher members of the series. Japp (1945) reported that the

TABLE 1.--VAGINAL RESPONSE OF OVARIECTOMIZED RATS TO ORALLY ADMINISTERED DIETHYLSTILBESTROL AND THREE DIETHERS OF DIETHYLSTILBESTROL
(Sondern, Sealey and Kartsonis, 1941)

Compound	Dose (Micrograms)	Per cent in Estrus
Diethylstilbestrol	3	60
Dimethyl Ether of Diethylstilbestrol	15	60
Diethyl Ether of Diethylstilbestrol	30	60
Dipropyl Ether of Diethylstilbestrol	100	25

estrogenic activity of the dimethyl ether of diethylstilbestrol and various other synthetic estrogens in fowl was greatly increased by solution in soybean oil before dispersion in the feed. However, Turner (1947) observed no increase in the comb stimulating potency of methyl testosterone in chicks when added to the feed in oil solution as compared to mechanical dispersion of the finely divided powder in the feed.

In the present work, the dimethyl ether of diethylstilbestrol was first dissolved in soybean oil. This solution was next incorporated into a small portion of the ground grain feed, and all lumps broken down by rubbing between the palms of the hands. This grain was next incorporated into the total amount of feed by a mechanical mixer.

In addition to estrogen alone, various steroids having progestational activity were administered orally in combination with dimethyl ether of diethylstilbestrol in an attempt to enhance alveolar development. These were progesterone, pregneninolone (ethinyl-testosterone or anhydro-hydroxy-progesterone), desoxycorticosterone acetate, pregnenolone, and a progesterone concentrate supplied by the Soya Products Division of the Glidden Company.

Progesterone, pregneninolone, and desoxycorticosterone acetate have been reported to be the most effective stimulants, in the order named, of both uterine progestational proliferation (Selye and Masson, 1943) and mammary alveolar proliferation (Mixner and Turner, 1942). With respect to alveolar proliferation (when administered with estro-

gen), pregneninolone and desoxycorticosterone acetate were one-half and one-third as potent, respectively, as progesterone. With respect to uterine proliferation, they were approximately one-tenth as potent as progesterone. Emmens and Parkes (1939) have likewise reported that pregneninolone is about one-tenth as active as progesterone in stimulating progestational proliferation when given by injection, but equally potent by mouth or by injection.

Mixner and Turner (1942) have reported that acetoxy-pregnenolone is approximately one-sixteenth as active as progesterone for alveolar stimulation. Since Selye and Masson (1942, 1943) have reported pregnenolone to be more than twice as active as acetoxy-pregnenolone for uterine proliferation, and in view of apparent correlation between uterine and mammary effect, pregnenolone was used in the present experiments.

The progesterone concentrate supplied by the Glidden Company was a very thick syrup containing a dispersion of very finely divided crystals, presumably progesterone. The manufacturer stated it contained from 200 to 250 International Units per gram by McPhail assay. It is apparently an intermediary product in the manufacture of crystalline progesterone.

RABBIT FEEDING EXPERIMENTS

Eleven albino rabbits of body weights ranging from 1855 to 3147 grams were divided into four experimental groups. Groups 1 and 2 were each composed of two males and two females, one of each sex being castrated and the other intact. Group 3 was composed of a male and a female, each castrated. Group 4 was composed of a single control rabbit with one testicle removed. The other testicle could not be found at the time of operation, but at the end of the experimental period was found in the abdominal cavity. At the start of the experiment mammary biopsies were removed from all animals. All males presented the typical rudimentary gland with the ducts confined to the area immediately beneath the teat. Of the females the mammary biopsies ranged from glands in which the ducts barely extended beyond the base of the teat to others with bare duct systems of good size. The female rabbit of Group 3 presented a few small clumps of alveoli.

Estrogen Alone. From four to seven days following removal of biopsies and castration (where performed), the rabbits were started on experiment. Body weights were recorded once weekly throughout the course of the experiments. Groups 1, 2, and 3 received 0.2, 0.6, and 1.8 milligrams respectively of the dimethyl ether of diethylstilbestrol in each kilogram of grain mixture, the sole source of feed. Group 4 received the same grain mixture without added estrogen.

(The dimethyl ether of diethylstilbestrol will be referred to simply as 'estrogen' throughout this section).

Average daily feed intake was estimated. This was approximately fifty grams per day per rabbit. This value undoubtedly increased during the six month period of the experiment as the animals increased in body weight. On the basis of fifty grams of feed intake per day the average daily intake per rabbit of the dimethyl ether of diethylstilbestrol would have been ten, thirty, and ninety micrograms for Groups 1, 2, and 3, respectively.

After fourteen weeks, a second biopsy was taken from a different mammary gland of each rabbit receiving estrogen. Since the biopsy did not always include the entire mammary gland, extension of the duct system could not always be determined, unless the original extent of the duct system was small enough to be entirely included in the area of the biopsy.

All results are presented graphically in Tables 2, 3, 4, and in Figures 1 to 19. After fourteen weeks, 0.2 milligram of estrogen per kilogram of feed produced no mammary growth in the male rabbits. In the castrate female, duct extension was not determinable because of the original large size of the duct system. In the non-castrate female, some extension of the duct system occurred; however, this may have been a result of the animals own ovaries. Since no growth was produced in the male rabbits, this level must be regarded as insufficient for mammary growth.

The 0.6 and 1.8 milligram per kilogram levels both produced definite extension of the duct system (Figures 1, 2, 3, 4, 8, 10). The higher level appeared to produce more complete stimulation with a fair amount of alveolar development being found in the castrate female which died after only seven weeks treatment. (Figure 10).

Since the animals of Group 1 showed no stimulation at the 0.2 milligram level, they were given 5.4 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed (estimated daily intake of 270 micrograms per rabbit). After thirty-two days, each animal of Group 1 had a third mammary biopsy removed. Good duct extension had occurred, but no alveolar development (Figures 11, 13, 15, 17).

Estrogen with Progesterone Concentrate. Within three to four days after Group 2 and Group 3 were biopsied (at the end of fourteen

(Continued on page 15)

TABLE 2.--MAMMARY RESPONSE OF ALBINO RABBITS TO THE DIMETHYL ETHER OF DIETHYLSTILBESTROL AND A PROGESTERONE CONCENTRATE* ADMINISTERED IN THE FEED -- GROUP 1

Animal	Biopsy taken at start of experiment.	Biopsy after 14 weeks of 0.2 mg. dimethyl ether of diethylstilbestrol per kg. of feed.	Biopsy after additional 32 days of 5.4 mg. dimethyl ether of diethylstilbestrol per kg. of feed.	Necropsy after additional 4 weeks of 5.4 mg. dimethyl ether of diethylstilbestrol plus 500 mg. of progesterone concentrate per kg. of feed.
Castrate male, No. 1	Ducts not extending beyond base of teat.	Ducts not extending beyond base of teat.	Extension of duct system. Figure 11.	Good extension of duct system with fair alveolar development and lactation in all glands. Figure 12.
Non-castrate male, No. 13	Ducts not extending beyond base of teat.	Ducts not extending beyond base of teat.	Extension of duct system. Figure 13.	Full extension of duct system with good alveolar development. Figure 14. Testes atrophic.
Castrate female, No. 5	Bare duct system.	Bare duct system. Extension not determinable.	Large duct system. Figure 15.	Full extension of duct system with good alveolar development and some lactation. Figure 16.
Non-castrate female, No. 9	Short bare ducts.	Some duct extension to bare duct system.	Large duct system. Figure 17.	Full extension of duct system with fair alveolar development. No lactation. Figure 18.

* 20 to 25 per cent progesterone.

TABLE 3.--MAMMARY RESPONSE OF ALBINO RABBITS TO THE DIMETHYL ETHER OF DIETHYLSTILBESTROL AND A PROGESTERONE CONCENTRATE* ADMINISTERED IN THE FEED -- GROUP 2

Animal	Biopsy taken at start of experiment	Biopsy after 14 weeks of 0.6 mg. dimethyl ether of diethylstilbestrol per kg. of feed.	Necropsy after additional 23 days of 0.6 mg. dimethyl ether of diethylstilbestrol plus 500 mg. of progesterone concentrate per kg. of feed.
Castrate male, No. 2	Ducts not extending beyond base of teat.	Sacrificed because of spinal injury. Very good extension of duct system. Figure 1.	
Non-castrate male, No. 12	Ducts not extending beyond base of teat.	Some extension of duct system. Figure 2.	Small duct system with fair alveolar development. Figure 5. Testes normal.
Castrate female, No. 6	Bare duct system.	Bare duct system. Extension not determinable. Figure 3.	Full extension of duct system with slight alveolar development. Figure 6.
Non-castrate female, No. 10	Ducts barely extending beyond base of teat.	Some duct extension to bare duct system. Figure 4.	Good duct extension with good alveolar development. Figure 7.

* 20 to 25 per cent progesterone.

TABLE 4.--MAMMARY RESPONSE OF ALBINO RABBITS TO THE DIMETHYL ETHER OF DIETHYLSTILBESTROL AND A PROGESTERONE CONCENTRATE* ADMINISTERED IN THE FEED -- GROUP 3

Animal	Biopsy taken at start of experiment.	Biopsy after 14 weeks of 1.8 mg. dimethyl ether of diethylstilbestrol per kg. of feed.	Necropsy after additional 23 days of 1.8 mg. dimethyl ether of diethylstilbestrol plus 500 mg. of progesterone concentrate per kg. of feed.
Castrate male, No. 3	Ducts not extending beyond base of teat.	Good extension of duct system. No alveolar development. Figure 8.	Full extension of duct system with good alveolar development and some lactation in all glands. Figure 9.
Castrate female, No. 8	Good duct system with a few small clumps of alveoli.	Died at 7 weeks. Duct extension not determinable. Some alveolar development. Figure 10.	

* 20 to 25 per cent progesterone.

Figures 1 to 4. Mammary glands of rabbits fed 0.6 milligrams of the dimethyl ether of diethylstilbestrol per kilogram of feed for fourteen weeks. (.8X).

Figure 1. Necropsy. Castrate male rabbit, No. 2. Sacrificed because of spinal injury. Very good extension of duct system.

Figure 2. Biopsy. Non-castrate male rabbit, No. 12. Some extension of duct system.

Figure 3. Biopsy. Castrate female rabbit, No. 6. Bare duct system. Extension not determinable.

Figure 4. Biopsy. Non-castrate female rabbit, No. 10. Some duct extension to bare duct system.

Figures 5 to 7. Mammary glands of rabbits fed 0.6 milligrams of the dimethyl ether of diethylstilbestrol per kilogram of feed for fourteen weeks, followed by 23 days of the same level of estrogen plus 500 milligrams of progesterone concentrate per kilogram of feed. (.8X).

Figure 5. Necropsy. Non-Castrate male rabbit, No. 12. Small duct system with fair alveolar development.

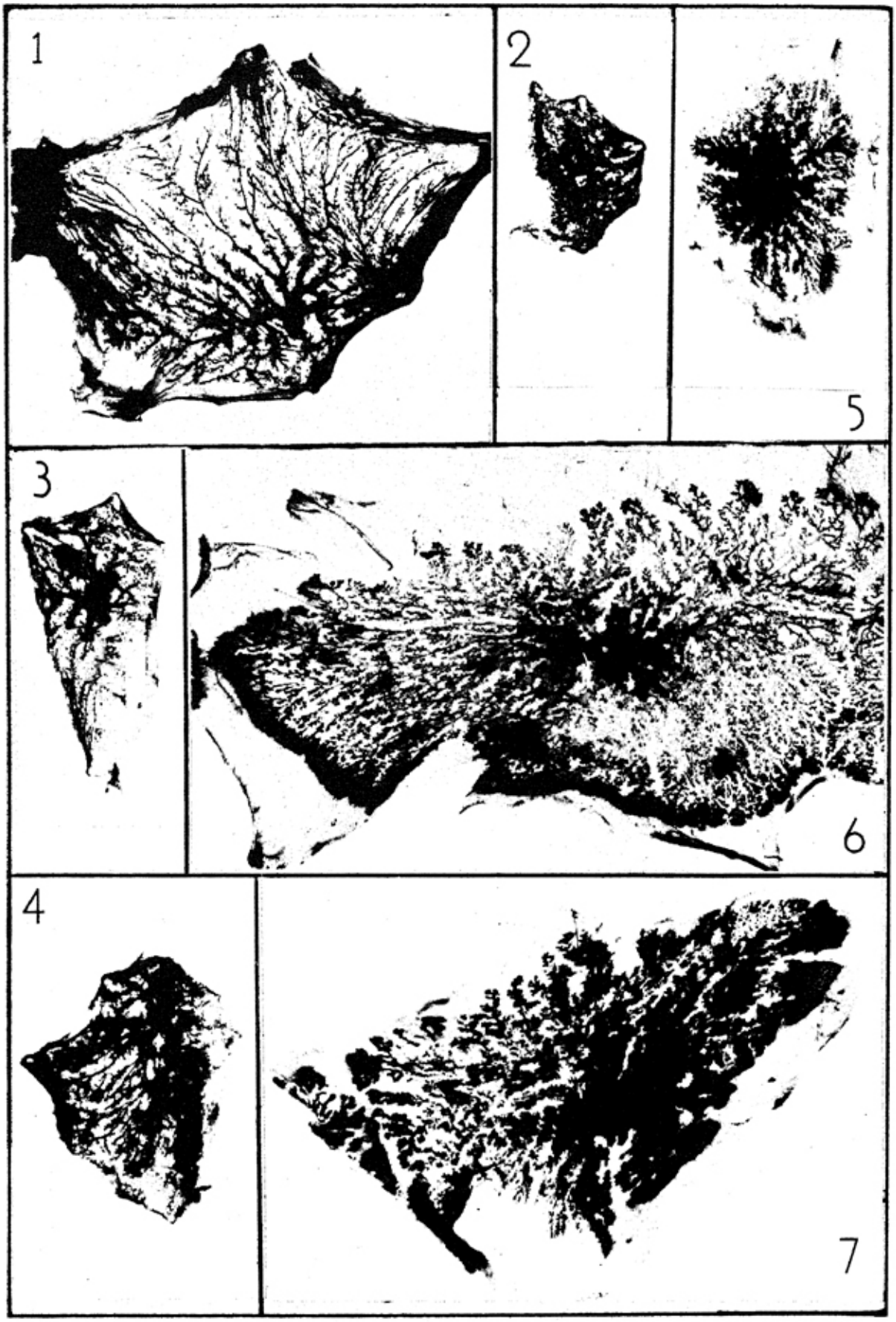
Figure 6. Necropsy. Castrate female rabbit, No. 6. Full extension of duct system with slight alveolar development.

Figure 7. Necropsy. Non-castrate female rabbit, No. 10. Good duct extension with good alveolar development.

Figure 8. Biopsy of mammary gland of castrate male rabbit No. 3 after 14 weeks of 1.8 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed. Good extension of duct system. No alveolar development. (.8X).

Figure 9. Necropsy of mammary gland of castrate male rabbit No. 3 after 14 weeks of 1.8 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed followed by twenty-three days of the same level of estrogen plus 500 milligrams of progesterone concentrate per kilogram of feed. Full extension of duct system with good alveolar development and some lactation in all glands. (.8X).

Figure 10. Necropsy of mammary gland of castrate female rabbit No. 8 at time of death after 7 weeks of 1.8 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed. Duct extension not determinable. Some alveolar development. (.8X).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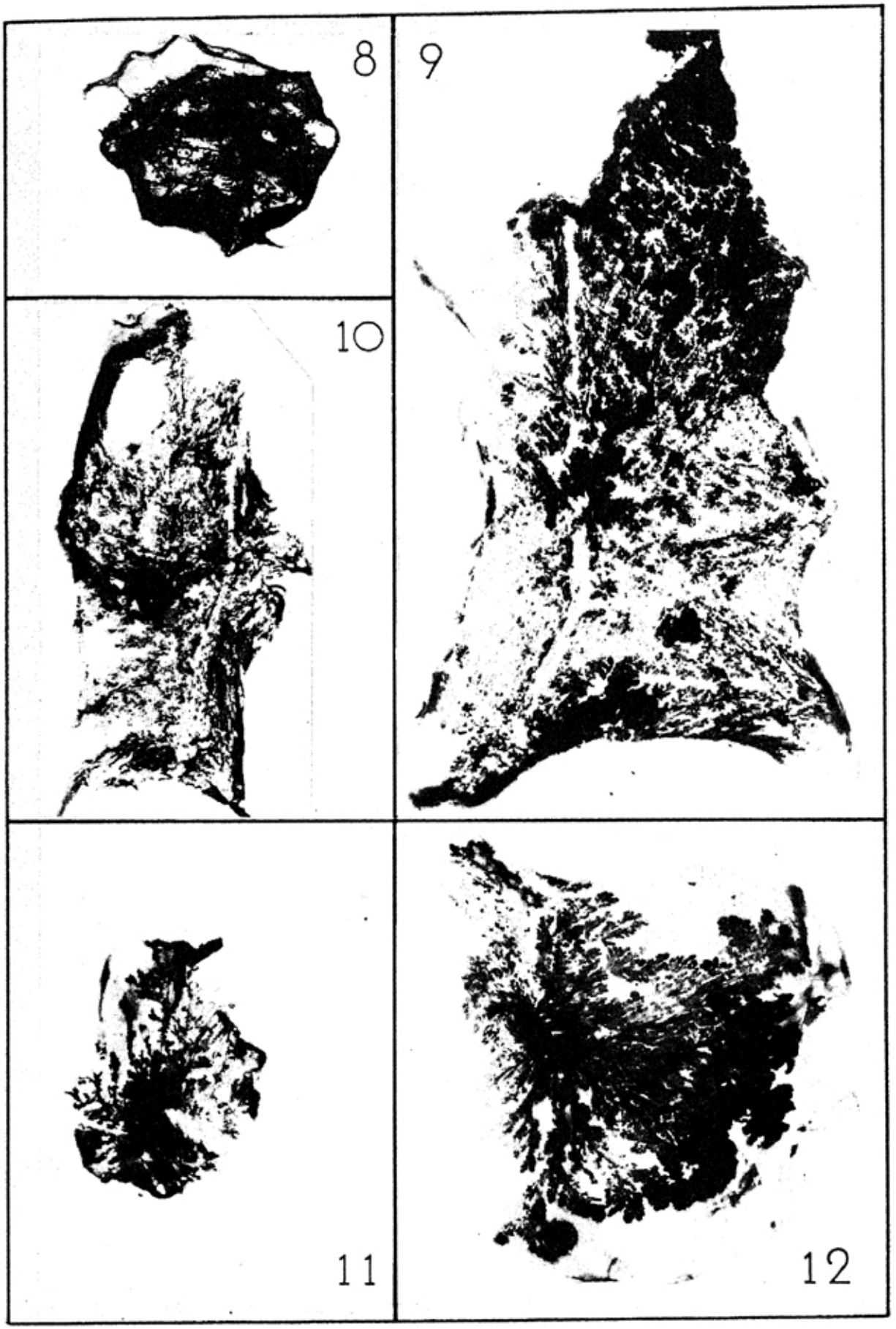
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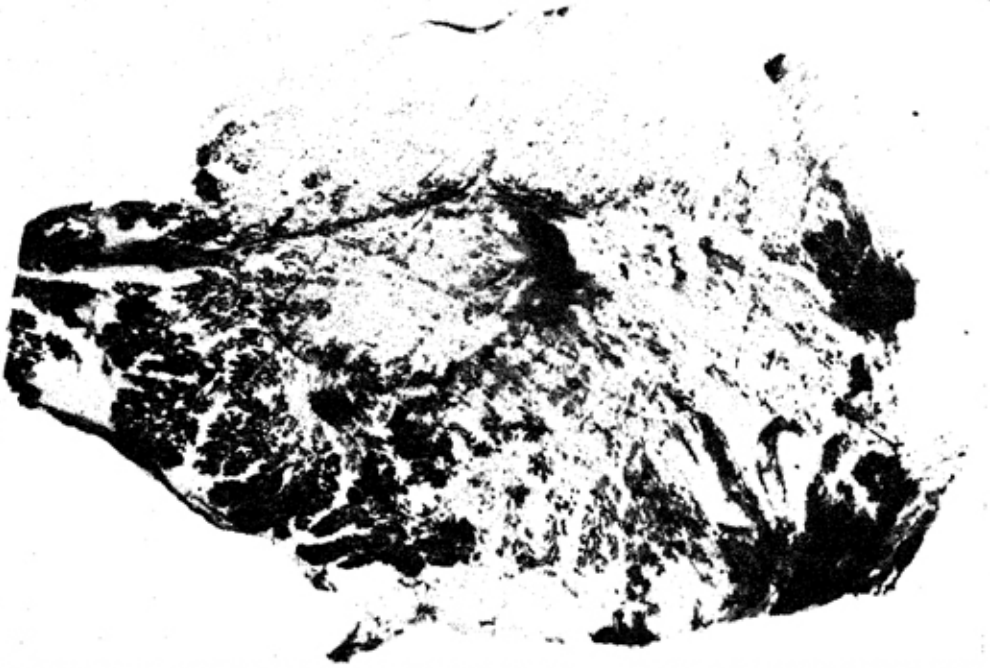

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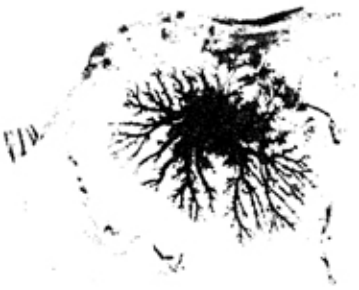


Figures 11, 13, 15 and 17. Mammary glands of rabbits fed 5.4 milligrams of the dimethyl ether of diethylstilbestrol per kilogram of feed for thirty-two days. (.8X).

Figure 11. Biopsy. Castrate male rabbit. No. 1. Extension of duct system.

Figure 13. Biopsy. Non-castrate male rabbit. No. 13. Extension of duct system.

Figure 15. Biopsy. Castrate female rabbit, No. 5. Large duct system.

Figure 17. Biopsy. Non-castrate female rabbit, No. 9. Large duct system.

Figures 12, 14, 16 and 18. Mammary glands of rabbits fed 5.4 milligrams of the dimethyl ether of diethylstilbestrol per kilogram of feed for thirty-two days, followed by four weeks of the same level of estrogen plus 500 milligrams of progesterone concentrate per kilogram of feed. (.8X).

Figure 12. Necropsy. Castrate male rabbit, No. 1. Good extension of duct system with fair alveolar development and lactation in all glands.

Figure 14. Necropsy. Non-castrate male rabbit, No. 13. Full extension of duct system with good alveolar development.

Figure 16. Necropsy. Castrate female rabbit, No. 5. Full extension of duct system with good alveolar development and some lactation.

Figure 18. Necropsy. Non-castrate female rabbit, No. 9. Full extension of duct system with fair alveolar development. No lactation.

Figure 19. Necropsy of mammary gland of control male rabbit, No. 4 with one testis removed and the other undescended. This represents a slight extension of the duct system over that usually found in normal male rabbits. (.8X).

weeks of estrogen feeding), and without having discontinued estrogen feeding, 500 milligrams of the progesterone concentrate was added to each kilogram of feed in addition to the same level of dimethyl ether of diethylstilbestrol as previously. Similarly, the day after removal of biopsies revealed that good duct extension had occurred in Group 1 as a result of thirty- two days of feeding at the 5.4 milligram estrogen level, progesterone concentrate was added to this ration. Again it was added at the rate of 500 milligrams per kilogram of feed in addition to the pre-existing level of estrogen. The three experimental groups were now receiving estrogen and progesterone at the following levels:

Group 1—5.4 milligrams of dimethyl ether of diethylstilbestrol plus 500 milligrams of progesterone concentrate per kilogram of feed.

Group 2—0.6 milligrams of dimethyl ether of diethylstilbestrol plus 500 milligrams of progesterone concentrate per kilogram of feed.

Group 3—1.8 milligrams of dimethyl ether of diethylstilbestrol plus 500 milligrams of progesterone concentrate per kilogram of feed.

Combined estrogen and progesterone feeding was continued for four weeks in the case of Group 1, and twenty-three days for Group 2 and Group 3. All animals including the control of Group 4 were sacrificed and whole mounts of the mammary glands prepared.

The added progesterone concentrate produced a definite improvement in mammary development over and above that produced by estrogen alone. As expected, this took the form of alveolar development, ranging from slight to good (Figures 5, 6, 7, 9, 12, 14, 16, 18). Since the progesterone concentrate was assayed by the manufacturer to contain approximately twenty to twenty-five per cent progesterone and since at the end of the experimental period each rabbit was consuming between fifty to one hundred grams of feed daily, the above effect was produced by the equivalent of only five to twelve and a half milligrams of progesterone daily, by mouth.

Testes of the normal male rabbit of Group 1 were very atrophic (856 milligrams). Germinal epithelium was only one or two cells in depth. The normal male rabbit on the lower estrogen level of Group 2 weighed 3980 grams and had testes weighing 6200 milligrams. This is only slightly below the normal testicular weight for rabbits of this size (Kibler, Bergman and Turner, 1943). The tubular epithelium appeared normal and numerous spermatids were present.

The uteri of the normal and castrate females of Groups 1 and 2 showed relatively little progestational proliferation. Evidence of estrogenic stimulation was apparent. The uteri of the rabbits on the higher estrogen level of Group 1 were particularly enlarged and hyperemic.

Ovaries of the two non-castrate females appeared smaller than normal. No formed corpora lutea or large follicles were present.

Mammary glands of the control rabbit killed at the end of the experiments showed slightly more duct extension than is typical of the normal male rabbit (Figure 19). An enlarged duct system in one old male rabbit has been reported (Turner, 1939a). It is equally probable that the control cage may have been given by mistake one of the estrogen containing experimental feeds at some time during the experiment.

Comparison of body weight curves revealed no retardation of growth of the estrogen fed rabbits as compared to the control animal, during the fourteen weeks on levels of 0.2, 0.6, and 1.8 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed. No valid comparison could be made of the 5.4 milligram level against the control, since the period of treatment was only four weeks, at the beginning of which time the estrogen fed animals had biopsies removed and the control did not.

Discussion. Lyons and McGinty (1941) and Scharf and Lyons (1941) have made a study of the effects of various doses of injected estrone and progesterone on mammary growth in male rabbits. These authors injected groups of male rabbits with 3, 6, 12, 24, 48, and 96 micrograms of estrone daily five days weekly for five weeks. The three and six microgram daily doses induced a duct extension that was somewhat less extensive than that obtained with the twelve, twenty-four and forty-eight microgram levels, which were all about equally effective with an optimum at twelve micrograms. The ninety-six microgram level was reported to be excessive as indicated by relatively less duct extension as well as cyst formation or sacculation in the larger ducts. The tendency toward cyst formation was evident even at the twenty-four microgram level.

In the present work, the daily oral intake of about ten micrograms of dimethyl ether of diethylstilbestrol for a period of fourteen weeks was ineffective as a mammary gland growth stimulant. The thirty and ninety microgram daily oral intake levels both produced good duct extension. The ninety microgram level also produced some alveolar development in one animal after seven weeks (Figure 10). The "cyst" formation referred to by Scharf and Lyons was not observed at the thirty and ninety microgram levels, but was present to a slight extent in at least one animal (Figure 11) after thirty-two

days at the 270 microgram daily oral intake level of synthetic estrogen. Cyst formation of the mammary ducts following estrogen treatment has also been reported in the rat (Burrows, 1935; McEuen, Selye and Collip, 1936; Astwood, Geschickter and Rausch, 1937). It is generally produced by prolonged treatment and higher dosages of estrogen than the minimum required for typical duct growth. It is associated with the initiation of secretory processes and the distention of the ducts with secretion.

Initiation of milk secretion in rabbits by estrogen administration has been dramatically demonstrated by Frazier and Mu (1935). These authors injected from twenty to sixty rat units of estrogen daily into six adult male rabbits for 250 days or more. Enlarged mammary glands and dilated superficial blood vessels became visible through the shaved skin. Milk could be expressed from the nipples of all animals. Four rabbits continued to secrete some milk for as long as 200 days. The lactating males willingly fostered young rabbits and in two instances suckled them. Milk secretion was apparently insufficient, however, to permit indefinite survival of the young.

Lewis and Turner (1941a) also reported alveolar development and the initiation of lactation in rabbits injected subcutaneously with diethylstilbestrol alone.

The relationship of estrogen to lactation is complex. It has been frequently demonstrated that administration of estrogen in proper amounts results in initiation of lactation or enhancement of established lactation (see Mixner, Meites and Turner, 1944, and Burrows, 1945, for review). On the other hand, large amounts of estrogen administered to animals in which lactation is already established results in an inhibition of lactation (see Meites and Turner, 1942a, and Burrows, 1945, for review).

Amounts of estrogen required to inhibit lactation are reported to be higher than the levels required for the enhancement of lactation (Walker and Stanley, 1941; Mixner, Meites and Turner, 1944). This relationship is somewhat comparable to the inhibition of mammary growth by the administration of excessive dosages of estrogen (Gardner, 1941a).

Initiation of milk secretion following estrogen administration is related to increased pituitary lactogen activity. An increased lactogenic hormone content of the pituitary following estrogen administration was first demonstrated by Reece and Turner (1936). Lewis and Turner (1941b) reported that initiation of secretion in the mammary glands of spayed rats by diethylstilbestrol treatment is accompanied by a similar rise in pituitary lactogenic hormone content. Meites and Turner (1942a) found that high doses of estrogen were

less effective in increasing pituitary lactogen content than lower dosages. They have also reported that the administration of estrone to male rabbits caused an increased lactogenic hormone content of the blood as well as of the pituitary gland (1942b).

Addition of the constant amount of progesterone concentrate to the pre-existing levels of synthetic estrogen in the feed resulted in an improved mammary development, particularly as regards alveolar development. The amount added represented approximately five to ten milligrams oral intake daily per rabbit. It appears that this level is below optimal for maximum alveolar proliferation. This is indicated by the presence of lactation in the mammary glands of some of the rabbits at necropsy, particularly those on the higher estrogen levels. Progesterone in adequate dosages inhibits the lactogenic hormone stimulating effect of administered estrogen (Meites and Turner, 1942c).

Scharf and Lyons (1941) obtained the greatest lobular proliferation in rabbits injected with ninety-six micrograms of estrone and one milligram of progesterone daily. The cyst formation (secretion) caused by this level of estrone alone was completely prevented by the progesterone.

Selye, Borduas, and Masson (1942) have similarly reported that in rats injected with varying doses of estradiol and progesterone, milk secretion appeared to be directly proportional to the amount of estrogen given, and that high levels of progesterone counteracted the secretory effects of a certain dose of estrogen.

Mixner and Turner (1943) injected virgin female goats with twenty to thirty milligrams of progesterone plus 100 or 150 micrograms of diethylstilbestrol daily. After sixty days mammary alveolar proliferation comparable to that at mid-pregnancy had been induced, but no secretion had been initiated. When progesterone administration was stopped and the daily level of diethylstilbestrol raised to 250 micrograms for twelve days, milk secretion similar to that seen at the time of parturition was initiated.

Further indication of the dominance of estrogen over progesterone in the present experiments is afforded by the condition of the uteri which showed estrogenic stimulation with but little progestational proliferation. The antagonistic effect of simultaneously administered high levels of estrogen on the progestational proliferation effect of progesterone has repeatedly been shown (see Burrows, 1945, for review).

In the present experiments, levels of estrogen which were capable of stimulating mammary growth and producing testicular atrophy were ineffective in causing growth retardation or body weight loss.

Similar results were obtained by Campbell and Turner (1942) who reported that ovariectomized rabbits treated with ten micrograms of estrone daily for five weeks and twenty micrograms daily for the next fourteen weeks actually outgrew the ovariectomized control. Mammary glands of the estrogen treated animals showed good lobule development and in some cases copious secretion.

The apparent failure of estrogen to inhibit the growth rate of rabbits is in striking contrast to the numerous reports showing an inhibitory effect of administered estrogen, both natural and synthetic, on body growth in the rat (Bugbee and Simond, 1926; Wade and Doisy, 1931; Spencer et al., 1932; Korenchevsky et al., 1939; Noble, 1939a; Page et al., 1941; Deanesly and Parkes, 1941; Richards and Peter, 1941; Griffiths and Young, 1941; Bogart et al., 1944), the mouse (Shimkin and Grady, 1940; Burrows, 1945), the pigeon (Riddle and Tange, 1928), the chick (Breneman, 1942), and of endogenous estrogen in the guinea-pig (Steinach and Holzknecht, 1916; Moore, 1922) and rat (Moore, 1919a).

In the rat, it has been shown that body growth inhibition is one of the first effects of estrogens. By administering solutions of increasing concentration of diethylstilbestrol as drinking water to mature rats, Noble (1939a) found that of the various effects of estrogen studied, the inhibition of growth required the smallest dosage. This was produced by as little as two to three micrograms daily intake per rat. The gonads remained normal until over seven micrograms per day were given. Adrenal and pituitary enlargement required still larger doses. Bogart et al. (1944) have reported that from 1 to 4 micrograms of estrone daily, subcutaneously, is required to retard the growth rate of ovariectomized post-pubertal rats to that of unoperated controls.

It would appear, therefore, that the rabbit is relatively immune to the body weight inhibiting effects of estrogen. This seems to conform with the fact that in the New Zealand White rabbit the growth rate and mature body weight of the male and female are similar. In most species, the female is not as large as the male, and this difference has been explained on the basis of gonadal activity. Thus castration had no effect or suppressed body growth in male rats, but caused females to grow larger than non-castrate controls (Stotsenburg, 1909; Moore, 1919a). Interchanging the gonads of young male and female guinea pigs caused the normally smaller females to exceed the body weight of normal males, while the castrate males with ovarian grafts showed a much smaller ultimate body weight than normal females (Steinach and Holzknecht, 1916; Moore, 1922).

Bergman and Turner (1941) have further found that in the

rabbit the thyrotrophic hormone content of the pituitary of males and females is quite similar, in contrast to the rat where the female pituitary contains a lower concentration. They have suggested that this relationship may be involved in the different mature body weights of male and female rats and the lack of such difference in rabbits.

Involvement of some factor in addition to endogenous estrogen secretion in explaining the different mature body sizes of the male and female is indicated by the work of Moore (1919b). In castrate male and female littermate rats, spayed females increased in weight relative to normal females and approached more nearly the weight of males. However, castrate males were still heavier than the ovariectomized females indicating a difference in the capacity of the two sexes to gain weight, over and above the detrimental body weight effect of the ovaries.

MALE MOUSE EXPERIMENTS

Estrogen Alone. In order to determine the optimal level of dimethyl ether of diethylstilbestrol to add to the feed for mammary stimulation in the male mouse, a grain mixture was prepared containing 100 milligrams of this synthetic estrogen per kilogram. Additional grain mixtures were prepared, each containing one-third as much estrogen as the former. In the first trial, groups of ten mice were fed at the 100, 33.3, 11.1, and 3.7 milligram per kilogram levels. At an estimated three gram daily feed intake per mouse this represented an average daily oral dose per mouse of 300, 100, 33.3, and 11.1 micrograms respectively. A fourth group of ten mice was run as a control. After three weeks, it was obvious from body weight and mortality data that the above range of dosages was too high. The animals were sacrificed, and the mammary glands examined.

Additional groups of ten mice were fed at the 3.7, 1.23, 0.41, and 0.14 milligram per kilogram levels (estimated daily oral dose of 11.1, 3.7, 1.23, and 0.41 micrograms per mouse). At the end of four weeks, four animals from each group were sacrificed, mammary glands examined, and testes weighed. After another nine days of estrogen feeding had elapsed, the remaining animals in each group were subdivided into two groups. One was continued for another four weeks at the same estrogen level, and the other had 100 milligrams of pregneninolone added per kilogram of feed in addition to the already existing estrogen level.

Results of the estrogen feeding are presented in Table 5 and Figures 20 to 32. Whereas the controls gained steadily in body weight, those mice on the three highest estrogen levels lost considerable body weight. At the 3.7 milligram per kilogram level, the mice essentially

maintained their original body weight over a four week period. Mice on the three lower levels appeared to gain as well or almost as well as the controls over a four week period. However, in the subgroups maintained on estrogen for a total of nine weeks and two days, those few animals on the 3.7 and 1.23 milligram per kilogram levels lost weight during the last four weeks. It would appear therefore that only the two lowest levels (0.41 and 0.14 milligrams per kilogram of feed) did not significantly affect body weight over a nine week period.

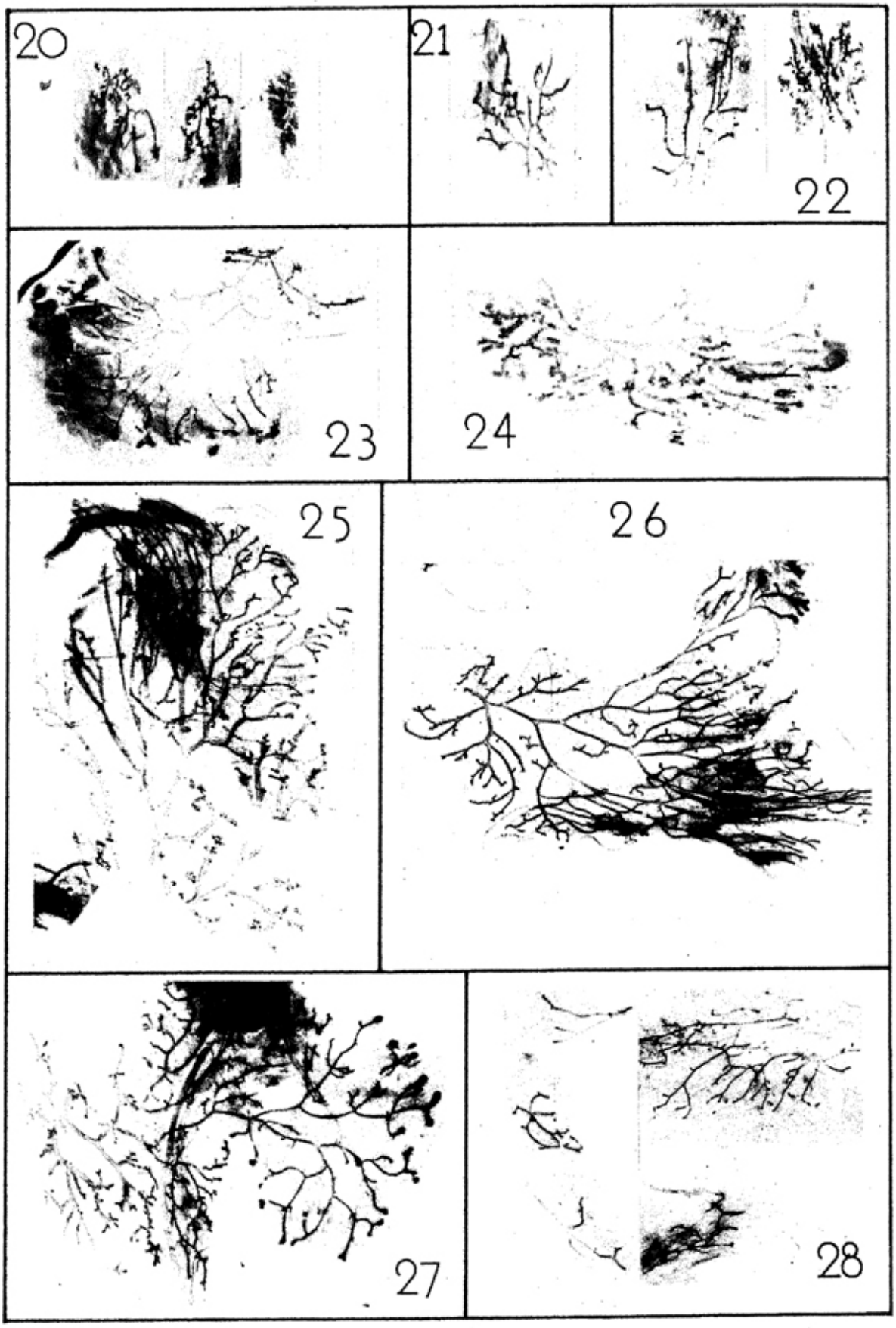
TABLE 5.--BODY WEIGHT, MORTALITY AND TESTICULAR WEIGHT OF MALE MICE FED VARIOUS LEVELS OF DIMETHYL ETHER OF DIETHYLSTILBESTROL IN THE FEED

Duration of Experiment	Number of Mice	Level of Estrogen (mg. per kg. of feed)	Average Body Weight Change (gm. per mouse)	Number of Mice Dead During Experiment	Average Testicular Weight Per Mouse (mg.)
3 Weeks	10	100.0	-3.4	6	Weights not recorded but testes observed to be atrophic
	10	33.3	-3.2	6	
	10	11.1	-1.6	5	
	10	3.7	-0.2	3	
	10	-----	+2.7	3	
First 4 weeks	10	-----	+2.8	0	166
	10	3.70	-0.2	0	38
	10	1.23	+2.4	1	121
	10	0.41	+2.4	0	152
	10	0.14	+2.8	0	148
5th to 9th Week	3	-----	+2.3	0	176
	2	3.70	-0.7	0	37
	2	1.23	-1.2	0	98
	3	0.41	+2.3	0	124
	3	0.14	+1.2	0	161

At levels of 11.1 milligrams per kilogram and above, mortality was greater than for the controls. Significant testicular atrophy was produced in four weeks by levels of 1.23 milligrams per kilogram and above; and in nine weeks by levels of 0.41 milligrams per kilogram and above.

At the end of nine weeks, urinary bladder distension was noted in one of two animals at the 1.23 milligram per kilogram level, and scrotal hernia was noted in two of three animals at the 0.41 milligram per kilogram level. Bladder distension and scrotal hernia are typical signs of long term estrogen treatment in the male mouse (see Burrows, 1945, for review).

With respect to mammary growth, the best extension of the duct system appeared to occur at the 1.23, 0.41, and 0.14 milligram per kilogram levels (Figures 25, 26, 27). Higher dosages resulted in a progressive decrease in the amount of duct extension (Figures 20, 21, 22, 23, 24). Stunted mammary development as a result of overdosage with estrogen has been reported in several species (Gardner, Smith and Strong, 1935; Astwood, Geschickter and Rausch, 1937; Van Heuverswyn, Folley and Gardner, 1939; Gardner, 1941a). At nine weeks no appreciably greater extension of the ducts was noted than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Figures 20 to 28. Mammary glands of control male mice and of male mice fed varying levels of the dimethyl ether of diethylstilbestrol in the feed. All figures 4.8X.

Figure 20. 100 milligrams per kilogram of feed for three weeks.

Figure 21. 33.3 milligrams per kilogram of feed for three weeks.

Figure 22. 11.1 milligrams per kilogram of feed for three weeks.

Figure 23. 3.7 milligrams per kilogram of feed for three weeks.

Figure 24. 3.7 milligrams per kilogram of feed for four weeks.

Figure 25. 1.23 milligrams per kilogram of feed for four weeks.

Figure 26. 0.41 milligrams per kilogram of feed for four weeks.

Figure 27. 0.14 milligrams per kilogram of feed for four weeks.

Figure 28. Control male mice. One gland is much larger than usual for male mice.

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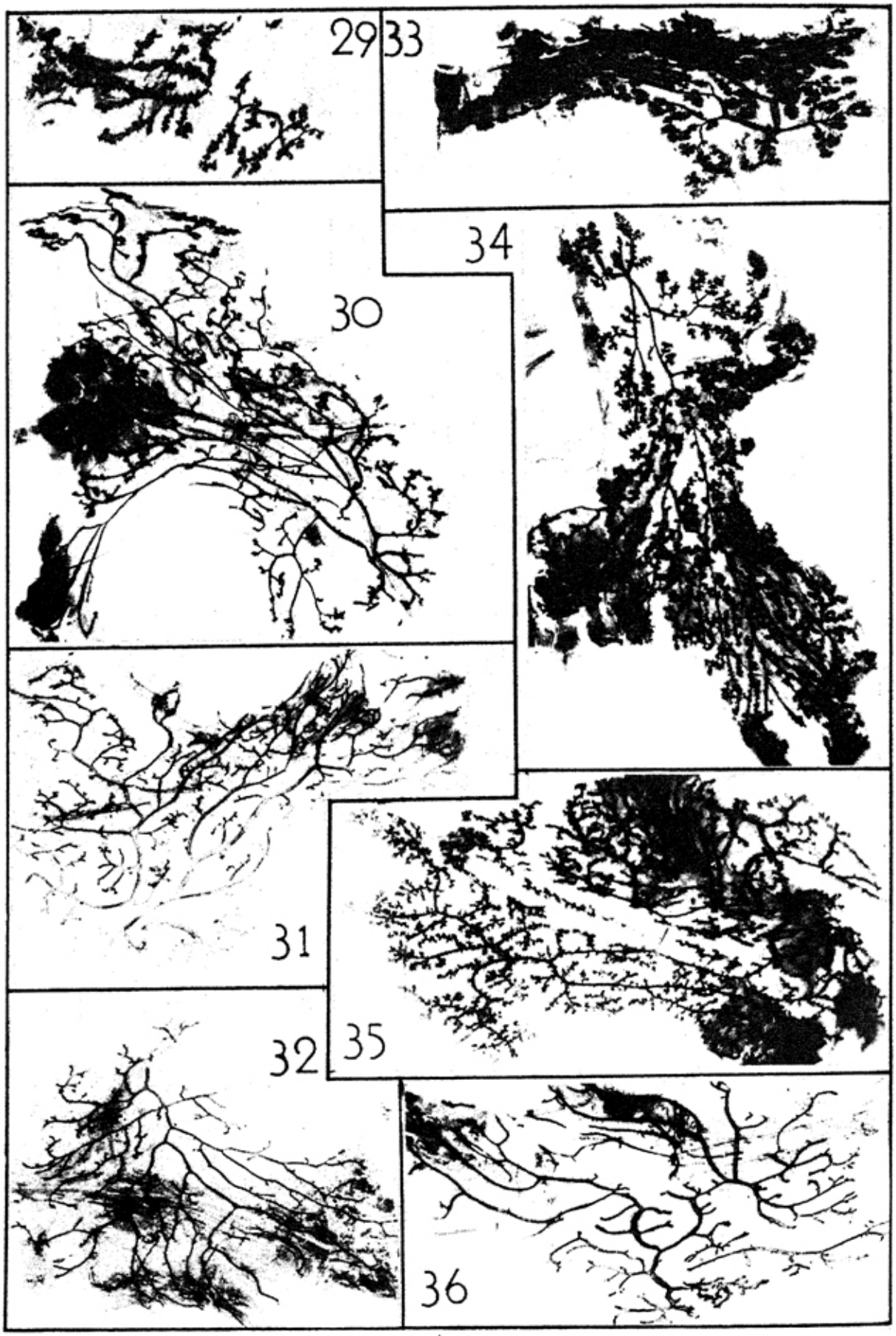
had already occurred by four weeks of treatment. Those lower levels of estrogen (1.23, 0.41, and 0.14 milligrams per kilogram of feed) which produced the best duct extension did not produce appreciable alveolar development. Alveolar development was most pronounced at the 3.7 milligram per kilogram level and higher.

Mammary glands of the control mice are shown in Figure 28. In some of the controls, larger duct systems were encountered than was considered normal for untreated male mice. Male mice generally possess a very rudimentary mammary gland. However Richardson and Cloudman (1947) have recently reported that male mice of certain strains show considerable extension of the mammary duct system. Since male mice from the source (Ed. Schwing, Harrison, Ohio) used in the present experiment had not been examined for mammary development for a period of four years, it was possible that some change had occurred in the breeding stock. Use of male mice from this source for mammary studies was therefore discontinued.

Estrogen with Pregneninolone, Progesterone, Pregnenolone and Desoxycorticosterone Acetate. Mammary glands of the male mice receiving 100 milligrams of pregneninolone per kilogram of feed in addition to the estrogen are shown in Figures 33, 34, 35 and 36. These mice were fed for a total of nine weeks and two days at the 3.7, 1.23, 0.41, and 0.14 milligram levels of estrogen per kilogram of feed. During the last four weeks, the constant amount of pregneninolone was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Figures 29 to 32. Typical mammary glands from male mice fed varying levels of the dimethyl ether of diethylstilbestrol for nine weeks and two days. All figures 4.8X.

Figure 29. 3.7 milligrams per kilogram of feed.

Figure 30. 1.23 milligrams per kilogram of feed.

Figure 31. 0.41 milligrams per kilogram of feed.

Figure 32. 0.14 milligrams per kilogram of feed.

Figures 33 to 36. Typical mammary glands from male mice fed varying levels of the dimethyl ether of diethylstilbestrol for nine weeks and two days. with 100 milligrams of pregneninolone added per kilogram of feed during the last four weeks. All figures 4.8X.

Figure 33. 3.7 milligrams of estrogen plus 100 milligrams of pregneninolone per kilogram of feed.

Figure 34. 1.23 milligrams of estrogen plus 100 milligrams of pregneninolone per kilogram of feed.

Figure 35. 0.41 milligrams of estrogen plus 100 milligrams of pregneninolone per kilogram of feed.

Figure 36. 0.14 milligrams of estrogen plus 100 milligrams of pregneninolone per kilogram of feed.

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added to the feed. Mammary glands of the male mice fed for the entire period with the same levels of estrogen alone are shown in Figures 29, 30, 31 and 32. No great improvement of alveolar development occurred except at the 1.23 and 0.41 milligram levels of estrogen. Of these two, the greatest amount of alveolar development appeared to occur at the 1.23 milligram level of estrogen. It was therefore decided to maintain this level of estrogen constant and add larger amounts of pregneninolone and of progesterone, pregnenolone, and desoxycorticosterone acetate.

Male albino mice obtained from White Rose Mousery, Billings, Missouri, were fed at the level of 1.23 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed for a period of six to seven weeks. After this pretreatment, the mice were divided into groups of four. One group continued to receive the same level of estrogen alone in the feed. In addition to this level of estrogen, the other groups had added to their feed (per kilogram) the following amounts of the following substances: pregneninolone, 300 and 900 milligrams;

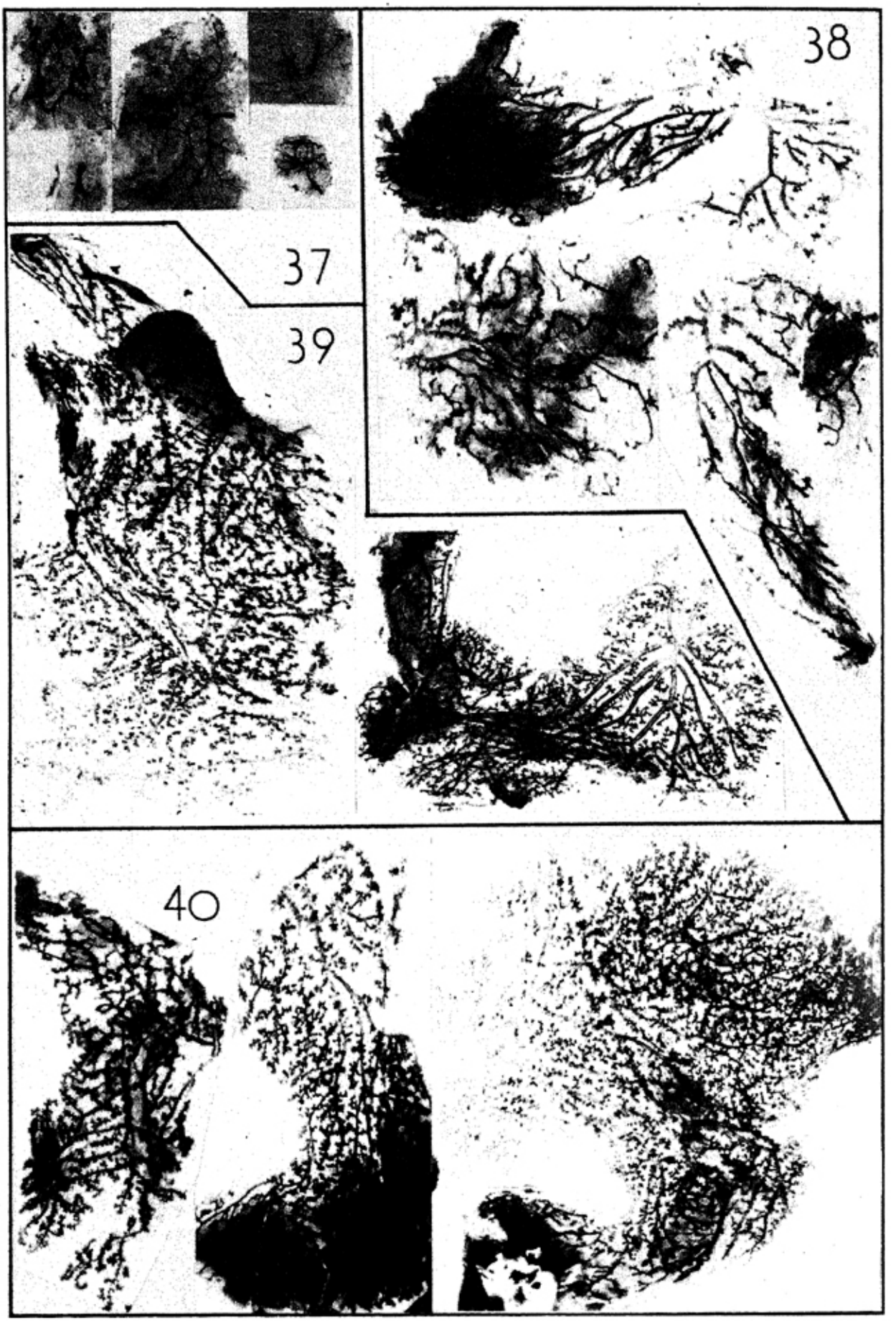
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Figure 37. Mammary glands of control male mice. (4.8X).

Figure 38. Mammary glands of male mice fed 1.23 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed for nine weeks. (4.8X).

Figure 39. Mammary glands of male mice fed 1.23 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed for nine weeks, with 900 milligrams of pregneninolone added per kilogram of feed during the last three weeks. (4.8X).

Figure 40. Mammary glands of male mice fed 1.23 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed for nine weeks, with 900 milligrams of progesterone added per kilogram of feed during the last three weeks. (4.8X).

progesterone, 100, 300, and 900 milligrams; pregnenolone, 1000 milligrams; desoxycorticosterone acetate, 2000 milligrams. Because of the difficulty experienced in dissolving pregneninolone, all of these steroids were added in the solid state to the feed. The finely ground crystalline steroid was first shaken thoroughly into a small quantity of feed in a tight container. When a good mixture had been achieved more feed was added and the process repeated until the total amount of feed was added. Estrogen was added to the feed as previously in oil solution.

After an additional three weeks of treatment, the animals were sacrificed and the mammary glands examined. The results are shown in Table 6 and Figures 37 to 42.

TABLE 6.--MAMMARY ALVEOLAR RESPONSE OF MALE MICE FED DIMETHYL ETHER OF DIETHYLSTILBESTROL WITH VARIOUS STEROIDS HAVING LUTEOID ACTIVITY

Estrogen		Additional Treatment During Last 3 Weeks		No. of Mice	Alveolar Response					
Mg. per kg. of feed	Weeks	Steroid	Mg. per kg. of feed		None	Very Slight	1+	2+	3+	4+
1.23	9	-	-	4	1	3				
1.23	9	Pregne-	300	4		2	2			
1.23	9	ninolone	900	4		1	1	2		
1.23	9	Proges-	100	4		3	1			
1.23	9	terone	300	3		2	1			
1.23	9		900	4			2	2		
1.23	10	Pregne-nolone	1000	4			1	2	1	
1.23	10	Desoxy-corticos-terone acetate	2000	4		2				2

The group receiving the standard level of estrogen alone showed good extension of the duct system (Fig. 38) as compared to the untreated male mice (Fig. 37). Dilation of the ducts was present, but very little alveolar development had occurred.

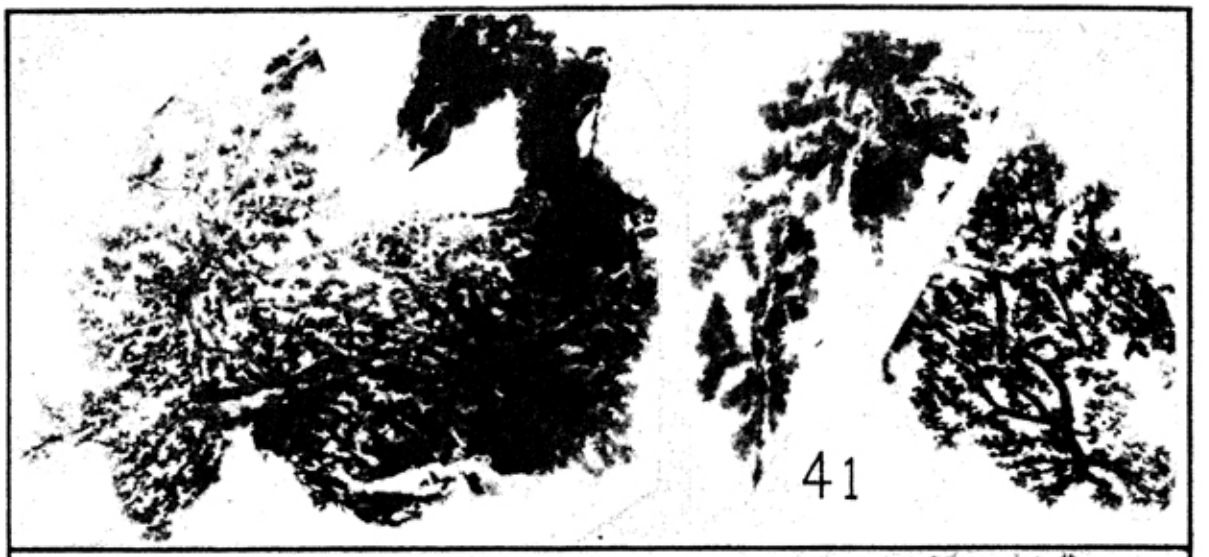
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Figure 41. Mammary glands of male mice fed 1.23 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed for ten weeks with 1000 milligrams of pregnenolone added per kilogram of feed during the last three weeks. (4.8X).

Figure 42. Mammary glands of male mice fed 1.23 milligrams of dimethyl ether of diethylstilbestrol per kilogram of feed for ten weeks with 2000 milligrams of desoxycorticosterone acetate added per kilogram of feed during the last three weeks. (4.8X).

Addition of pregneninolone increased the degree of alveolar development in two of four animals at the 300 milligram level and in three of four animals at the 900 milligram level.

Progesterone likewise increased the degree of alveolar development. At the 900 milligram level progesterone and pregneninolone appeared equally effective as alveolar stimulants. Pregnenolone at the 1000 milligram level produced somewhat better results.

Response to desoxycorticosterone acetate and estrogen was striking. Of four animals, two showed no significant improvement of mammary gland growth over that produced by estrogen alone. The other two however, had developed mammary glands equal to those found in female mice during advanced pregnancy. As compared to a control series of pregnancy mammary glands, one was rated from twelve to fourteen days pregnant and the other sixteen to nineteen days pregnant. (Figure 42.)

Discussion. No adequate explanation can be given for this great variability in response to desoxycorticosterone. It is not necessarily associated with desoxycorticosterone treatment per se, but appears to be characteristic of the mammary response of male mice in general. Considerable variation in the response to estrogen has also been observed, not only from one animal to another but also between different glands on the same animal. Nor is the variation necessarily associated with exogenous hormone therapy, for in those strains of mice in which large mammary duct systems may be found in the males, all the glands do not develop uniformly, even within the same animal (Richardson and Cloudman, 1947). It is possible that the variation may be due to some such factor as a variation in the vascular supply to the mammary glands.

From the present experiments, it is not possible to distinguish any great difference in the oral effectiveness of pregneninolone, progesterone, pregnenolone, and desoxycorticosterone acetate for alveolar growth stimulation in the male mouse. Pregneninolone and progesterone, the only two tested at identical levels, appeared to be equally effective. Pregnenolone, although evoking somewhat better alveolar

response, was administered at a slightly higher level. Similarly, desoxycorticosterone acetate, although evoking a much stronger response in some animals, was administered at a much higher level.

The present experiments demonstrate the feasibility of growing the mammary glands of experimental animals by oral administration of hormonally active substances.

SUMMARY

Addition of 0.2 milligrams of dimethyl ether of diethylstilbestrol to each kilogram of the grain ration of rabbits was insufficient to stimulate mammary growth. Levels of 0.6, 1.8, and 5.4 milligrams per kilogram were all effective in promoting duct growth. Alveolar growth was also stimulated in at least one instance. The 0.6 milligram per kilogram level did not cause noticeable testicular atrophy or cessation of spermatogenesis, while the 5.4 milligram per kilogram level caused marked atrophy of the testes and cessation of spermatogenesis. No significant body weight depression was observed.

Addition of a 20 to 25 per cent progesterone concentrate at the rate of 500 milligrams per kilogram of feed to the rations of the rabbits receiving the 0.6, 1.8, and 5.4 milligram levels of estrogen enhanced the alveolar development of the mammary gland, but was not sufficient to inhibit the lactation stimulating effect of the estrogen.

Dimethyl ether of diethylstilbestrol added to the grain ration of male mice in levels of 3.7 milligrams or over per kilogram of feed caused retardation of growth or loss of body weight over a period of four weeks. Over a nine-week period, 1.23 milligrams per kilogram appeared to retard body weight gain. At levels of 11.1 milligrams or over per kilogram of feed the mortality was greater than for the controls. Significant testicular atrophy was produced in four weeks by levels of 1.23 milligrams or over per kilogram of feed and in nine weeks by levels of 0.41 milligrams or over. The best mammary duct extension occurred at the 0.14, 0.41, and 1.23 milligram levels. Higher dosages resulted in a progressive decrease in the amount of duct extension. Alveolar development was most pronounced at the 3.7 milligram level and above. Enhanced mammary development with formation of varying degrees of alveolar development was induced by the addition of pregneninolone, progesterone, pregnenolone, and desoxycorticosterone acetate to the feed in addition to the 1.23 milligram level of estrogen. On feeding the synthetic estrogen with desoxycorticosterone acetate, mammary development in one male mouse equalled that in sixteen to nineteen day pregnant females. Considerable variation in the responsiveness of different animals was observed.

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