THE THYROID AND REPRODUCTIVE PERFORMANCE IN THE ADULT FEMALE GUINEA PIG, WITH A STUDY OF PLACENTAL PERMEABILITY

by

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INTRODUCTION

The study here reported was started as an investigation of the influence of hypo- and hyperthyroidism on the reproductive performance of the adult female guinea pig. During the course of the work it was found, in confirmation of earlier work (Albrieux, Estefan and Gonzalez, 1946; Webster, 1949a), that treatment of pregnant guinea pigs with antithyroid drugs leads to the development of hyperplastic thyroids in the newborn. It was felt that the clarification of the manner in which these goiters are produced would be a contribution to the knowledge of the pharmacology of the antithyroid drugs. To this end a study of the problem of the premeability of the placenta for thyrotrophin, propylthiouracil and thyroxine in the guinea pig was undertaken. In the interests of clarity these two studies will be discussed separately.

Part I. The Thyroid and Reproductive Performance in the Adult Female Guinea Pig

The general belief that the thyroid is related to reproductive function in the human female arose from the observations that the thyroid enlarges at puberty, at menstruation and during pregnancy (Garnier, 1921; Marine, 1935; Neumann, 1937; and others). This opinion was strengthened 1) by reports that sterility is frequently associated with a basal metabolic rate of -10 or lower (Litzenberg, 1926; Litzenberg and Carey, 1929) and 2) that pregnancy often occurs after treatment with thyroid preparations (Hertoghe, 1914; Veil, 1917; Huntington, 1929; Breckenridge, 1932; Mussey, 1938; King and Herring, 1939; Mason, 1939; Nicodemus and Ritmiller, 1945). Hypothyroidism has been incriminated as a causative factor in secondary amenorrhea and menorrhagia (Hertoghe, 1914; Garnier, 1921; Fluhmann and Murphy, 1941). These and other menstrual irregularities are often alleviated by thyroid therapy (Breckenridge, 1932; Mussey and Haines, 1934; Mason, 1939; Mussey 1939; Foster and Foster, 1941; Lisser, 1942; Thompson, Thompson and Jeppson, 1947). Current clinical opinion with respect to the relationship between the thyroid and reproduction in the human female is expressed by Means (1948): "Thyroid should be prescribed in any case of infertility not traceable to some definite local cause. This

applies whether the basal metabolic rate is substandard or standard." Davis (1951) states: "Thyroid medication should be tried in all women with ovarian failure for, at the present time, it is the most useful endocrine preparation we have clinically."

Despite the abundant evidence in support of the belief that satisfactory reproductive performance depends on a normally functioning thyroid, there are conflicting reports. Baumgartner (1942) states: "Although the incidence of sterility is high among hypothyroid women the fact remains that they do become pregnant." Parkin and Greene (1943) cite five cases in which pregnancy occurred in myxedematous women without benefit of thyroid therapy. Hamblen (1940) states that there is no evidence for the effectiveness of thyroid substance in correcting ovarian failure and continues: "..., thyroid substance, ..., has received less critical study of its gynecologic role than any of the various gonadotropins or sex sterols ...."

Hyperthyroidism in the human female is not considered incompatible with pregnancy but pregnancy often makes the hyperthyroid condition worse, in some cases necessitating therapeutic abortion (Frazier and Ulrich, 1932; Taussig, 1936; Baumgartner, 1942; Davis, 1944). When disturbances in ovarian and menstrual function are associated with elevated metabolism they are sometimes favorably affected by thyroidectomy or antithyroid therapy (Wilson and Bourne, 1922; Bram, 1936; Astwood, 1951).

The relation between the thyroid and reproduction in laboratory and other domesticated mammals has received much attention. Many investigators have reported that thyroidectomy causes ovarian degeneration, arrested folliculogenesis and failure of ovulation (rat-- Hammett, 1923; Salmon, 1936; Ross, 1938; Scow and Simpson, 1945; Leathem, 1951; rabbit-- Hofmeister, 1894; Tatum, 1913; Kunde, Carlson and Proud, 1929; Chu, 1945; Fredrikson and Rydin, 1947; guinea pig-- Williams, Phelps and Burch, 1941). As many or more report no effect on the ovary following thyroid removal or suppression with antithyroid drugs (rat-- Bodansky and Cooke, 1937; Leonard and Leonard, 1937; Folley, 1938; Jones, Delfs and Foote, 1946; Krohn and White, 1950; rabbit-- Bensen, 1902; Sax and Leibson, 1937; Krohn, 1951; dog-- Dragstedt, Sudan and Phillips, 1924; Friedmann, 1933; Binswanger, 1936). Cyclic activity becomes irregular, prolonged or even abolished after thyroidectomy or administration of the antithyroid drugs (rat-- Lee, 1925; Richter, 1933; Van Horn, 1933; Freedman, Wright and Webster, 1935; Ross, 1938; Smithcors, 1945; Krohn and White, 1950; mouse-- Krohn, 1947; guinea pig-- Williams, Phelps and Burch, 1945; cow-- Brody and Frankenbach, 1942; monkey-- Engle, 1944; Aranow, Engle and Sperry, 1946). Evans and Long (1921a) and Tobin (1942) indicate that an anestrous period after the operation is followed by the resumption of normal cycles.

The implication contained in much of the clinical literature, that the hypothyroid female is usually sterile, does

not received complete support from the results of experiments on laboratory mammals. Over-all fertility may be reduced, but many of the females reproduced in spite of thyroidectomy and, in instances when measurements were made, a lowered metabolic rate (rat-- Hammett, 1922; Bodansky and Cooke, 1936; Nelson and Tobin, 1936; Folley, 1938; Ross, 1938; Prenheim, 1940; Karnofsky, 1942; Krohn and White, 1950; rabbit-- Parhon and Marza, 1924; Patterson, Hunt and Nicodemus, 1938; Krohn, 1951; guinea pig-- Knaus, 1924; Marza and Marza, 1929). The antithyroid drugs apparently have a more serious effect, at least in the rat (Goldsmith, Gordon, and Charipper, 1945; Jones, Delfs and Foote, 1946; Barker, 1949). Nevertheless litters were born even after 10 months of treatment (Hughes, 1944; Krohn and White, 1950; Leathem, 1951).

Reports of the effects of hyperthyroidism on the ovary are contradictory. Halpern and Hendryson (1935) and Ershoff (1945) state that there was a decrease in ovarian weight in the rat. Herring (1917) and Korenschvesky, Hall and Clapham (1943) noted that they were hypertrophied. The discrepancies may be explained by differences in dosage and length of treatment inasmuch as Matsumoto (cited by Hayashi, 1929) found that when small doses of thyroid were fed, an initial increase in ovarian weight was followed by atrophy.

Experimental hyperthyroidism leads to the development of irregular cycles (rat-- Reiss and Perény, 1928; Weichert, 1930; Richter, 1933; Halpern and Hendryson, 1935; Drill,

Overman and Leathem, 1943; mouse-- Cameron and Amies, 1926; Reiss and Perény, 1928). The irregularity appears to be part of a general metabolic effect inasmuch as it occurred only when large doses were given (Evans and Long, 1921b; Hayashi, 1929). Treatment with dessiccated thyroid or thyroxine during pregnancy is reported to lead to a high incidence of abortion and fetal death in the rat, rabbit and guinea pig (Hoskins, 1910; Gudernatsch, 1915; Döderlein, 1928, 1929; Kunde, Carlson and Proud, 1929; Weichert, 1930; Thérèsa, 1939). Chidester and Insko (1929) and Kraatz (1939), on the other hand state that pregnancy was not prevented or interrupted.

When the subject was considered as a whole the need for clarification was obvious. It seemed however that any new investigation should be attempted by procedures that are somewhat different from many that have been used previously. They should involve the effects of hypo- and hyperthyroidism on all aspects of reproduction from mating behavior through gestation, and both sexes should be studied simultaneously. Most important would be the relating of the reproductive performance of individuals to the level of thyroid activity as estimated from the rate of oxygen consumption and heart rate or some other measure of thyroid activity. Provided extremes of hypo- and hyperthyroidism were found to be incompatible with reproduction, such a study would lead not only

to the identification of the part or parts of the reproductive process that are most closely associated with thyroid function, but also to the establishment of the range of thyroid activity within which reproduction is normal. Data for the male guinea pig have been summarized (Young, Rayner, Peterson and Brown, 1952; Young and Peterson, 1952). Those obtained for the female guinea pig are presented here.

MATERIALS AND METHODS

For any study of total reproductive performance, the guinea pig presents advantages that are not possessed by other small laboratory mammals. The character of the estrous cycle and the patterns of mating behavior are well known, as they are in the rat and other species (Young, 1941; Hartman, 1945). The guinea pig is unique, however, in two important respects. Ovulation is spontaneous and, without the stimulus of coitus, is followed by the development of a fully functional corpus luteum. Except for the primates, the gestation period of 68 to 70 days is longer than that of any other laboratory mammal. About the end of the first trimester there is a transition from the ovarian to the placental control of pregnancy (Herrick, 1928, Nelson, 1934; Ford, Webster and Young, 1951), as there is even earlier in the monkey and human female (Hartman, 1939). The result is a sequence of events from folliculogenesis through fertilization and the long gestation period which bears a close resemblance to that in the primates. Theoretically, therefore, the relationship of such a factor as the level of thyroid activity to the phases of reproduction characteristic of primates can be better tested with the guinea pig than with any other commonly used subprimate species.

The animals used in this study were healthy sexually mature females, bred in our colony and weighing at least

500 gm. at the beginning of the experiments. The temperature of the laboratory was maintained between 70° and 75°F. Food and water were available at all times. The diet consisted of a mixture of oats and rabbit pellets supplemented with alfalfa hay and fresh green vegetables daily.

In one part of the investigation, reproductive capacity under conditions of hypo- and hyperthyroidism was studied. Surgical thyroidectomy was performed on a group of females at least 30 days prior to their use experimentally. The operation was carried out under nembutal anesthesia (30 mg/kg) with the aid of a binocular dissecting microscope. An electric cautery was used in the dissection. Care was taken to avoid injuring the recurrent laryngeal nerve. No attempt was made to dissect the parathyroids free from the thyroid, but quite often external parathyroids were seen at some distance from the thyroid and were left intact. As a precaution against the development of tetany, 1% calcium lactate was placed in the drinking for 2 weeks following the operation. Fifty-nine females survived the operation and contributed data. At autopsy 45 of these were found to be free of visible thyroid rests.

The effect of propylthiouracil-induced hypothyroidism on reproductive performance was investigated in 39 females. The propylthiouracil was administered as a 0.1% solution in very dilute ammonia. From records of the daily water consumption, the average consumption of the drug was found to be 25 mg. per

animal per day. In previous work (Brown and Young, 1952) it was found that the ammonia had no toxic effects. The drug was administered for an average of 7 months. Tests of reproductive capacity were started 60 days after the beginning of treatment by which time the thyroids were enlarged.

Hyperthyroidism was simulated in 38 animals by subcutaneous injections of thyroxine. The animals were weighed daily and the interval between doses adjusted individually so that the weight was maintained at about 85 to 90% of the initial weight except that allowance was made for weight-gains during pregnancy. One tenth milligram d-l thyroxine, dissolved in saline, injected every 3 to 4 days was a satisfactory dose level and was continued for 8 months. Experiments were started after 30 days of treatment. Thirty-seven untreated females served as controls.

Most of the animals were observed daily for behavioral signs of estrus (Young, Dempsey, Hagquist and Boling, 1937). When a female was found in heat she was placed with a normal male and observed until copulation occurred (Young and Grunt, 1951) or until she was no longer responsive to the male. Mated females were segregated and in order that the fertility of the mating could be established and any abortions detected, the females were observed at frequent intervals during pregnancy. Experimental treatment was continued throughout pregnancy. Thirteen thyroidectomized females and 14 controls

comprising the balance of the animals were confined with males of known fertility from 2 to 4 months.

Determinations of the rate of oxygen consumption and heart rate were made on some animals in each group at the end of the experiments. The rate of oxygen consumption was measured by use of the apparatus shown in figure 1, which is a modification of that described by Williams, Phelps and Burch (1941). The unit of comparison was the cc. of oxygen consumed per 100 gm. of body weight per hour, corrected to standard conditions. The average of three consistent determinations was taken as the rate of oxygen consumption for each animal. The protocol used in recording and calculating the rate of oxygen consumption is shown in Table 1. Heart rates were calculated from electrocardiograms taken after the animals were anesthetized with nembutal (30 mg/kg). Loops of stainless steel wire fastened snugly around the distal portion of each limb served as electrodes. The records were taken with a Sanborn Viso-Cardiette.

RESULTS

Alterations in the measures of thyroid activity that resulted from the experimental treatments are summarized in Table 2. Although the oxygen consumption rate was depressed only 6.4% below the control value by thyroidectomy and propylthiouracil treatment, it was considered that a hypothyroid condition existed inasmuch as the heart rate was decreased about 16% by both treatments. The degree to which the measures of thyroid activity were elevated by thyroxine treatment clearly indicates that a condition of severe hyperthyroidism was achieved.

Cyclic activity as indicated by the frequency of vaginal openings was not significantly affected by the treatments, but it was lowest in the thyroidectomized animals (Table 3). The per cent of animals found in heat was reduced by thyroidectomy. Of the control and hypothyroid females found in heat, approximately 90% mated. The animals given thyroxine tended to be excitable and sometimes became refractory to the male early in the test. Possibly because of this, only 80.6% of the thyroxine-treated females found in heat mated. There were no sterile matings except among the thyroidectomized females.

The per cent of pregnancies terminating the sixty-sixth day or later was not influenced by hypo- or hyperthyroidism (Table 4). The length of gestation and litter size were

likewise unaffected. The per cent of offspring born alive was markedly reduced by thyroidectomy of the mother thus indicating further impairment in the ability of such animals to reproduce. On the other hand, the per cent of young born alive to the thyroxine-treated mothers was significantly higher than in the controls. This increase is of interest in connection with a study by Chu (1945). He found that the administration of desiccated thyroid to thyroidectomized rabbits in which superovulation had been induced by chorionic gonadotrophin was effective in retaining the supernormal number of fetuses in a normal condition until term. Without the thyroid treatment the number of fetuses at term was normal but a high per cent were born dead.

Propylthiouracil, although suppressing oxygen consumption and heart rate to points comparable with these measures of thyroid activity in surgically thyroidectomized animals, was without effect on reproduction except for the condition of the young. The newborn had extremely enlarged thyroids similar to those described by Albrieux, Estefan and Gonzalez (1946) and Webster (1949a). The development of these goiters is attributed to the placental passage of propylthiouracil (demonstrated in a separate study, Part II of this thesis pp (19 - 30).

In the analysis of the data as a whole it seemed desirable to compare the level of thyroid activity in females that reproduced with the level in females which did not

reproduce (Table 5). When the oxygen consumption and heart rate of the animals that failed to reproduce are compared with these measures of thyroid activity in animals that reproduced, it is seen that they were often very similar. Clearly, therefore, individuals react differently to given levels of thyroid hormone. The data also reveal something of the range of thyroid activity within which reproduction is possible.

In the 14 thyroidectomized females in which thyroid rests were found at autopsy, oxygen consumption and heart rate were intermediate between the controls and the thyroidectomized animals that were free of thyroid tissue. They retained this intermediate position with respect to the per cent of cyclic vaginal openings, the per cent found in heat, and the per cent of fertile matings. In other respects they were more nearly like the controls.

DISCUSSION

The data presented above are believed to have a significance for the problem of the relationship between the thyroid and reproductive performance in the female guinea pig that can be summarized briefly. The degree of hyperthyroidism that was attained, which was close to the limits of tolerance for many animals, seems to have had no deleterious action on any phase of reproduction from folliculogenesis through mating and the course of gestation. On the other hand, the per cent of young born alive to females given thyroxine was much higher than in any other group.

Impairment of reproduction was seen in many but not all hypothyroid females, although the nature of the effect depended on the manner in which hypothyroidism was produced. In the surgically thyroidectomized animals the frequency of cyclic vaginal opening was low, the per cent of animals found in heat was reduced, the per cent of sterile matings was increased, and the per cent of young born dead was high. The only detectable effect of propylthiouracil-induced hypothyroidism, which depressed thyroid activity to the same level as surgical thyroidectomy, can be attributed to the placental passage of the drug.

Comparison of these results with those obtained during work with the male (Young, Rayner, Peterson and Brown, 1952; Young and Peterson, 1952) is of interest. In neither sex

did the degree of hyperthyroidism produced have any effect on over-all reproductive performance, but in both sexes fertility was decreased following surgical thyroidectomy. The decrease was greater in the female than in the male, although thyroid activity was depressed more in the male. This would seem to indicate that reproductive processes are more dependent on normal thyroid function in the female.

The results obtained when the male was being studied indicated that the range of thyroid activity within which reproduction can occur is wide, and that individuals react differently to low levels of thyroid hormone. Within the limit that the level of thyroid activity has been varied in the female the same conclusions are suggested, but we cannot be certain of the reaction of the female to the extreme hypothyroidism produced in the male (Young and Peterson, 1952) until comparable experiments have been performed.

A point for point comparison of the results obtained during this study with those reported by other investigators would be difficult, but certain generalization is suggested. The conclusion that in the guinea pig chronic hyperthyroidism is without effect whereas hypothyroidism impairs reproductive performance is consistent with much of the experimental and clinical literature we have cited. We are impressed, however, by the reports that hypothyroid females of other species reproduce (Folley, 1938; Krohn and White, 1950; Krohn, 1951; Leathem, 1951). These reports and the numerous older contradictory claims cannot all be ascribed to inadequate controls

and species differences. Were attempts made in a comparative study of a number of species to define the limits of thyroid activity beyond which reproduction is impaired or fails, and to determine the nature of the impairment as these limits are approached, it is believed that clarification of the problem might be achieved.

SUMMARY AND CONCLUSIONS

The reproductive performance of adult female guinea pigs under conditions of hypo-and hyperthyroidism was studied. The degree of alteration in the level of thyroid activity was estimated from oxygen consumption and heart rate determinations.

Surgical thyroidectomy which decreased the rate of oxygen consumption 6.4% and heart rate 18% decreased the frequency of cyclic vaginal openings, the per cent of animals found in heat, the per cent of fertile matings, and the per cent of young born alive.

Propylthiouracil-induced hypothyroidism, which reduced the measures of thyroid activity to a degree comparable with that following thyroidectomy, was without detectable effect on reproductive performance except that large goiters were present in the young born to such females.

The reproductive performance of females made hyperthyroid by doses of thyroxine close to the limits of tolerance was if anything better than in the controls, particularly with respect to the per cent of young born alive.

There is a wide range of thyroid activity compatible with reproduction, and individuals react differently to given levels of thyroid activity.

Part II. The Problem of Placental Permeability for Thyrotrophin, Propylthiouracil and Thyroxine in the Guinea Pig[1]

Many investigators have reported that the thyroids are enlarged in offspring from mothers treated with antithyroid agents during pregnancy (mouse-- Kaufman, Hurst and Turner, 1948; rat-- Hughes, 1944; Goldsmith, Gordon and Charipper, 1945; guinea pig-- Albrieux, Estefan and Gonzalez, 1946; Webster and Young, 1948; goat-- Schultze and Turner, 1945; man-- Davis and Forbes, 1945; Eaton, 1945). This has been taken as evidence for the placental passage of an antithyroid effect.

The placental passage of the antithyroid effect could occur in one or more of three ways (Goldsmith, Gordon and Charipper, 1945). Thyrotrophin (TSH) from the maternal pituitary might cross the placenta to affect the fetal thyroid. D'Angelo and Gordon (1950) have since shown that administration of propylthiouracil is followed by increased concentration of TSH in the serum. Second, the goitrogen might pass the placenta to inhibit thyroid hormone production in the fetus. This would lead to stimulation of the fetal thyroid by TSH secreted by its own pituitary. The third alternative is suggested by the fact that the antithyroid

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drug decreases production of thyroid hormone by the maternal thyroid. The fetal thyroid might then undergo hyperplasia and supply the hormonal needs of the mother.

The literature concerning the passage of thyrotrophin across the placenta is difficult to interpret. Döderlein (1933), using the guinea pig, and Thérèsa (1939), using the rat, state that TSH passes the placenta and stimulates the fetal thyroids. Aron (1930), Schittenhelm and Eisler (1935) and Grumbrecht and Loeser (1938), on the other hand, found no consistent evidence for stimulation of the thyroids in guinea pigs born to mothers treated with TSH-active pituitary extracts during pregnancy.

The permeability of the placenta of the rat for methylthiouracil has been proved by bioassay of the fetuses (Freiesleben and Kjerluf-Jensen, 1947). The passage of other antithyroid agents across the placental barrier has been suggested but not conclusively demonstrated (Hughes, 1944; Goldsmith, Gordon and Charipper, 1945; Albrieux, Estefan and Gonzalez, 1946).

Though it is not concerned with the transmission of the antithyroid effect to the fetus, we felt that passage of thyroxine from mother to fetus should be investigated. There are reports relative to the transfer of thyroid hormone, but they are contradictory. Courrier and Aron (1929) concluded that the placenta of the dog is impermeable to thyroid hormone. Grumbrecht and Loeser (1938) and Thérèsa (1939),

however, state that this hormone passes the placenta in the guinea pig, rat, and rabbit.

In a single study we have attempted to clarify the problem of the permeability of the guinea pig placenta for thyrotrophin, propylthiouracil and thyroxine.

MATERIALS AND METHODS

Working under the assumption that the placental passage of the antithyroid effect is due to one or more of three possibilities, the following series of experiments was performed.

Thyroidectomy of the mother removes the endogenous supply of thyroid hormone. It is presumed from the work of D'Angelo and Gordon (1950) on the female guinea pig that production of TSH by the maternal pituitary would thus be released from inhibition and elevate the level of circulating TSH. The level of TSH can be further raised by injection of this hormone. If TSH were to pass the placenta of a pregnant animal so treated, the thyroids from the offspring should show some change. To test this hypothesis, 8 female guinea pigs were thyroidectomized at least 30 days prior to mating. Beginning the 38th day after copulation they were given daily subcutaneous injections of 0.5 mg. of purified thyrotrophic hormone from a preparation containing 10 Junkmann-Schoeller units per mg. If there were no changes in the fetal thyroid, the placental passage of antithyroid effect could not be due to the transfer of TSH from mother to fetus. Furthermore, the possibility that it had undergone hyperplasia and compensated for the deficiency of thyroid hormone in the mother would be eleminated.

It is known that the thyroids of offspring from propylthiouracil-treated guinea pigs are hyperplastic (Webster

and Young, 1948; Webster, 1949a). The hyperplasia could be shown to be due to the placental passage of propylthiouracil, provided there were no hyperplasia of the fetal thyroids following treatment of thyroidectomized mothers with TSH. In order to make this comparison, 11 pregnant guinea pigs were given approximately 25 mg. propylthiouracil per day in the drinking water from the 38th day of pregnancy until parturition.

The problem of the passage of thyroxine from mother to fetus was investigated in another series of experiments. Dempsey and Astwood (1943) demonstrated that the effects of thiouracil on the thyroid of the adult rat can be counteracted by small daily doses of thyroxine. If it were shown that propylthiouracil acts directly on the fetus, an amelioration of the antithyroid effect by thyroxine administered to the mother during gestation would be convincing evidence that thyroxine can enter the fetal circulation from the mother. To test this hypothesis, 4 pregnant animals were given 25 mg. of propylthiouracil per day in the drinking water and injected daily with 0.025 mg. thyroxine in saline beginning the 38th day of pregnancy.

The placental passage of thyroxine might be shown in another way. The administration of thyroid hormone to an intact adult animal causes flattening of the thyroid epithelium and enlargement of the follicles (Courrier, 1928; Adams and Jensen, 1944). If thyroxine passes the placental barrier, the administration of this substance to pregnant guinea pigs

might cause similar changes in the fetal thyroids. This possibility was tested in two groups of 5 females each. Both were treated with different doses of thyroxine for at least 30 days before mating and throughout pregnancy. One group received subcutaneous injections of 0.10 mg. thyroxine every 4 days. The other was injected with 0.15 mg. thyroxine every 3½ days.

The offspring from 13 untreated pregnant guinea pigs served as controls for all of the above experiments.

On the day of delivery the thyroids and pituitaries from 71 offspring from treated and 38 offspring from untreated mothers were removed, dissected free of excess fat, weighed on a torsion balance, and fixed in Dawson's fixative (Dawson and Friedgood, 1938). The thyroids were dehydrated and embedded by the dioxan-paraffin method, sectioned at 7 micra, stained with hematoxylin and eosin, and examined microscopically.

The guinea pigs used in this study were from our own stock kept under the following conditions: The temperature of the laboratory was maintained between 70° and 75°F. Food and water were available at all times. The diet consisted of a mixture of oats and rabbit pellets supplemented with alfalfa hay and fresh cabbage once daily.

RESULTS

Three of the 8 thyroidectomized-TSH-injected females delivered living litters. On gross examination at autopsy no regenerated thyroid tissue was found in these mothers. The thyroids of their offspring showed neigher an increase in weight (Table 6), nor any histological evidence of TSH stimulation (compare Figs. 2 & 3). Thus, there is no evidence that the antithyroid effect in the fetus is due to passage of TSH from the mother or to hyperplasia of the fetal thyroid which might have supplied thyroid hormone to the mother.

As in other work (Webster and Young, 1948; Webster, 1949a), administration of propylthiouracil to the mother during pregnancy caused extreme hypertrophy and hyperplasia of the fetal thyroid (Table 6 & Fig. 4). Since no such effect was found in offspring from thyroidectomized females treated with TSH, the thyroid enlargement in offspring from propylthiouracil-treated mothers must have been due to placental passage of the antithyroid drug. The stimulation of the fetal thyroid by thyrotrophin from its own pituitary is borne out by the fact that the fetal pituitaries showed a simultaneous increase in weight (Table 6).

When propylthiouracil and thyroxine were administered simultaneously during pregnancy, the fetal thyroids were larger than normal, but significantly smaller than in the group receiving the antithyroid drug alone (Table 6). Histologically they showed less drastic effects. Although

the epithelium was high, colloid-containing follicles were formed (compare Figs. 4 & 5). The pituitaries were of normal weight. All of this indicates a partial inhibition of the production of TSH by the fetal pituitary in consequence of the passage of the injected thyroxine across the placenta. Theoretically, if sufficient thyroxine were administered with the propylthiouracil, the fetal thyroids should be normal.

Further evidence for the passage of thyroxine across the placenta is considered to be given by data from the offspring of thyroxine-treated mothers. The thyroid epithelium was not flattened nor was there enlargement of the follicles as we might have expected from the work of Courrier (1928) and Adams and Jensen (1944), but the thyroids were significantly smaller than those of control newborn (Table 6). The greater the dose of thyroxine, the smaller were the thyroids. The pituitaries showed corresponding changes in weight. Histologically, the thyroids of offspring from thyroxine-treated mothers were similar to the controls except for the presence of smaller follicles and relatively more interfollicular cells and connective tissue (compare Figs. 2, 6, & 7). Since the pituitaries and thyroids of these offspring were significantly smaller than those of the control newborn, it is assumed that the TSH production by the fetal pituitary exerts an influence on the normal prenatal development of the thyroid.

DISCUSSION

The data indicate that the enlargement of the thyroids in offspring from propylthiouracil-treated female guinea pigs is due neither to passage of TSH from the mother to the fetus nor to hyperplasia of the fetal thyroid which might compensate for the deficiency of thyroid hormone in the mother. It has been concluded, therefore, that of the three hypotheses which might have accounted for the transmission of the antithyroid effect to the fetus, the placental passage of propylthiouracil is most in accord with the data.

This conclusion is consistent with the opinions of several investigators who have studied one or more aspects of the subject (Freiesleben and Kjerluf-Jensen, 1947, for the goitrogen methylthiouracil in the rat; Aron, 1930, Schittenhelm and Eisler, 1935, Grumbrecht and Loeser, 1938, for TSH in the guinea pig; Grumbrecht and Loeser, 1938, Thérèsa, 1939, for thyroid hormone in the guinea pig and rabbit).

An explanation for the contradictory reports of the placental passage of thyrotrophin and thyroxine is not easy. Döderlein (1933) stated that TSH passes the placenta to cause hypertrophy of the fetal thyroid in the guinea pig. In our opinion, his data do not support this conclusion. From a comparison of his figures of alleged TSH stimulation with his control figure, it might seem that the conclusion was justified. Close examination of the thyroid of the newborn control he chose for comparison (his Fig. 2a) reveals,

however, that the epithelium is squamous and that the follicles are distended. This is a condition we have not seen in any one of a large number of normal newborn guinea pigs. Moreover, the appearance of the fetal thyroids which he believed to have been subjected to TSH stimulation was not different from that in our controls. Species differences may account for the reported passage of TSH through the placenta of the rat (Thérèsa, 1939), and for the non-passage of thyroid hormone across the placenta of the dog (Courrier and Aron, 1929).

Since it is clear that thyroid hormone and at least two antithyroid agents pass the placenta, the long term effects on the young from females treated with these substances during pregnancy become of interest. Rather severe chronic hyperthyroidization of the pregnant rat has been reported to produce cretenoid progeny (Cunningham, 1941). The retarded growth of the cretenoid rats was overcome by the administration of thyroxine. We have seen no such condition in the guinea pig. The weight of newborn animals from mothers given thyroxine during pregnancy was not less than that of the controls (Table 6). Following another study of thyroxine-treated pregnant guinea pigs (Peterson, Rayner, Brown and Young, 1950), the development of 9 male and 18 female offsping was observed for 3 to 4 months after birth. Their growth and sexual maturation were not different from the controls.

Changes in the goiters in the offspring of propylthiouracil-treated female guinea pigs have been followed (Webster, 1949a,b). The thyroids underwent a rapid involution, but were still enlarged even after 8½ months. The presence of these goiters had no apparent effect on growth and sexual maturation. Thus, even though the weight of the thyroids and pituitaries in offspring from animals treated similarly differed significantly from normal (Table 6), there was no other effect on postnatal development.

Antithyroid agents have been used in the treatment of hyperthyroidism complicating pregnancy in the human female. Varied effects on the fetus and newborn have been reported. Davis and Forbes (1945) found an enlarged thyroid gland in a six-month fetus from a mother treated with thiouracil. An infant observed by Eaton (1945) had a grossly enlarged thyroid which regressed in size until it was no longer visible by the third month. Whitelaw (1947) found little difference from normal in the thyroid of a male monster born to a mother who had received thiouracil during the latter part of pregnancy. Caren (1949) states that there were no ill effects of thiouracil on the fetus or its subsequent development. We suggest from our work on the guinea pig that the thyroid enlargement in some infants born to mothers treated with antithyroid drugs can be attributed to placental passage of the drug.

SUMMARY AND CONCLUSIONS

The thyroids and pituitaries of offspring from thyroidectomized female guinea pigs treated with thyrotrophin during pregnancy did not differ in weight from those of controls, nor was there any histological sign of thyrotrophic stimulation of the fetal thyroid. Administration of propylthiouracil during pregnancy causes hyperplasia of both the fetal thyroid and pituitary which can be counteracted by simultaneous administration of thyroxine. Both the fetal thyroid and pituitary are supressed in development when thyroxine alone is injected into pregnant animals. Such results indicate that the placenta of the guinea pig is impermeable to thyrotrophin, but is permeable to both propylthiouracil and thyroxine.

Table 1 demonstrates, with the results of an actual determination, the method used for recording the data and calculating the rate of oxygen consumption. In the first three columns are recorded the chamber temperature (C T) in degrees centigrade, the barometric pressure (B) in mm Hg., and the volume of water (V_1) introduced from the burette, respectively. The stopwatch reading is converted to its equivalent decimal part of an hour (T). The water vapor pressure (V P) is subtracted from the barometric pressure to obtain the corrected pressure (P_1). With the correction factor F, the oxygen volume (V_1) is reduced to its corresponding volume (V_2) at standard conditions. The oxygen consumption rate (O C) in cc/100 gm/hr. is calculated according to the last equation shown.

TABLE 1.

METABOLISM RECORD

DATE 12/13/51

NUMBER 3304 T

TREATMENT UNTREATED FEMALE

CYCLE 6 DAYS AFTER HEAT

AGE 18 MO.

OBSERVER R.R.P.

FASTED NO

INTO CHAMBER 9:00 AM

OUT OF CHAMBER 9:50 AM

WEIGHT

PRE-FAST ——

PRE-TEST 1170

POST-TEST 1170

2 | 2340

W 1170

C T	B	V_1	TIME	T	V P	B - V P = P_1	F	$(V_1)(F)=V_2$	$\frac{(V_2)(100)}{(W)(T)}=0C$	REMARKS
29.0 29.0 29.0	736.4 736.4	100.0	7:52.4 7.87	0.131	30.0	706.4	0.8402	84.0	54.8	NO MOVEMENT
29.0 29.5 29.25	736.4 736.4	100.0	7:50.2 7.83	0.130	30.5	705.9	0.8389	83.9	55.2	" "
29.5 30.0 29.75	736.4 736.4	100.0	7:53.4 7.89	0.132	31.4	705.0	0.8368	83.7	54.2 3 | 164.2 54.7	" "

TABLE 2. MEASURES OF THYROID ACTIVITY IN HYPO- AND HYPERTHYROID FEMALE GUINEA PIGS

Treatment	Number of females	O_2 consumption in cc/100 gm/hr	Heart rate in beats/min.
Controls	37	53.0 ± 1.0	271 ± 5.2
Thyroidectomy	22	49.6 ± 2.4	228 ± 9.7**
Propylthiouracil	6	49.6 ± 1.7	231 ± 13.9**
Thyroxine	10	93.8 ± 2.0**	319 ± 6.8**

The ± values are the standard errors of the means.

** The difference from the control value is significant at 1% ("t" test).

TABLE 3. EFFECT OF HYPO- AND HYPERTHYROIDISM ON CYCLIC ACTIVITY AND FERTILITY OF THE FEMALE GUINEA PIG

Treatment	Number of females	Number and % showing vaginal openings		Number and % showing heat responses		Number of matings	Per cent fertile matings
Controls	39	37	94.9%	33	84.6%	30	100%
Thyroidectomy	45	36	80.0%	26*	57.8%	23	69.6%**
Propylthiouracil	39	37	94.9%	35	89.7%	31	100%
Thyroxine	38	38	100%	36	94.7%	29	100%

* The difference from the control value is significant at 2% (X^2 test).

** The difference from the control value is significant at 1% (X^2 test).

TABLE 4. EFFECT OF HYPO- AND HYPERTHYROIDISM IN FEMALE GUINEA PIGS ON THE COURSE OF GESTATION AND THE NUMBER AND CONDITION OF THE OFFSPRING

Treatment	Number and % pregnancies terminating 66th day or later		Average length of gestation in days	Average litter size	Number of young	Per cent born alive
Controls	26	92.8%	68.8	3.2	110	73.6%
Thyroidectomy	8	88.9%	66.8	3.1	47	48.9%**
Propylthiouracil	27	90.0%	69.4	2.7	92	67.4%
Thyroxine	24	82.8%	67.4	3.4	100	98.0%**

** The difference from the control value is significant at 1% (X^2 test).

TABLE 5. MEASURES OF THYROID ACTIVITY IN FEMALE GUINEA PIGS THAT REPRODUCED AND IN THOSE THAT FAILED TO REPRODUCE

Treatment	Reproduced			Failed to reproduce		
	No.	O_2 consumption cc/100 gm/hr	Heart rate beats/min.	No.	O_2 consumption cc/100 gm/hr	Heart rate beats/min.
Controls	28	52.9 ± 1.2	272 ± 6.6	9	53.3 ± 2.0	268 ± 6.4
Thyroidectomy	9	50.0 ± 2.5	258 ± 12.0	13	49.3 ± 2.4	202 ± 6.9**
Propylthiouracil	4	51.0 ± 2.3	238 ± 25.1	2	46.7 ± 0.7	222 ± 5.2
Thyroxine	6	93.5 ± 3.0	316 ± 8.3	4	94.1 ± 2.7	330 ± 1.2

The ± values are the standard errors of the means.

** The difference from the corresponding value for animals that reproduced is significant at 1% ("t" test).

TABLE 6. EFFECT OF TREATMENT OF THE MOTHER ON THE WEIGHT OF THE THYROID AND PITUITARY OF THE NEWBORN GUINEA PIG

Treatment	Number of mothers	Number of young	Birth weight	Thyroid weight (mg/100gm)	Pituitary weight (mg/100gm)
Untreated controls	13	38	93.8	28.7 ± 1.0	5.2 ± 0.1
Thyroidectomized plus 5 J-S units TSH daily for 30 days	3	5	112.4	27.1 ± 1.1	5.0 ± 0.6
Propylthiouracil, 25 mg daily for 30 days	11	20	94.9	2090.3 ± 301.3*	8.4 ± 0.4*
Propylthiouracil, 25 mg and 0.025 mg thyroxine daily for 30 days	4	14	97.7	267.9 ± 50.8*	5.7 ± 0.3
Thyroxine, 0.10 mg every 4 days throughout pregnancy	5	20	94.8	21.5 ± 0.8*	4.8 ± 0.2
Thyroxine, 0.15 mg every 3½ days throughout pregnancy	5	12	101.1	17.4 ± 1.1*	4.3 ± 0.3*

The ± values are the standard errors of the means.

* The difference from the control value is significant at 1% ("t" test).

Figure 1. Diagram of respiration apparatus. An animal is placed in the chamber (C) which contains soda lime in perforated copper baskets (D). The pyrex baking dish (N) is sealed against a sponge rubber gasket (O) by tightening web straps K. Oxygen is admitted from rubber balloon E by opening stopcocks F and G. The amount of mercury (M) in the indicating manometer (A) is so adjusted that it makes contact with electrode P when both arms of the manometer are open to atmospheric pressure. The mercury completes the circuit through the transformer (T) which lights the signal lamp (L). After a rest period of 10 minutes, time trials are taken with more than 100 ml. of oxygen in the pressure bottle (J) and with stopcocks F and H closed and G open. When the signal lamp comes on a stopwatch is started and 100 ml. of water is allowed to slowly run from the burette (B) into the pressure bottle. As pressure in the system rises the singal lamp turns off. When the lamp again comes on the stopwatch is stopped and the time required to consume a volume of oxygen equal to the volume of water added is recorded. The volume of oxygen is reduced to standard conditions from local barometric pressure and the temperature inside the animal chamber (from thermometer I). Determinations may be repeated several times without disturbing the animal. When the pressure bottle becomes full, water may be forced out by closing stopcock G and opening F and H.

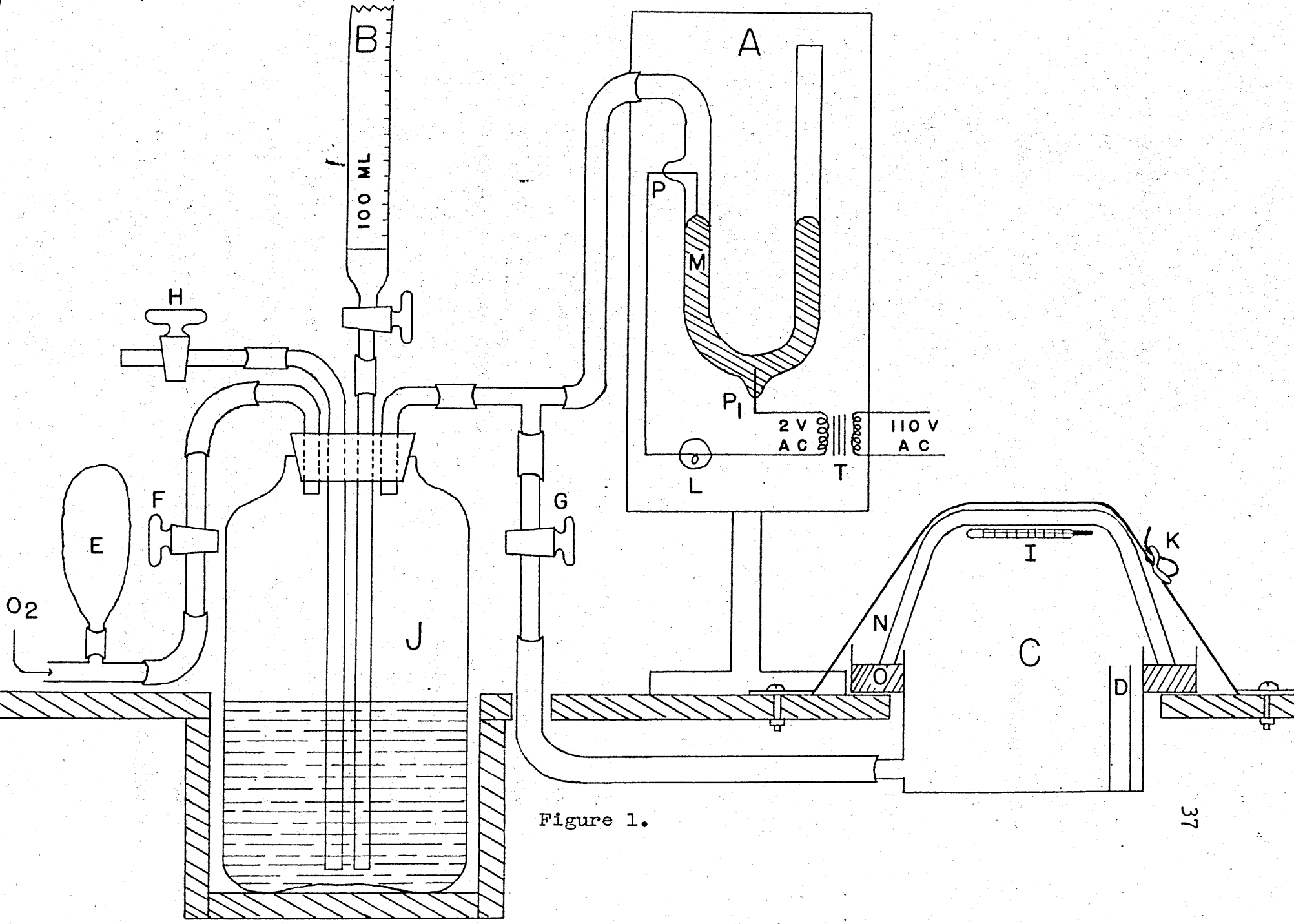

Figure 1.

Figure 2. Thyroid of an offspring from an untreated control female. X 150.

Figure 3. Thyroid of an offspring from a thyroidectomized female injected with 5 J-S units TSH daily the last 30 days of gestation. X 150.

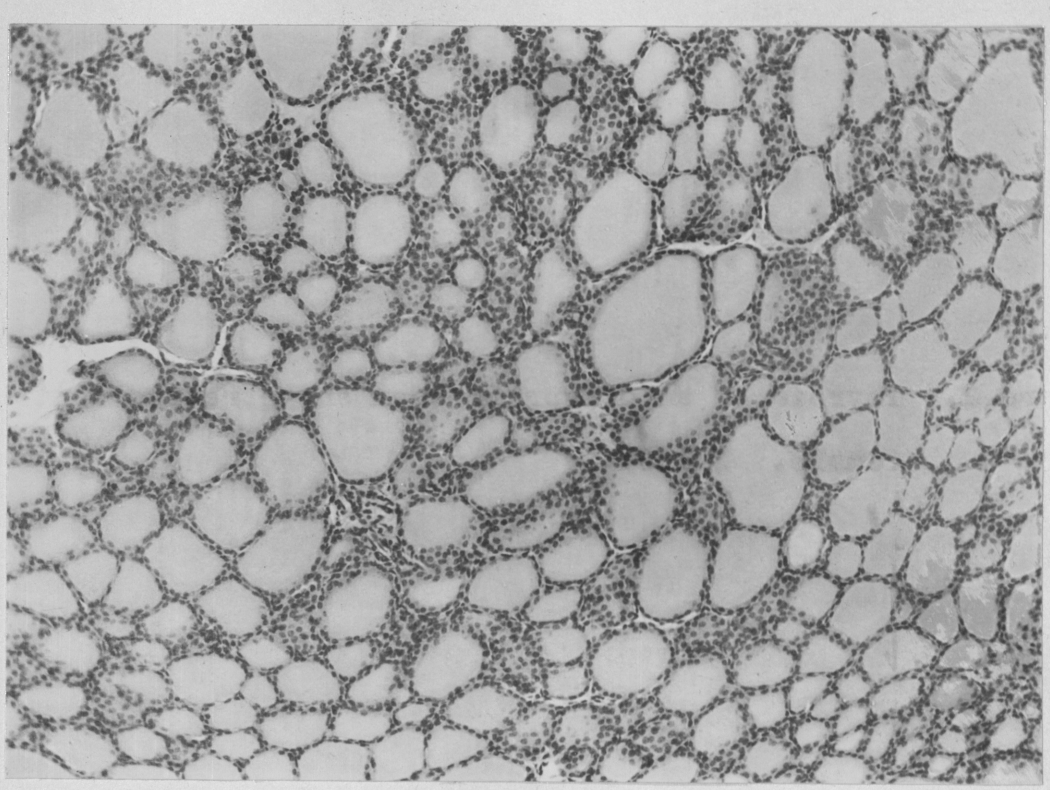

Figure 2.

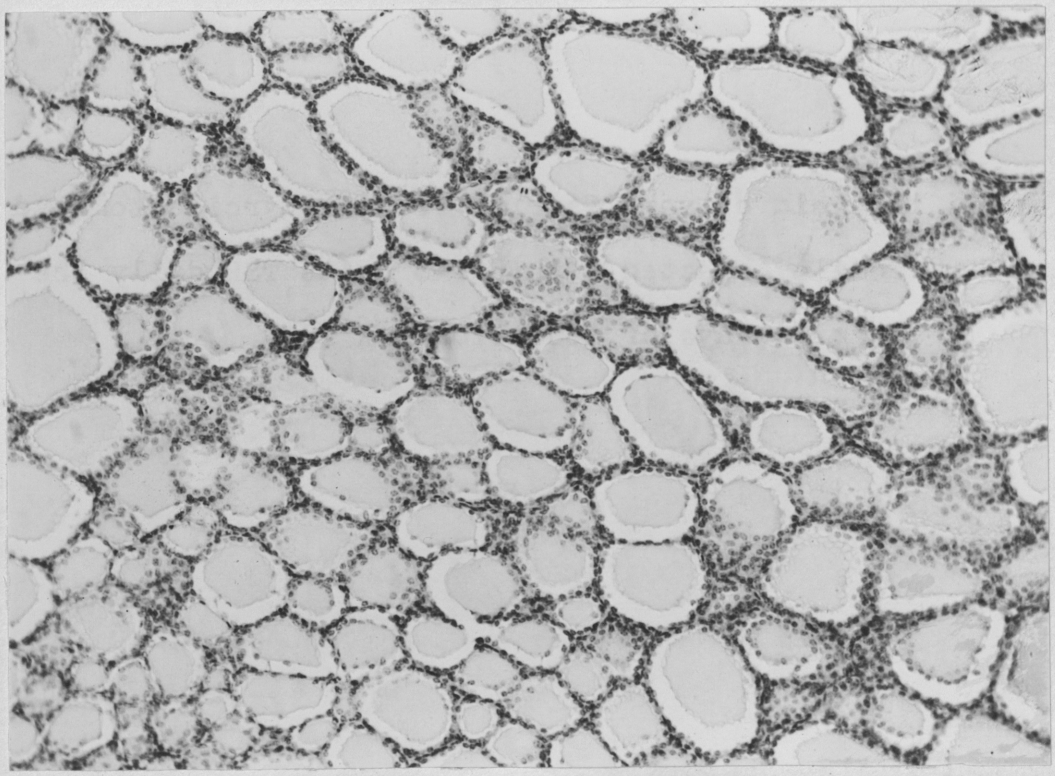

Figure 3.

Figure 4. Thyroid of an offspring from a female treated with 25 mg. propylthiouracil daily the last 30 days of gestation. X 150.

Figure 5. Thyroid of an offspring from a female treated with 25 mg. propylthiouracil and 0.025 mg. thyroxine daily the last 30 days of gestation. X 150.

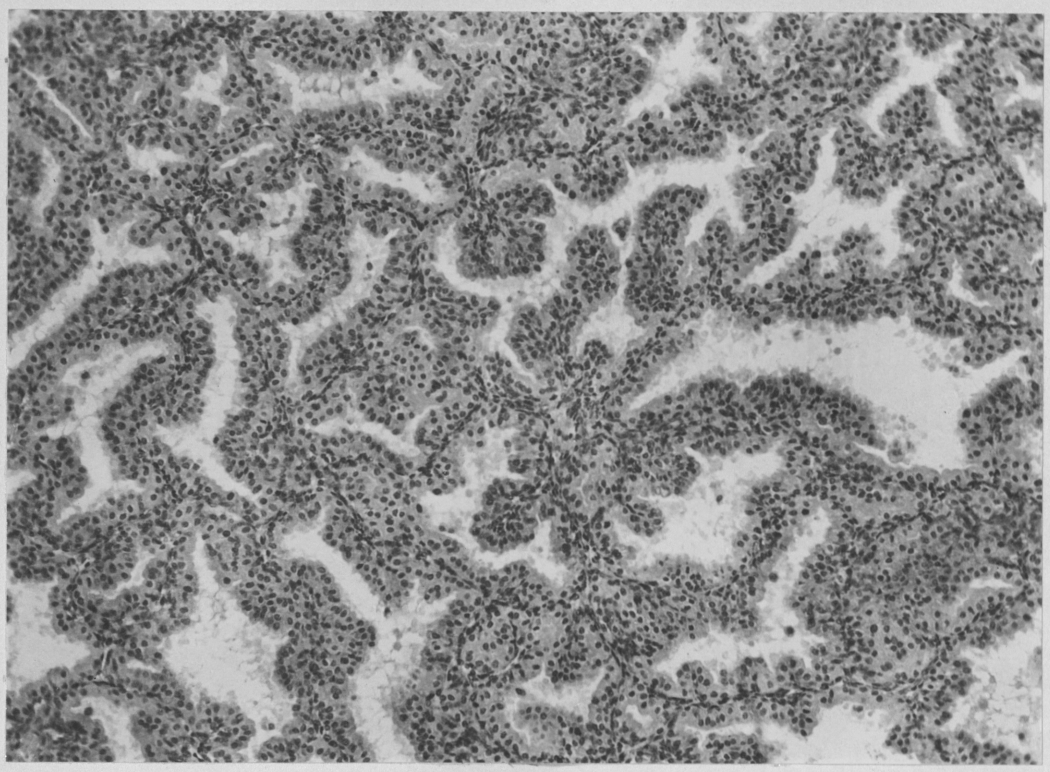

Figure 4.

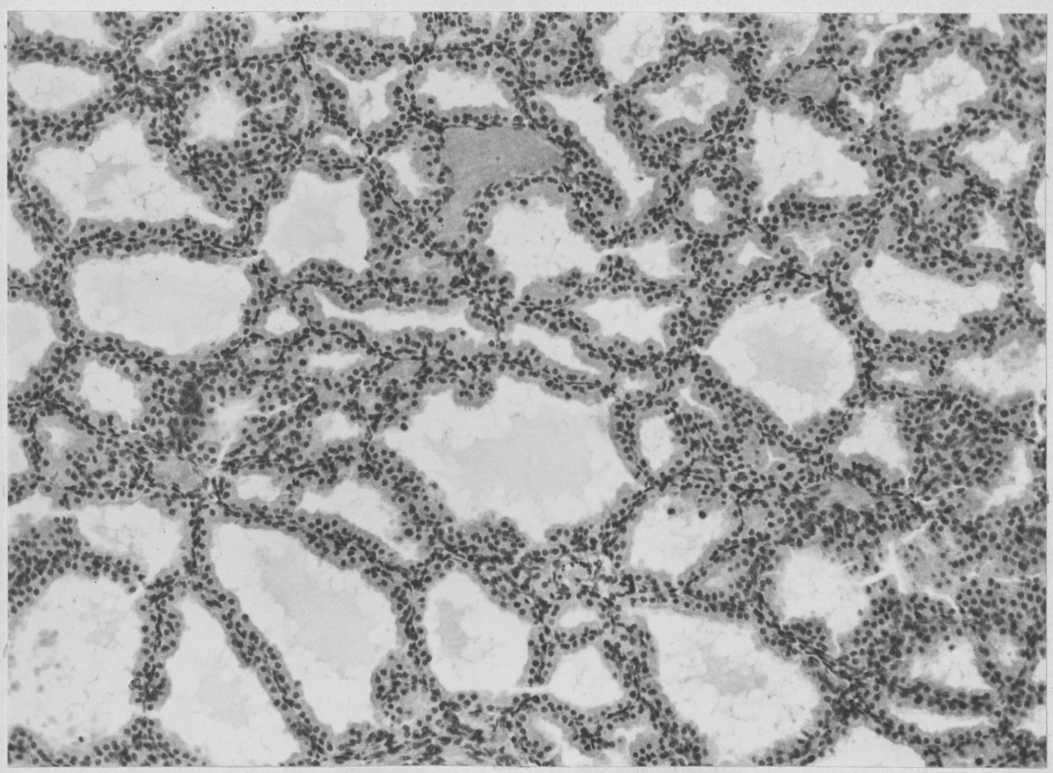

Figure 5.

Figure 6. Thyroid of an offspring from a female injected with 0.10 mg. thyroxine every 4 days throughout gestation. X 150.

Figure 7. Thyroid of an offspring from a female injected with 0.15 mg. thyroxine every 3½ days throughout gestation. X 150.

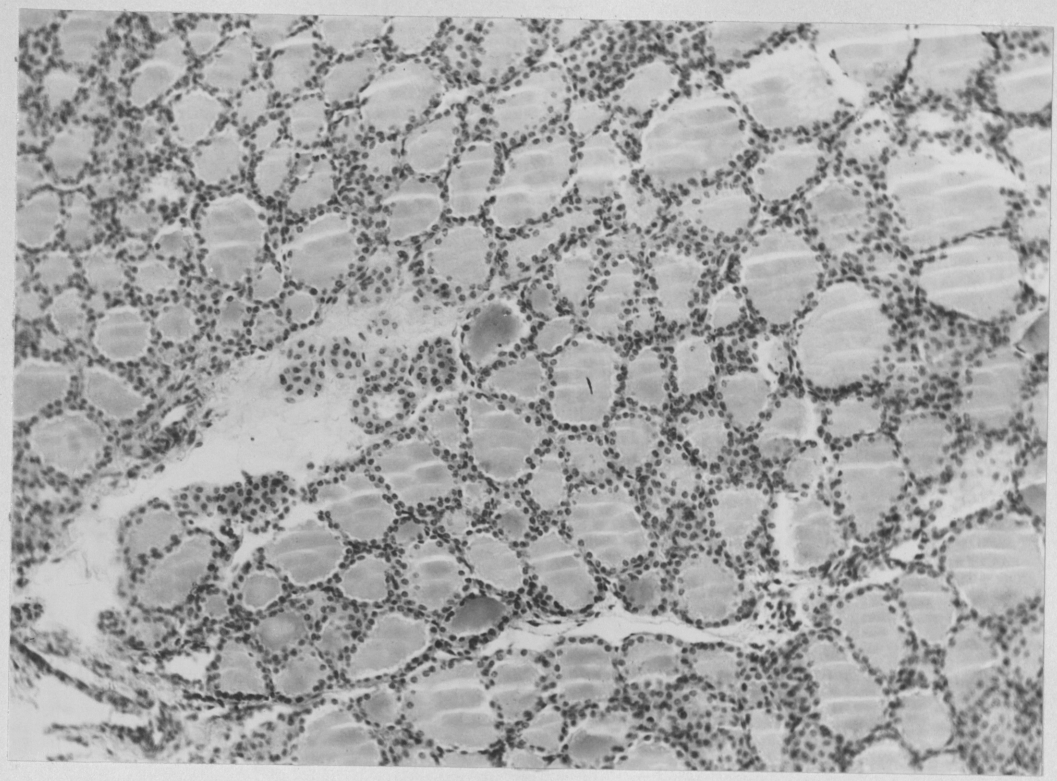

Figure 6.

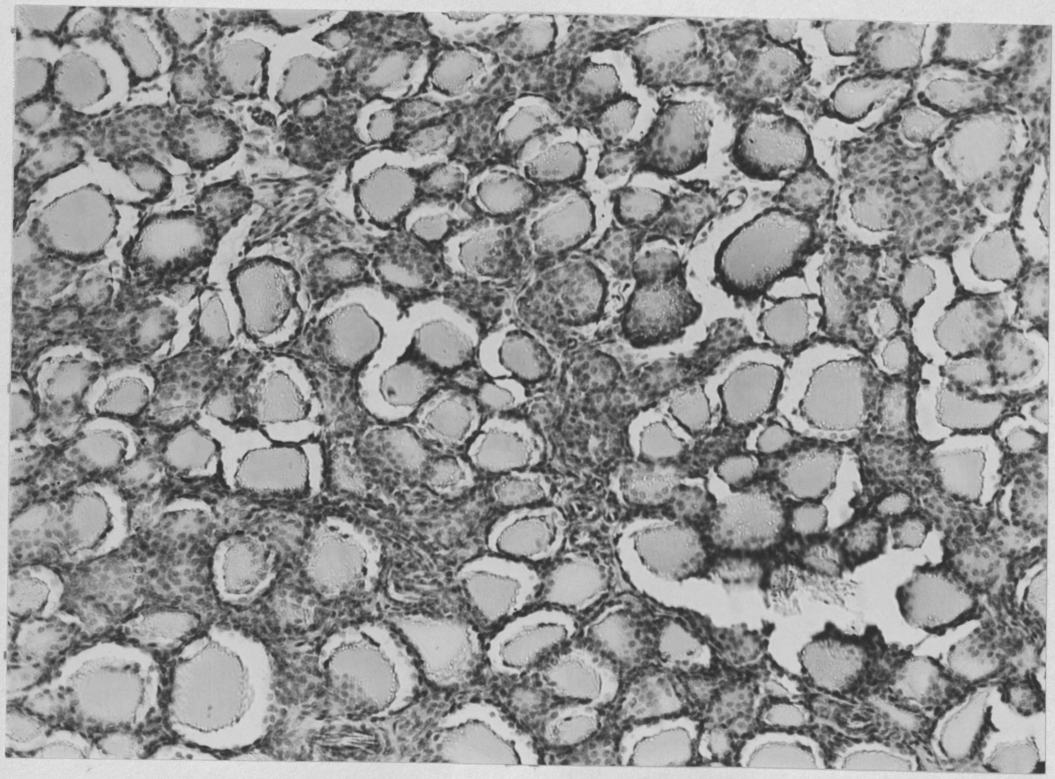

Figure 7.

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