THE EFFECT ON BOTH MOTHER AND OFFSPRING OF ARTIFICIAL REGULATION OF THE METABOLIC RATE OF PREGNANT AND NURSING GUINEA PIGS.

by

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ABSTRACT

Fifty-six animals were used in an experiment designed to study the effect of feeding protamone and thiouracil to guinea pigs. A modified type of oxygen consumption apparatus was described. Observations were made on the weight, oxygen consumption, feed and water intake and respiration rate of the animals during pregnancy and lactation. The outcome of pregnancy, post mortem findings and histological changes in both the mother and offspring were noted, as well as the birth weight and gains of the young. Thiouracil (40 mg./day) was without effect on the guinea pigs. The two levels of protamone used (800 ug. and 1600 ug./day) had an equal effect. Although protamone brought about an increase in oxygen consumption per unit of body weight and a decrease in rate of gain of adult female guinea pigs, no detrimental effect on either the mother or the young was observed.

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INTRODUCTION

The use of thyroproteins for increasing milk secretion in dairy cattle was first introduced on a large scale in the British Isles, because of the great demand for milk during the recent war. Thyroprotein is the name given to iodinated proteins (usually casein) which increase the basal metabolic rate of lactating cows and by so doing, increase the milk production.

Whether a similar increase is to be expected in sows has not yet been established. The problem with swine is the length of time required to bring the young pigs to market weight. Presumably an increase in milk production by the sow would lead to an increase in the weight of the young, if the increase came at a time when the young pigs were almost wholly dependent on their mother's milk. Any damage to the

sow, however, which might result in the production of fewer litters, would prove less economical in the long run, even though these litters were marketed earlier.

Since thiouracil, in practice, should have on animals an effect opposite to that of thyroprotein it seemed of interest to observe the results when it was fed alone and in conjunction with thyroprotein. In that thiouracil reduces the B.M.R., it also permits more rapid gains from a given amount of feed. This is desirable in swine, however, only if such extra weight is deposited as muscle rather than fat, since the Canadian bacon standard requires uniform but narrow back fat.

Because of the difficulties involved in experimental work with sows, it was felt that preliminary tests should be run with guinea pigs to determine if possible the results to be expected from feeding thyroprotein and thiouracil throughout pregnancy and lactation. Since it is easier to control the environment in the laboratory than in the barn, conditions can be kept more constant. Post mortem examination of large numbers of sows would entail great expense. Guinea pigs can be spared more easily. Guinea pigs were chosen in preference to rats, because

their physiological age at birth is more nearly like that of pigs.

Consequently a study was planned in which protamone (an iodinated casein) and thiouracil were fed to both pregnant and nursing guinea pigs. The specific object of this experiment was to observe the gross and microscopic changes resulting from the prolonged feeding of thyroprotein and thiouracil to guinea pigs.

REVIEW OF LITERATURE

The physiology of the thyroid gland has been a matter of great interest for several centuries. Originally it was thought that one function of the gland was to beautify the neck. It was not until about the nineteenth century that the metabolic rate of animals and man came to be associated with the thyroid gland.

In 1895 Magnus-Levy published the first report on the effect of feeding desiccated thyroid material to a normal individual. He found a rise in oxygen intake and in carbon dioxide output. In 1941, Boettiger demonstrated that the oxygen consumption of dwarf mice was 30 - 40% below that of normal mice of approximately the same weight. This could be brought up to normal by thyroid powder or thyroxin.

THE MODE OF ACTION OF THE THYROID GLAND

The lobes of the thyroid gland are located on either side of the trachea, just behind the cricoid cartilage. They are embedded in fatty tissue from which they can be distinguished by their red

color. In order to remove the gland from the ventral side in the guinea pig, two layers of muscle have to be folded back. Several large lymph glands lie in the same region. These are usually larger than the lobes of the thyroid. The isthmus between the lobes is visible in only 33% of normal glands.

The thyroid is composed of many groups of cells known as acini. The height of these cells varies with the activity of the gland (Rawson and Starr, 1938). During the quiescent state the cells are cuboidal, whereas during periods of active secretion the cells elongate, becoming columnar in type. Colloid, containing the hormone itself or a precursor thereof, collects within the acinar cavity.

The exact nature of the hormone is unknown. It is a thyroglobulin containing thyroxin as one of its amino acids. That thyroxin is not the hormone per se is suggested by the work of Means, Lerman and Salter (1933) who found that the activity of whole thyroid containing a standard amount of thyroxin iodine is much greater than that of thyroxin polypeptide containing the same amount. Harington (1935) proposed the theory that the iodine which reaches the thyroid is first introduced into the

molecule of tyrosine to form 3:5 diiodotyrosine. The latter then fulfils a dual role. Part of it is converted into thyroxin and another part is linked with the thyroxin so formed, together with other amino acids, to form the true active principle of the gland. Palmer and Leland (1935), using intact guinea pigs as test animals, came to the conclusion that thyroxin iodine rather than total organic iodine determines physiologic potency. This discrepancy could be explained by the fact that in herbivorous animals there might be considerable destruction of ingested thyroid due to stasis of the food in the animal's stomach. Further research will be required to settle this question. It is, however, of more theoretical than practical value, since synthetic thyroxin can be used wherever thyroid medication is indicated.

The anterior pituitary gland influences and in most cases directly controls all the other glands of internal secretion. Its control over the thyroid is through the thyrotropic hormone (Aron, 1929). As the blood level of thyroid hormone drops, (hereinafter referred to as thyroxin) the anterior pituitary is

stimulated to secrete its tropic hormone. Under normal conditions, this thyrotropin stimulates the release of colloid from the thyroid. This action is, in turn, probably responsible for the induced activity on the part of the acinar cells to produce more colloid. The increased thyroxin level of the blood obviates the necessity of further secretion by the anterior pituitary and the cycle of events is broken. If the action of the tropic hormone on the thyroid gland does not result in an increased thyroxin level in the blood, either because of the surgical removal of the thyroid gland or because all the colloid has been dissipated, then the anterior pituitary continues to secrete its thyrotropic hormone.

ACTION OF THIOURACIL

When thiouracil is administered to intact animals (rats, dogs, sheep etc.) for long periods of time, a typical hyperplasia of the thyroid gland results. In the hypophysectomized animal this does not occur (Higgins and Ingle, 1946). The colloid in the gland is first used up and then the cells begin to elongate in an effort to replenish the depleted stores of colloid. The thiouracil present in the blood stream inhibits the synthesis of thyroxin by

the gland. That the thiouracil prevents the synthesis of thyroxin rather than destroying the preformed hormone is illustrated by the work of Malkiel (1946) on white mice. The susceptibility of white mice to acetonitril poisoning was increased by the administration of thiouracil. The simultaneous injection of thyroxin, however, completely protected the animals. This confirmed the earlier work of MacKenzie and MacKenzie (1943), who found thiouracil ineffective against preformed thyroxin.

Several investigators have recently thrown some light on the question of how thiouracil interferes with thyroxin production. Although thiocyanate goitre can be prevented by added iodine, the latter is ineffective against thiouracil goitre (Astwood, 1943). The difference in action is best explained by the work of Vander Laan and Bissell (1946), in which they show that thiocyanate interferes with the uptake of iodine, whereas thiouracil has no effect on the iodine trapping mechanism but rather with the thyroid hormone synthesis. For this reason the work of Franklin, Chaikoff and Lerner (1944) is of interest. They showed that thiouracil strongly inhibited the conversion of the radioactive iodine

of Ringer's solution to thyroxin and diiodotyrosine.

Then De Robertis and Gonçalvez (1945) observed a wide difference between the oxidation - reduction potential of the follicle cells and of the colloid in the thyroid gland. This difference was altered when the gland was activated. Thiourea produces a drop in the oxidation-reduction potential of the cells.

One more piece was added to the puzzle when Schachner, Franklin and Chaikoff (1943) concluded from their in vitro studies that the formation of both diiodotyrosine and thyroxin by the thyroid gland is linked with aerobic oxidations, in which the cytochrome-cytochrome oxidase system is concerned. However, Lerner and Chaikoff (1945) showed that thiouracil does not interfere with this oxidase system in the thyroid gland.

Moreover, McShan, Meyer and Johansson (1946) found no inhibition of succinic dehydrogenase of the thyroid tissue.

Workers in Europe had also been experimenting along these lines and in 1945, Jensen and Kjerulf-Jensen also suggested that thiouracil prevented the transformation of tyrosine into

thyroxin by inactivation of the oxidation enzyme. They showed, however, that it was a more specific reaction than a copper antagonism.

About the same time, Harington and Rivers (1945) showed that hydrogen peroxide increased the yield of thyroxin from diiodotyrosine in vitro. Sumner and Somers (1943) had already stated that thiourea had an inhibitary effect on peroxidase. This led up to the discovery by Dempsey (1944) of peroxidase activity in the thyroid cells of the normal rat. This activity was inhibited by thiouracil. This observation was confirmed in 1946 by De Robertis and Grasso. They summarized their conclusions by stating that the inhibitary effect of thiourea on thyroxin formation in the thyroid gland is probably due to direct inhibition of the peroxidase system responsible for the liberation of iodine from iodide, thus preventing the iodination of tyrosine to 3:5 diiodotyrosine.

Before considering the changes in metabolic rate brought about by experimentally imposed conditions, it is advisable to be aware of the changes which normally occur at different stages of the life cycle. For this reason, observations on normal animals have been discussed under three separate headings: growth, pregnancy and lactation.

NORMAL CHANGES IN BASAL METABOLIC RATE

(a) During growth.

When Belasco and Murlin (1941) measured the B.M.R. of rats of different ages, they concluded that the basal metabolic rate of the normal rat declines rapidly during the first few months of life and then levels off.

(b) During pregnancy.

The B.M.R. of human subjects was measured before, during and after pregnancy. Comparison of the values obtained just preceding the birth of the baby with the pre-pregnant figures show increases in calories per 24 hours ranging from 23 - 30% (MacLeod, Taylor, Robb, Baker, O'Donahoe and McCrery, 1939).

Spiga-Clerici (1937) used oxygen consumption as an index of B.M.R. He noted a 20% increase after the fourth month of pregnancy.

Thyroid secretion rate is another means of estimating B.M.R. Monroe and Turner (1946) determined the thyroid secretion rate of pregnant rats between the 15th and 23rd day of gestation. They found that it did not differ from that of non-pregnant females of the same body weight. They concluded that pregnancy does not alter the activity of the thyroid gland of the

rat.

Hewitt and Van Liere (1941) support this concept in that they observed no change in the thyroid-body weight ratio of guinea pigs during any stage of pregnancy. It appears, therefore, that any increase in oxygen consumption, thyroxin secretion or thyroid weight in pregnant animals only compensates for the increase in weight. If this hypothesis is correct, further research will be required to elucidate the finding of Scheringer (1933) that the blood picture responded to injection of adrenalin during pregnancy in a manner similar to injections of the same substance in patients with Graves' disease, in contrast to the response of normal or myxedematous patients. Moreover the work of Phatak, Zener and David (1940) is also difficult to explain. When they used iodine medication during pregnancy the oxygen consumption tended to remain close to the normal range of non-pregnant individuals, although the weight increased.

(c) During lactation.

It appears from a review of the published literature that just as the increased oxygen consumption during pregnancy can be accounted for by

changes in body weight, so also can the subsequent post partum drop in B.M.R. be accounted for by loss of weight when the young is born.

Monroe and Turner (1946) came to this conclusion from their determination of the thyroxin secretion rate of lactating rats. They found that the values obtained were the same per unit body weight, as those for the non-lactating animal.

Moreover Hewitt and Van Liere (1941) using different criteria came to the same conclusion. They showed that the ratio of thyroid weight to body weight in guinea pigs does not change in the post partum period. What is true of laboratory animals is not necessarily true of larger animals. However, Schultze and Turner (1945) noted no significant difference in the thyroid secretion rate between goats producing 1.8 pounds of milk daily and non-lactating goats of approximately the same body weight.

Since we now have some idea of how thyroxin exercises its control over the body and what changes one might expect under normal conditions, it is reasonable to look into the changes brought about when the gland is regulated artificially.

EFFECT OF ARTIFICIAL REGULATION OF THYROID SECRETION

(a) Effect of hypothyroidism on growth.

The effect of the thyroid hormone on body weight is in all probability an indirect result of its effect on metabolism. This is suggested by the work of Boettiger (1941) on dwarf mice. He found that associated with the dwarfism, was an inability of the mice to keep up the body temperature. A high environmental temperature had the same effect on the dwarfs as thyroxin.

Scow and Simpson (1945) also show this relationship in their work with rats. The principle effect of thyroidectomy at birth was manifested as a marked retardation in growth and maturation but the rats also had a reduced oxygen consumption.

Growth as measured by body weight is more dependent upon the presence of the thyroid in very young animals than it is in older ones. This is well illustrated by Simpson (1924) in his work with lambs. When thyroidectomy was performed two or three weeks after birth, marked stunting resulted but if the operation was delayed until the animal was three or four months old retardation of growth was only slight.

Thyroidectomy in other species results in a retardation of growth as shown by Salmon (1938) and Binswanger (1936) working with rats and dogs, respectively. Since thiouracil acts in such a way that it reduces the effectiveness of the thyroid gland, it is interesting to note that when thiouracil was added to the ration of chickens at levels up to 0.2% of the ration, growth was retarded at all levels (Andrews and Schnetzler, 1946). When it was fed to lambs, the gain was significantly decreased (Andrews, Beeson, Barrick and Harper, 1947). Gordon, Goldsmith and Charipper (1945) found the same result when they added 0.2% thiouracil to the diet of rats.

In contrast to these findings Andrews and Bullard (1940) observed that all steers gained at an increased rate following partial thyroidectomy. The first rapid gains were probably the result of compensation for the loss of weight and decreased gains immediately following the operation. Moreover, Van Der Noot, Reece and Skelley (1947) fed thiouracil to hogs and found the average daily gains higher than for the controls. The hogs in this experiment weighed 200 pounds at the start of the test. It is possible that they were depositing fat rather than muscle,

but no mention is made of carcass quality. Muhrer and Hogan (1945) had also recorded more rapid gains in pigs on a 28-day thiouracil feeding test but their pigs were pair-fed. This resulted in limiting the feed intake of the controls to that of the test animals.

Aranow, Engle and Sperry (1946) fed thiouracil to adult female monkeys for 14 months and found no significant change in the body weight, although menstrual irregularity became more pronounced. Beeson, Andrews and Brown (1947) also found no growth effect when steers were fed thiouracil. Adult humans appear to be more dependent upon the presence of the thyroid gland than other animals as evidenced by the work of Danowski, Man and Winkler (1946), who found that total thyroidectomy (or thiouracil) did not produce in the dog, any syndrome comparable to human myxedema.

(b) Effect of hyperthyroidism on growth.

Robertson (1928) fed desiccated thyroid tissue to white mice and noted an accelerated growth rate but no modification of maximum weight. In contrast, the feeding of iodinated casein to cows (Hibbs and Krauss, 1947) or pigs (Braude, 1947)

resulted in a loss of weight. The feeding of desiccated thyroid to guinea pigs (Durrant, 1928) or of injecting thyroxin into rats (Bodansky and Duff, 1936) although not causing a loss of weight, did result in a less rapid gain than was found in the controls. Belasco and Murlin (1941) demonstrated that the body weight lost during the administration of thyroxin is influenced by the age of the animal. The younger the animal, the more resistant it is to body weight changes.

It therefore appears reasonable to postulate that the thyroid gland secretes a hormone which controls the metabolic processes of the body. If too little hormone is present during the growth period, stunting results; if too much is present, catabolism takes place at a greater rate than anabolism and rate of growth is also decreased. An optimum amount for growth is essential. After the animal has grown to maturity, the need for the hormone appears to drop. Complete removal of the hormone (by thyroidectomy or thiouracil) therefore causes little effect. Surplus thyroxin on the other hand speeds up the catabolic processes, resulting in a loss of weight.

(c) Effect of hypothyroidism upon the outcome of pregnancy.

Prolonged thiouracil administration, associated with hypothyroidism in the adult female rat, does not cause sterility but does interfere with continuation of gestation, causing resorption of embryos in 100% of the cases. If the drug is given over a period of less than 100 days, some rats do deliver litters which are normal in growth and development and reproduce normally. It seems unlikely that resorption is due to a direct effect of thiouracil upon the fetuses, since in this experiment the young born alive had been exposed to the same amount of drug as the resorbed fetuses. The difference lay in the length of time during which the mother had received thiouracil (Jones, Delfs and Foote, 1946). This confirms the work of Goldsmith, Gordon and Charipper (1945), in which they found that young rats given thiourea for seven months produced no litters until placed on a normal diet. Conception took place but no young were born alive. Jones suggested that the resorption might have taken place because hypothyroidism reduced the utilization of

estrogen and progesterone, thus rendering them inadequate for the maintenance of late pregnancy. The experiments of Freiesleben and Kjerulf-Jensen (1947) demonstrate that, even if resorption of fetuses is not due to direct action of thiouracil on the fetus, nevertheless, thiouracil does reach the young. They fed a thiouracil derivative to pregnant rats for four days. The fetuses were then removed, dried and fed to normal rats. The glands of these normal rats became hyperplastic. Administration of thyroid hormone concurrently with methylthiouracil prevented the development of infantile myxedema. Thus they concluded that it was not the thiouracil derivative but the thyroid deficiency that was detrimental to the quickly growing organism.

From this it may be assumed that since thiouracil passes across the placenta, and since thyroxin counteracts the effect on the young, then thyroxin must also cross the placenta. Further evidence of this is given by Zondek (1940) in his case report of a pregnant myxedematous woman. She had received 0.4 grams "thyreodin" daily for two years before she became pregnant. About the third month of her pregnancy myxedematous symptoms showed up. Treatment was

discontinued, but remarkably enough no further symptoms appeared up to the period after delivery. At that time typical symptoms reappeared. It is concluded that the fetal thyroid was supplying the thyroxin requirement of the mother.

Young animals can tolerate much larger quantities of thyroxin than can adults of the same species. It is quite possible that the hyperthyroidism which sometimes accompanies human pregnancies is less harmful to a developing fetus than a hypothroid condition in the mother, which results in a cretinous infant.

(d) Effect of hyperthyroidism upon pregnancy.

Rats given thyroid for three to five days before mating showed greater litter size during cooler weather and smaller litter size during hot weather. Hyperthyroidism in hot weather caused a greater loss of weight and reduced survival of litters (Kraatz, 1939).

Two interesting cases of hyperthyroidism in humans have been recorded. A primipara with recurrent hyperthyroidism was treated with thiouracil for the last 55 days of her pregnancy (Strouse and Drabkin, 1946). Although the baby did not appear

normal at birth (hypothyroid) she developed into a normal child by four months of age. In the other case, reported by Vogt (1946), a hyperthyroid pregnant woman was given enough thiouracil to cause a marked goitre, but there was no thyroid enlargement in her child. The effect of thiouracil on the metabolic rate of humans is reflected rather slowly. In the first case, the thyroxin level may have dropped lower than the B.M.R. indicated, whereas in the second case, the goitre which developed in the mother may not necessarily have indicated a low thyroxin secretion. Goldsmith, Gordon and Charipper (1945) showed that the hyperplastic, activated glands of the young rats born to thiourea treated mothers were transient in nature. When these young animals were transferred to a normal diet these effects disappeared.

The conclusion seems justified that both thiouracil and thyroxin can cross the placenta.

(e) Effect of hypothyroidism upon lactation.

Although experiments outlined above have shown that there is no increase in B.M.R. during lactation, still, changes in the metabolic rate of lactating animals have a profound influence on the rate of milk secretion. Many studies show that

thyroidectomy depresses lactation in several species [cows (Graham, 1934), goats (Ralston, Cowsert, Ragsdale, Herman and Turner, 1940) and rats (Folley, 1938)] . Moreover Schultze and Turner (1945) found that the oral administration of three grams of thiouracil per day to goats for 14 days, resulted in a reduction of 35% in milk yield. Apart from reducing milk production in the mother, thiouracil also has an undesirable effect on the young.

When methylthiouracil was administered to suckling rats immediately after parturition, there was a slight retardation in the growth rate of the rats and thyroid hyperplasia could be traced in the young after one week (Freiesleben and Kjerulf-Jensen, 1947). Other workers have also shown this to be true (Hughes, 1944), (Williams and Kay, 1944) and (Williams, Weinglass, Bissell and Peters, 1944).

These experiments indicate that thiouracil is able to pass through the mammary gland into the milk.

(f) Effect of hyperthyroidism upon lactation.

In that a lowered B.M.R. has a depressing action on milk secretion, it is not surprising to find the opposite effect when the B.M.R. is raised

above normal. Graham (1934) showed that the addition of small amounts of thyroid material to the diet of normal cows, when the curve of lactation was falling, caused a rapid rise in milk production. Herman, Graham and Turner (1938) also showed the same to be true. Then in 1940, when Ralston et al injected thyroxin into cows and goats, they made the same observations.

Unlike thiouracil, thyroxin apparently does not pass through the mammary gland into the milk. Metabolic studies in children, fed milk from cows receiving synthetic thyroprotein, showed that the thyroxin was not transmitted through the mammary gland into the milk. No changes were observed in the B.M.R. of the children (Bruger and Silberbush, 1946).

Moreover, Monroe and Turner (1946) gave thiouracil and thyroxin to lactating rats and weighed the thyroids of the young. Because the thyroids showed hypertrophy in spite of the thyroxin administered to the mother, they concluded that thiouracil can pass into the mother's milk, whereas the mammary gland is impermeable to thyroxin.

Since there appears to be a species difference between guinea pigs and other mammals, at

least in terms of amount of thyroid secretion, if not also in the manner of its control, the effect of artificial regulation of the B.M.R. of guinea pigs has been considered separately.

REACTION OF GUINEA PIGS TO ARTIFICIAL REGULATION OF B.M.R.

(a) Effect of hypothyroidism.

When seventeen guinea pigs were successfully thyroidectomized a drop of 10% in the B.M.R. was observed (Williams, Phelps and Burch, 1941). Because thyroidectomy resulted in a lowered B.M.R., MacKenzie and MacKenzie (1943) expected to produce the histological alterations and weight increase in the thyroid glands of guinea pigs fed 1% sulfapyridine or 1.5% sulfaguanidine but unlike rats, guinea pigs showed no such changes. The possibility exists that the results do not represent a true species difference but rather that the dietary level of sulfaguanidine employed was just insufficient to elicit a response. However, since higher dietary levels caused a lack of appetite, loss of weight and death, no further work was attempted on guinea pigs.

It has been shown by several investigators

that the response of rats to changes in metabolic rate is at least in part dependent on the diet. Thyroidectomy in rats maintained on a diet containing meat meal causes only moderate reduction in pituitary thyrotropin and acidophil substance (Griesbach and Purves, 1943). Moreover Drill, Overman and Leathem (1943) found that rats on a synthetic diet were protected against hyperthyroidism if sufficient B vitamins in the form of yeast were fed along with the desiccated thyroid.

The unusual response of guinea pigs to thiouracil might conceivably be related to the diet used at the time.

Apparently the chief difference between guinea pigs and rats in their response to thiouracil is due to the fact that the anterior pituitary in the guinea pig secretes very little thyrotropic hormone. Aron (1931) was unable to demonstrate the presence of the tropic hormone in guinea pig blood. For this reason, the guinea pig is the most commonly employed test object for thyrotropic hormone.

Billingsley (1937) summarized the results of his work as follows. "A number of thyrotropic extracts have been simultaneously assayed by the

weight and histology of the guinea pig thyroid and by the metabolic rate. No correlation can yet be expressed between these, except that increase in weight and hyperplasia apparently are associated more often than are either of these responses with an increased metabolism".

(b) Effect of hyperthyroidism.

Because of the low secretion of the tropic hormone, it is postulated that the level of thyroxin in guinea pig blood is also low. In a private communication to the writer, Dr. Turner, of the University of Missouri, stated, "I feel quite certain that the guinea pig secretes very little thyroxin and therefore, its requirement for thyroprotein is very low". Koger and Turner (1943) state that preliminary experiments (not reported) showed that guinea pigs were more sensitive to thyroid materials than rats and that 0.02% thyroactive casein was all that guinea pigs would tolerate. Krogh and Lindberg (1945) noted that iodine and diiodotyrosine prevented the rise in B.M.R. normally brought about by administering thyrotropic hormone to guinea pigs. However, neither of these substances had any effect on the hyperthyroidism induced by feeding thyroid preparations. When Carlson,

Rooks and McKie (1912) fed desiccated sheep's thyroid to fifteen guinea pigs, there was a rapid and progressive loss of body weight in all cases, which resulted in death in 12 days.

No references are available concerning the feeding of protamone to pregnant guinea pigs. The reason is not known. It may be that since it was without effect, it was not of sufficient interest to publish. It is more likely, however, that since protamone is a comparatively new product, no work of this kind has previously been attempted.

The present study is therefore of particular interest. Since non-pregnant animals have been used as controls, the results can be compared with those already discussed.

EXPERIMENTAL PROCEDURE

An experiment was designed in order to study the ultimate effect on both mother and young of feeding thyroprotein and thiouracil to the pregnant and lactating guinea pig. This was a preliminary study to be repeated at some future date on swine. Guinea pigs were chosen as the laboratory animal because the length of the gestation period is such that they are born at approximately the same physiological age as the pig. Rats are born in a helpless condition. Guinea pigs and pigs on the other hand are much farther advanced.

I. Animals.

The guinea pigs used in this study came from the colony maintained by the Department of Pensions and National Health at Hull, Quebec. They were kept at Macdonald College for six weeks on the same diet as that used in this experiment. Forty-eight mature females were used. Three quarters of them were young, virgin females. The rest had previously had one or more litters. The animals were allotted to test at random according to the following experimental design (4 guinea pigs per lot):-

Table 1. Allotment Plan.

Level of protamone	Pregnancy	Thiouracil	None
800 ug/day	Pregnant	Lot 1*	7
	Non-pregnant	2	8
1600 ug/day	Pregnant	3	9
	Non-pregnant	4	10
None	Pregnant	5	11
	Non-pregnant	6	12

* 8 Replacements were also used, as follows:-

Lot 1	3	pigs
3	1	"
5	2	"
7	1	"
11	1	"

making a total of 56 animals on test.

II. Diet.

The basal diet consisted of the Macdonald College guinea pig mixture No. 6, which has the following composition:-

Ground oats	15%	Fishmeal	5%
Ground wheat	13	Molasses	5
Beet pulp	20	Brewer's yeast ...	10
Linseed oilmeal ..	12.5	Bone char	4
Skimmilk powder ..	15	Salt (iodized) ...	0.5

III. Supplements.

In addition to the basal diet, each test animal received in a single dose 0.4 ml. of corn oil per week in which was dissolved:-

3000 i.u. vitamin A

21 mg. alpha tocopherol

300 i.u. vitamin D_2.

Each animal also received a weekly dose of

35 mg. ascorbic acid

dissolved in 0.4 ml. orange juice. During the winter each guinea pig received

1 oz. cabbage per day

1 oz. hay per week (approx.)

When lawn clippings became available, three ounces of grass replaced the hay, cabbage, ascorbic acid, alpha

tocopherol and vitamin A.

IV. Test materials.

(a) Thiouracil.

The thiouracil was prepared by the Calco Chemical Division of the American Cyanamid Co. It was supplied by Dr. Noble, formerly of McGill University. 40 mg. of thiouracil was fed per day. 10 parts of the dry powder were mixed in a mortar with 15 parts of purified casein. A glass tube with a metal plunger was calibrated in such a way that it delivered a dose equivalent to 100 mg. of the mixture (40 mg. thiouracil). This dry mixture was put directly into the mouth of the guinea pig.

(b) Protamone.

Protamone is an iodinated casein prepared by Cerophl Laboratories Inc. Kansas City, Missouri. We are indebted to them for this gift.

The protamone solution was made up by dissolving 800 mg. protamone in 200 ml. distilled water. One pellet of sodium hydroxide was added (final pH 11.5). The protamone went into solution, if allowed to stand overnight. Thompson, Nadler, Thompson and Dickie (1934) showed that an alkaline solution of thyroxin was nearly three times as

effective on the average as an equivalent dose of the monosodium salt, when both compounds were administered by mouth. The protamone solution was kept in a cool place. 0.2 ml. (800 ug) or 0.4 ml. (1600 ug) per day was put directly into the mouth of the guinea pig.

V. Management.

(a) Mating period.

The non-pregnant animals were kept throughout the test in individual wire bottom metal cages. They acted as the controls for the experiment. The test animals were placed in a group pen, with a perforated floor, in six lots of four pigs to a lot. The males were rotated among the pens each day. The pens were bedded with hay and the animals supplied with food and water ad libitum.

(b) Pregnancy period.

After a period of at least 16 days, the females were removed to the individual metal cages. They remained here until the litters were dropped.

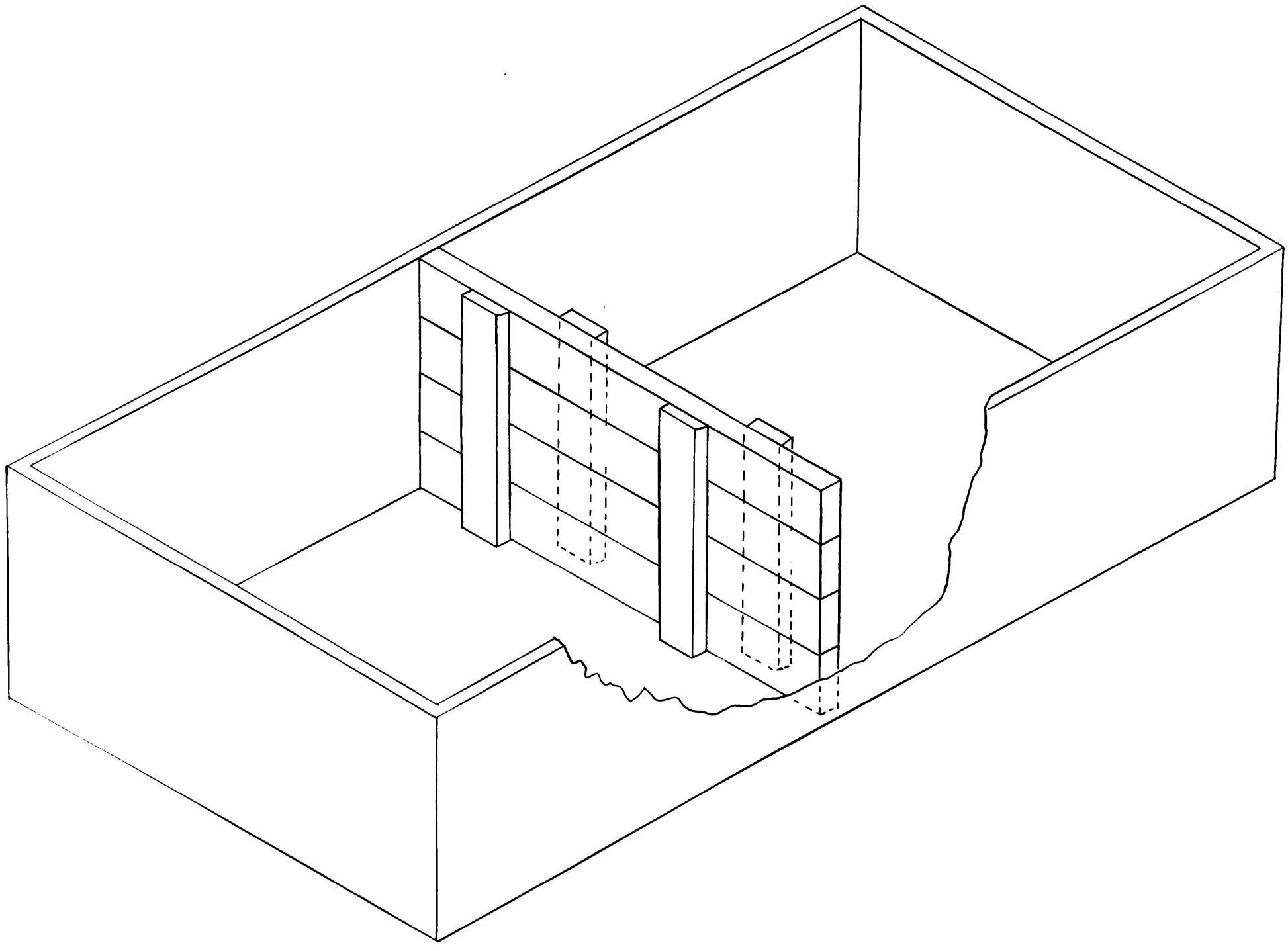
(c) Lactation period.

At this time the mother and young were transferred to individual pens, bedded with shavings. Each pen was partitioned in such a way that the mother could reach the food, by jumping, whereas the young

pigs could not. Eventually the young learned to jump also. Then the partition was made higher each time they went over. By the time the young pigs were 14 days old, they could jump as high as the mother. It was then necessary to hobble the little pigs by tying a front and hind leg together. Although the guinea pigs learned to jump over an eleven inch partition in search of food, they never jumped onto the floor, even though the pens were not covered and the divisions only twelve inches high.

Figure 1 shows a drawing of the pen used during the lactation period.

FIGURE 1.



PEN USED FOR GUINEA PIGS
DURING LACTATION.

RECORDS

1. Observations on the living animals.

(a) Adult females.

(1) Body weight.

The weight in grams of all animals was determined weekly throughout the test. This weight was always recorded after a 12 - 20 hour fast.

(2) Feed record.

The feed record for each animal was determined during pregnancy and lactation. No record was possible during the mating period. A container holding enough feed for the experiment was weighed in grams at the beginning of the test. At weekly intervals the amount consumed was determined by difference. The animals had access to feed at all times except during the fasting period (once a week) when it was removed overnight. No record was kept of the weight of cabbage, hay or grass eaten.

(3) Water intake.

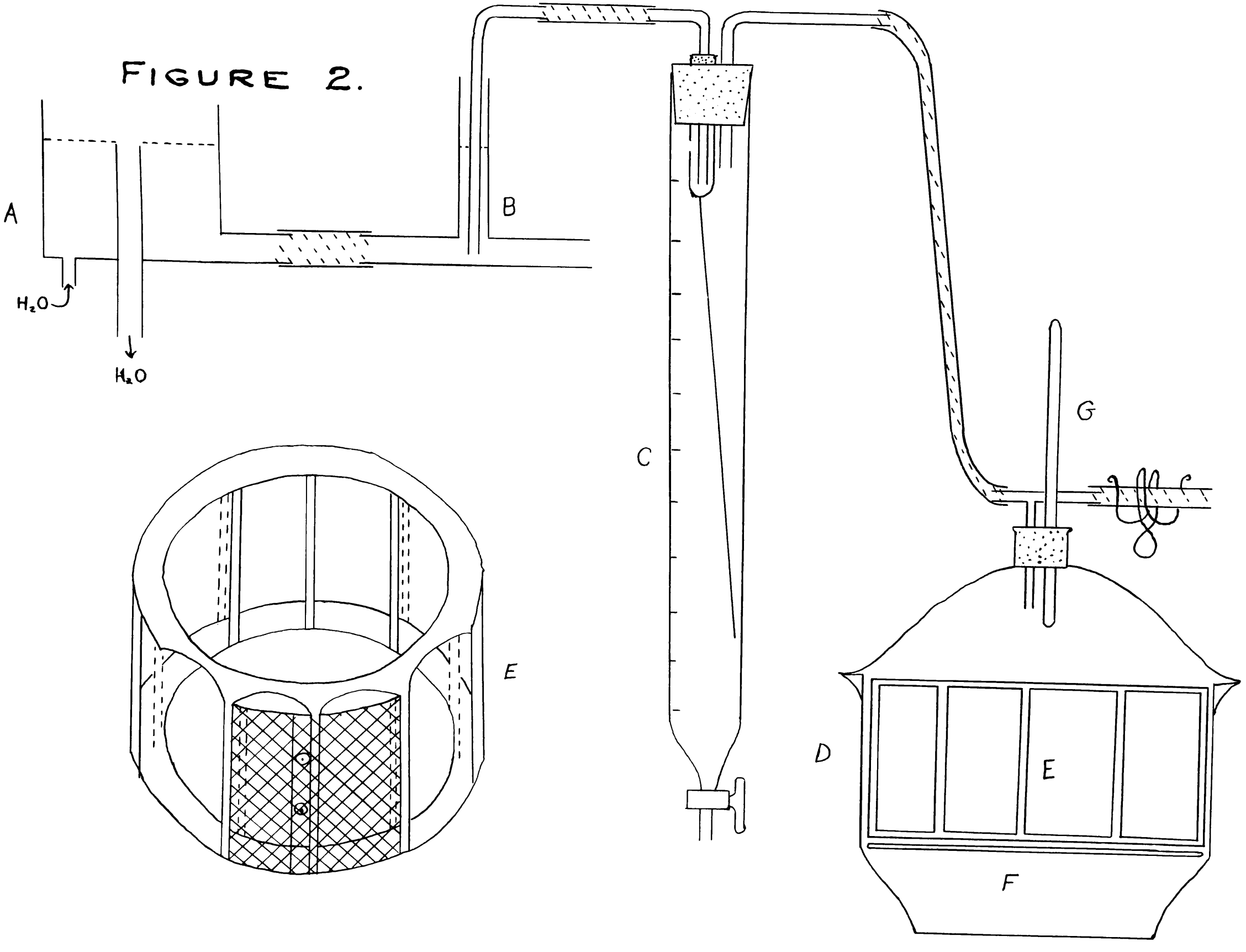
A record of the water consumed was kept for each animal during pregnancy. A beaker was filled with water, weighed in grams and placed in the pen. About three times a week, the beaker was reweighed,

emptied and filled again with fresh water. The amount of water used was determined by difference. In this way the animals had water before them at all times. No record was kept during the mating or lactation period.

(4) Oxygen consumption.

The oxygen consumption for each guinea pig was determined weekly throughout the test. The apparatus used was designed in this laboratory. It is a modification of the Teitelbaum and Harne apparatus (1940). Figure 2 is a drawing showing the details of construction. Figure 3 shows four units in operation.

FIGURE 2.



APPARATUS FOR MEASURING OXYGEN CONSUMPTION IN GUINEA PIGS.

A. A water leveling device made of chromium plated brass.

B. A "T" tube joined to A by rubber tubing. There are four in the series, each unit capable of working alone or with the others.

C. A 1000 ml. dispensing burette fitted with a rubber stopper. Hung from the stopper is a test-tube with a hole in it. The glass tubing (syphon from B) is below the level of the hole, so that the syphon can be maintained. The height of the burette is adjusted until the hole is at the level of the water in the "T" tube (B). Thus no water flows into the burette unless the air-pressure is reduced. The string hanging from the test-tube prevents splashing.

D. A desiccator fitted with a rubber stopper. A "T" tube not only allows air to pass from the burette C into D, as the pressure in D drops, but also permits the operator to suck the air out of D to start the syphon from B to C at the beginning of the test.

E. A two-walled wire basket holding soda lime. A frame of brass (chromium plated) is made of two discs held together by rods. The inner screening

is threaded between the two sets of rods and secured permanently. The outer screening (copper) surrounds the outer rods and is fastened with two snaps. It can be removed to change the soda lime. The top disc is cut away between each set of rods, to allow for filling the basket by means of a spoon.

F. A perforated floor (chromium plated brass).

G. A thermometer.

<u>To run the test.</u>

1. Turn on the water until the level is constant in B.
2. Suck on "T" tube in D until the water rises in B and syphons over into the test tube in C. Continue sucking until water reaches some mark in C to be used as starting level.
3. Remove lid from desiccator and put guinea pig in. Replace lid and seal. Seal all other joints with stop-cock grease if necessary.
4. Seal off the "T" tube in D with a pinch-cock.
5. The test is continued until a given amount of water (300 ml.) has been drawn over.

<u>CAUTION</u>

All connections must be air-tight.

FIGURE 3.

The oxygen consumption is expressed in ml./min, calculated according to the following formula:-

$$\text{ml./min} = \frac{P_1 V_1 T_2}{P_2 T_1 \ \text{time}}$$

where P_1 = pressure in mm.

V_1 = 300 ml.

T_1 = temp. in ^oA

P_2 = 760 mm.

T_2 = (20°C) 293°A

time = time in minutes, required to use 300 ml. oxygen.

(5) Respiration rate.

The respiration rate, expressed as respirations per minute, was determined weekly. This was done by counting the rise and fall of the abdomen for 30 seconds. This reading was made just at the end of the metabolism test, while the animal was usually at rest.

(b) Young guinea pigs.

A daily record was kept of the weight of the young guinea pigs from birth to eighteen days of age. Only the two heaviest pigs in each litter were kept after the fourth day. The general thrift of the animals was noted throughout the nursing period.

II. Post Mortem Examination.

At the end of the experiment all the guinea pigs were killed with chloroform and examined for any gross changes in the internal organs (lungs, kidney, uterus, ovary, adrenal, thyroid and liver). The adrenal glands were removed and the fresh weight determined (two adrenals weighed together).

III. Histological Examination.

The thyroids, gonads and adrenals in all pigs (including young ones) were removed. A section of the mammary gland in all the lactating animals was also kept. These tissues were fixed for three hours in Bouin's solution, dehydrated, embedded in paraffin, sectioned on a microtome and stained with hematoxylin and eosin. The tissues were then examined under oil immersion on the microscope.

RESULTS

Biological work involving the use of animals presents problems of variability not encountered in lifeless objects. By careful selection much of the individual variability among animals of the same species can be eliminated. The guinea pigs used in this test were of the same sex and age and had been subjected to the same previous dietary regime (except one replicate of older animals). By randomization of these pigs among different treatments, according to a definite experimental design, it was possible to measure statistically variation which could not be removed. The necessary difference between treatments decreases as the number of animals involved increases. It is unfortunate that this experiment was limited to four animals per lot, the limiting factor being the number of determinations of oxygen consumption that could be made in a week.

I. Effect of protamone and thiouracil treatment of guinea pigs during pregnancy.

Wherever thiouracil and protamone were fed together, as in Lots 1, 2, 3 and 4, the groups reacted

as if no thiouracil had been present. For this reason, the results will be interpreted as if they had received protamone alone.

Eight of the pregnant guinea pigs produced no living young. Replacements for these were started so that equal numbers of young pigs would be available for the statistical analysis. The conclusions arrived at of the effect of treatment upon the outcome of pregnancy were based on both the original animals and the replacements (32 in all).

(a) Outcome of pregnancy.

The effect of protamone and thiouracil administration on the outcome of pregnancy is shown in Table 2.

Table 2. Effect of Treatment Upon the Outcome of Pregnancy

Lot number	Number of animals mated	Number of animals pregnant	Number of litters	Number of abortions
1 T* p	7	7	4	3
3 T P	5	5	4	1
5 T	6	4	3	1
7 p	5	4	3	1
9 P	4	4	4	0
11 control	5	4	4	0
Total	32	28	22	6

*T Thiouracil 40 mg/day.
P Protamone 1600 ug/day.
p Protamone 800 ug/day.

Of the 32 animals mated, all but four conceived. Although abortion did not occur, there was no way of telling whether or not resorption had taken place. The animal in the control treatment (Lot 11) was found, on post-mortem examination, to have a non-functional horn on the right side of the uterus. The protamone treated animal (Lot 7) became extremely thin and eventually died of a lung condition. The stomach and part of the intestine showed muscle degeneration often seen in an old animal. The other two pigs (Lot 5) were receiving thiouracil without any additional protamone. This is known to cause resorption in rats (Jones, Delfs and Foote, 1946). However, the animals used as replacements on this same treatment conceived after the regular mating period. For this reason no significance is attached to a failure to conceive.

Of the 28 animals which conceived, 22 produced litters (see Table 2). Thus 78% of the pregnancies resulted in normal litters. This is only slightly lower than the 80% - 90% found by Farmer (1944), when normal guinea pigs were kept on a diet supplemented with lawn clippings. Since cabbage and hay were used for the first two months of the present

experiment, this might have accounted for the slight difference. Moreover it is interesting to note that, of the six animals which aborted, five showed abnormalities on post mortem examination. The word abortion is used here to include all pregnancies terminating in the birth of dead young, as well as the expulsion of fetuses at any time before term. In one of the animals in Lot 1, the placental sac was expelled at least twelve hours before the young were born. Then three young were born dead. There was extensive hemorrhage, following which the mother died. On examination, the uterus was found to contain two more fetuses and one very much enlarged placental attachment, which had undergone degeneration. The two other guinea pigs in Lot 1 showed abnormalities of the spleen. In one, the spleen was adhered to the abdominal wall more than usual and in the other, a large growth, the size of the kidney, was found at one end of the spleen. In this animal the liver was badly adhered to the abdominal wall. The pig which aborted in Lot 3 had a large growth on the uterus. In the remaining animal (Lot 7), pus was found where the uterus and intestines had adhered to the abdominal wall. Since the abortions were not confined to any

one treatment and since they were no greater in number than is usual in the stock colony, it is assumed that neither protamone nor thiouracil affected the number of abortions.

Of the 22 litters produced three contained some young which were dead at birth. This is shown in Table 3.

Table 3. Birth Weight of Young Pigs

Lot number	Litters		Average No. of young born alive per litter	Average birth weight in grams
	All pigs alive	Some pigs dead		
1 T* p	3	1	3.0	94
3 T P	3	1	1.5	94
5 T	2	1	3.0	90
7 p	3	0	3.3	81
9 P	4	0	3.0	90
11 control	4	0	3.0	105

*T Thiouracil 40 mg/day.
P Protamone 1600 ug/day.
p Protamone 800 ug/day.

All three of the animals producing the dead young (among living ones) were receiving thiouracil. No conclusion concerning an ill-effect of thiouracil can be based on these results, since the animals in the stock colony normally produce 28% of litters containing some dead young. The reason is unknown (Farmer, 1944).

Table 3 also shows the average number of pigs born alive per litter. Previous work in this laboratory has shown that litters of one are rare. In that two litters of one occurred in Lot 3, the average for the group is low. This is assumed to have happened by chance and treatment appears to have had no effect upon the size of the litters in this experiment.

The birth weight of young guinea pigs varies greatly according to the size of the litter. Only 22 litters were available for the comparison of the effects of thiouracil and protamone on birth weight and therefore the differences shown in Table 3 are not statistically significant. They are attributed to chance variation rather than treatment.

It is concluded that neither protamone nor thiouracil, in the amounts fed, had any effect on conception, abortion, birth of dead pigs among living

ones, size of litter or birth weight of the young.

(b) Body weight.

Three animals lost weight rapidly soon after being put on test. After two weeks, they had lost an average of 116 grams. Although these animals lived for at least seven weeks, all three eventually died in an emaciated condition. These animals were receiving protamone, two with and one without additional thiouracil. However, since all the animals treated thus did not respond in a similar manner, the loss of weight must have resulted from an individual susceptibility to thyrotoxicosis. Because protamone increased the oxygen consumption per unit of body weight (as discussed later) it is not surprising to find that animals receiving protamone did not gain as rapidly as the controls. The average initial weights for treatments ranged from 367 to 487 grams. Table 4 shows the average gain in body weight for seventy days before parturition relative to the group controls (pregnant and non-pregnant).

Table 4. Gain in Body Weight for Seventy Days Before Parturition

Group	Lot	Average gain for 70 days	Gain relative to group control		
			Protamone	Thio-uracil	No protamone No thio-uracil
		gms.	%	%	%
Pregnant	1	456	93	94	100
	3	340	69		
	5	460			
	7	418	85		
	9	391	80		
	11	490			
Non Pregnant	2	105	63	130	100
	4	105	63		
	6	215			
	8	137	83		
	10	46	28		
	12	166			

Protamone treated animals gained less rapidly than did the controls in all cases. On the whole pregnant animals fared better than the non-pregnant ones. This is probably accounted for by the fact that the body weight of the mother is only a portion of her total weight, during pregnancy.

Differences between the gains made by thiouracil treated animals and the controls were not significant.

(c) Oxygen consumption.

The values for oxygen consumption show a high degree of variability (see Appendix Table 1).

By the use of Fisher's Summation Method of Fitting Polynomials (Gouldon, 1939), trends in oxygen consumption can be emphasized. These values were used in interpreting the results. Because the rate of oxygen consumption was not the same for all lots at the start of the test, values are expressed as an increase rather than an absolute oxygen consumption. The original value for each lot (ten weeks before term) was assumed to be normal. This value was determined three to ten days after the treatments were started. If protamone exerts its effect on oxygen consumption within 72 hours, the initial rise is not shown. Figure 4 shows

the increase in oxygen consumption in millilitres per minute for pregnant guinea pigs for ten weeks before term. Figure 5 represents a similar period for non-pregnant animals.

FIGURE 4.

INCREASE IN OXYGEN CONSUMPTION
OF PREGNANT GUINEA PIGS.

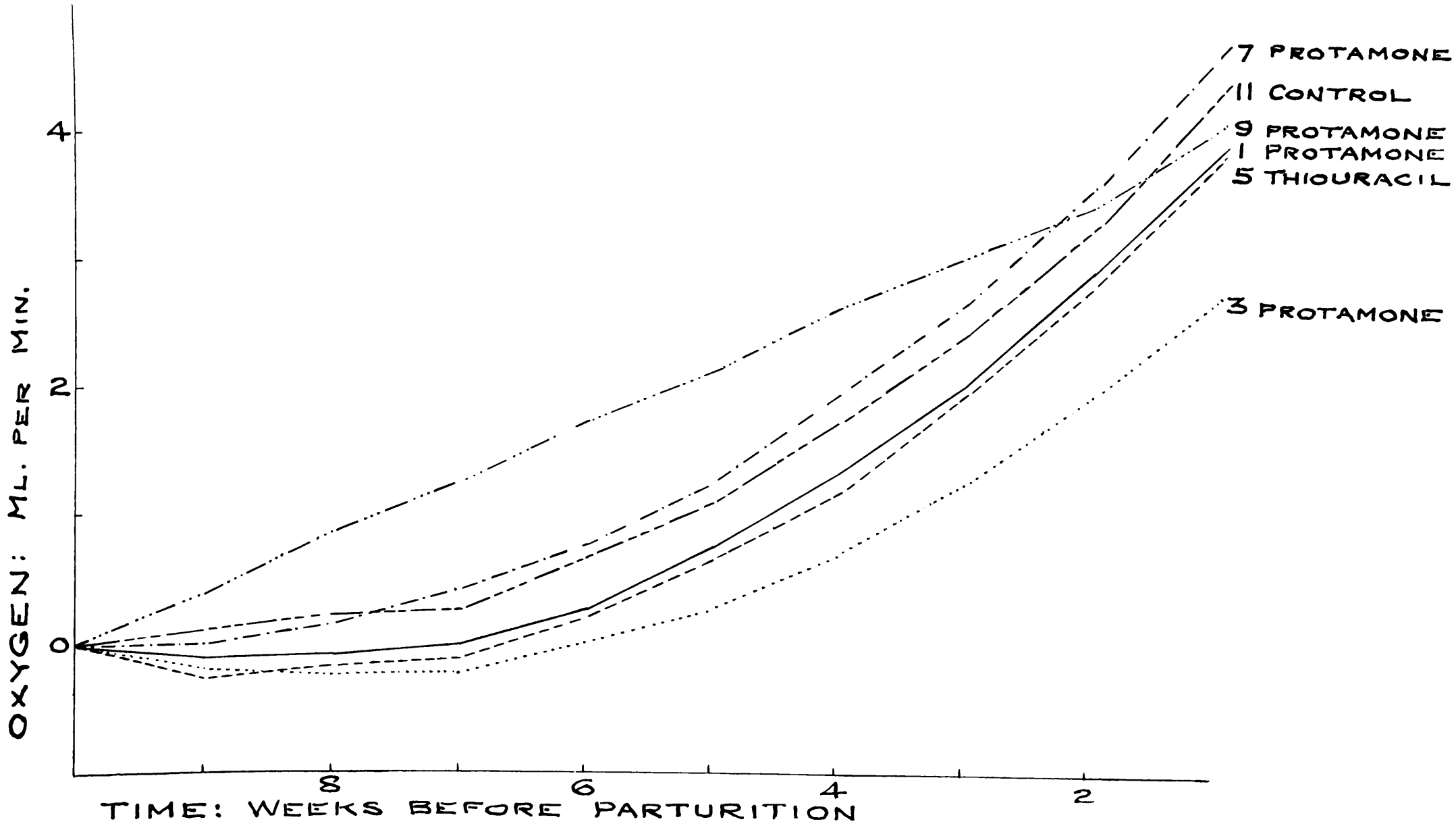
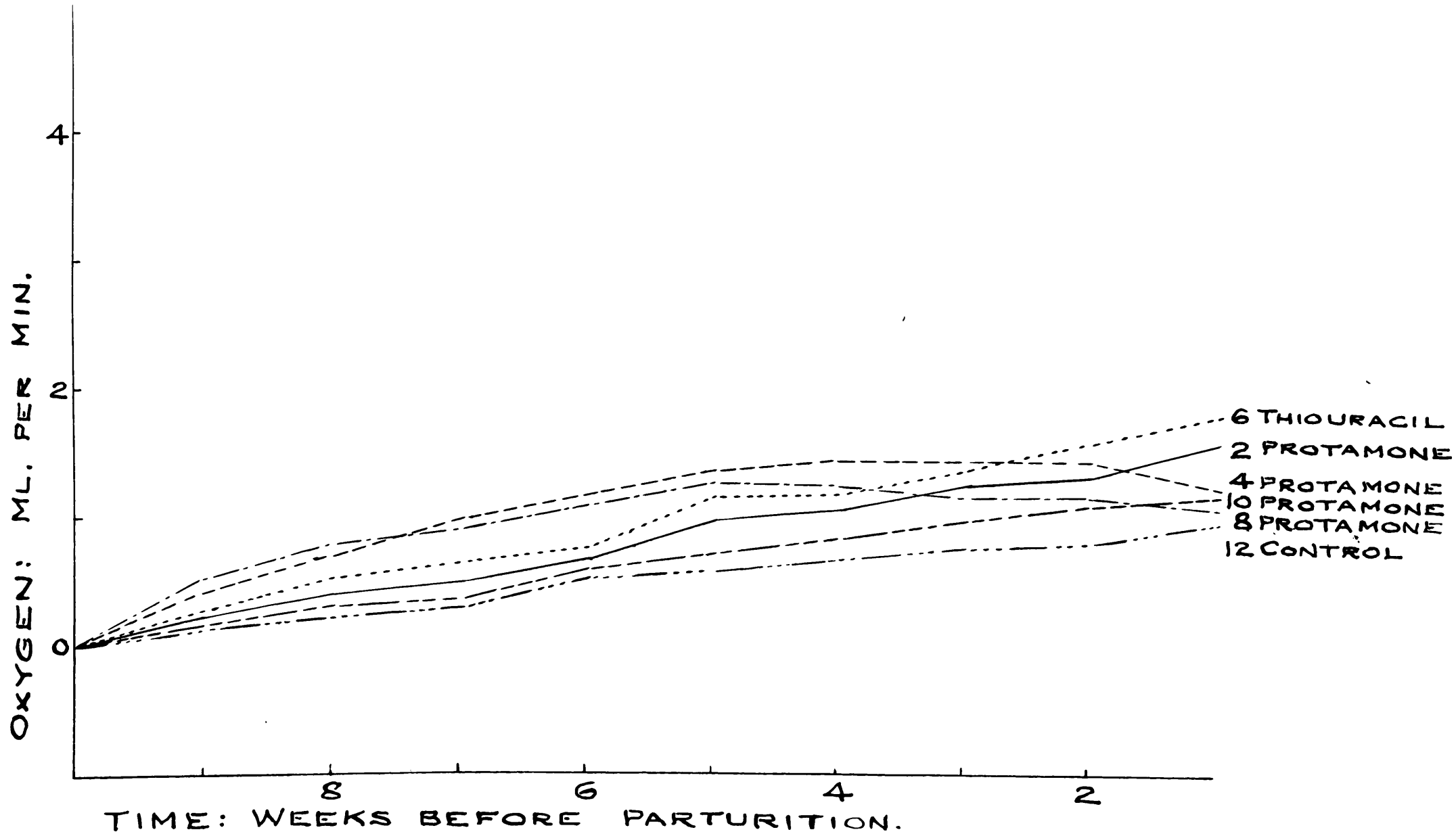


FIGURE 5.

INCREASE IN OXYGEN CONSUMPTION
OF NON-PREGNANT GUINEA PIGS.

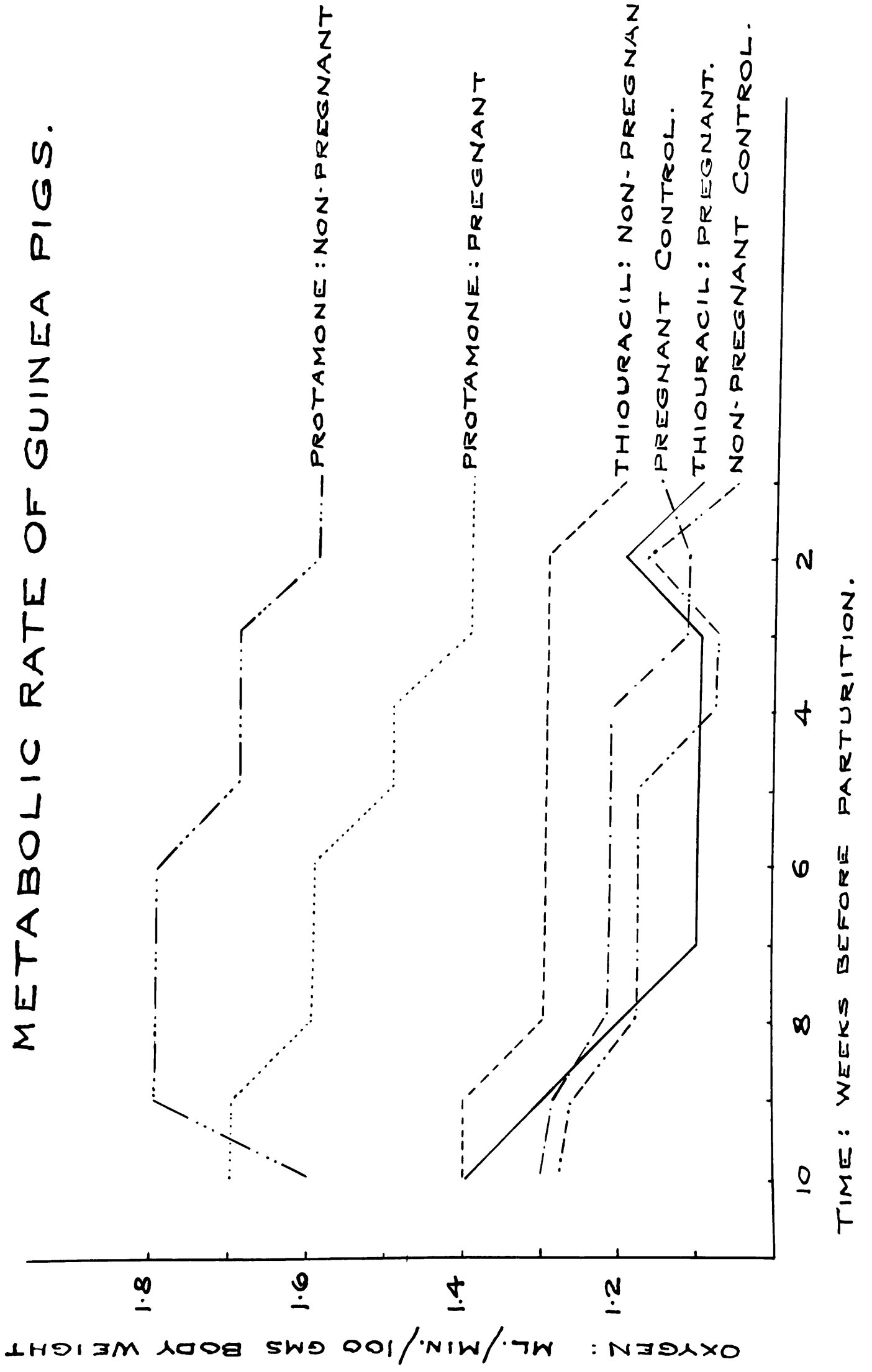


From a comparison of Figures 4 and 5, it may be seen that the pregnant animals showed a greater increase in oxygen consumption than the corresponding non-pregnant animals. Because the non-pregnant animals were growing, their oxygen consumption rose 21% during the ten week period. The oxygen intake of the pregnant guinea pigs increased 55% during the same time. If the pregnant animals had not also been growing we can assume that they would have showed only 34% increase in oxygen consumption due to pregnancy. This supports the work of MacLeod et al (1939) who found a 23 to 30% increase and Spiga-Clerici (1937), who found a 20% increase in oxygen consumption during pregnancy in humans.

In spite of this actual increase in oxygen consumption during pregnancy, the B.M.R., when expressed as the millilitres of oxygen per minute per 100 grams of body weight, appears to drop as pregnancy proceeds. When this is compared with the non-pregnant values, however, it is seen that the two curves are almost identical. This is illustrated graphically in Figure 6.

FIGURE 6.

METABOLIC RATE OF GUINEA PIGS.



The thiouracil treated animals, both pregnant and non-pregnant, showed a total increase in oxygen consumption and a metabolic rate practically identical with that of the untreated controls.

Similar to these findings, protamone has no effect on the absolute oxygen intake but, in contrast to thiouracil, it raises the metabolic rate of both pregnant and non-pregnant animals above that of the controls. The fact that the increase in oxygen consumption remains the same in protamone treated animals as in the controls, in spite of an increase in metabolic rate, is best explained by the fact that protamone causes a decreased rate of gain which compensates for the increased metabolic rate. Table 5 shows numerically the average metabolic rate of protamone and thiouracil treated animals, expressed as a percentage of the average B.M.R. of the controls during pregnancy or a corresponding period in the non-pregnant animals.

Table 5. Average Oxygen Consumption of Pregnant Guinea Pigs in ml. per Minute per 100 gms. of body weight for 10 weeks before parturition.

Group	Lot	Average oxygen consumption per 100 gms. body wt.	% Increase above group control		
			Protamone	Thiouracil	No protamone. No thiouracil.
		ml/min	%	%	%
Pregnant	1	1.44	21		
	3	1.59	34		
	5	1.17		-2	
	7	1.54	29		
	9	1.52	28		
	11	1.19			0
Non-pregnant	2	1.79	50		
	4	1.72	45		
	6	1.31		10	
	8	1.60	34		
	10	1.70	43		
	12	1.19			0

(d) Feed intake.

Although no record was kept of the amount of cabbage, hay or grass eaten by the guinea pigs, it is assumed that the animals in different lots ate on the average, the same amount of these feeds daily. Table 6 shows a record of the average daily consumption of basal diet, in grams, for the last three weeks of pregnancy. A corresponding period is given for non-pregnant controls.

Table 6. Average Daily Consumption of Basal Diet in grams for Three Weeks Before Term.

Group	Lot	Protamone	Thio-uracil	No Prot-amone. No thio-uracil.
		gms.	gms.	gms.
	1	36		
	3	34		
Pregnant	5		26	
	7	37		
	9	43		
	11			36
	2	28		
	4	30		
Non-	6		31	
pregnant	8	31		
	10	30		
	12			24

Pregnant animals showed an average feed intake greater than that of the controls (except in Lots 5 and 6). No difference could be shown between the protamone or thiouracil treated animals and the controls in this experiment. Table 7 shows the gain, in grams, per 100 grams feed intake for three weeks before term.

Table 7. Gain in Grams per 100 gms. Feed Intake for Three Weeks Before Term.

Group	Lot	Protamone	Thio-uracil	No prot-amone. No thio-uracil
		gms.	gms.	gms.
	1	34		
	3	27		
Pregnant	5		44	
	7	30		
	9	24		
	11			34
	2	9		
	4	10		
Non-	6		11	
pregnant	8	12		
	10	4		
	12			5

As might be expected, the pregnant animals made greater gains per unit of food intake than non-pregnant guinea pigs. This is probably explained by the fact that the efficiency of food utilization is greater when water and protein are being laid down (for fetal development) than when fat is being stored, since the latter is of much higher caloric value per unit of weight. The thiouracil treated animals made significantly greater gains than the controls, per unit of feed intake, whereas the protamone treated guinea pigs made smaller gains.

(e) Consumption of water.

In many laboratories throughout the country, it is customary to raise guinea pigs without water. In the management of the colony at Macdonald College, guinea pigs have access to water even when fresh grass forms a part of their diet. Just how much water is required under these conditions is not known. It was interesting, therefore, to measure the water intake in the test animals, whose metabolic rate was being altered artificially. Table 8 shows the average daily water consumption (in grams) of pregnant animals for the three weeks before term. For

comparison, a similar record was kept on the non-pregnant controls.

Table 8. Average Daily Water Intake in Grams for Three Weeks Before Parturition.

Group	Lot	Protamone	Thio-uracil	No prot-amone. No thio-uracil.
		gms.	gms.	gms.
	1	72		
	3	69		
Pregnant	5		43	
	7	76		
	9	79		
	11			60
	2	104		
	4	127		
Non-	6		61	
pregnant	8	101		
	10	112		
	12			70

All the animals receiving protamone drank more water than the controls. No difference was shown between the thiouracil treated animals and the controls. A particularly interesting and unexpected finding is that pregnant guinea pigs drank less water than the non-pregnant controls for the last three weeks of pregnancy. This was true, in spite of the fact that the pregnant animals drank 63 grams per day and the controls only 55 grams per day for the first week of the test. Observation of the test animals suggests that pregnant animals, especially as they get very heavy towards the end of the gestation period, run about in the cage less than the controls. It is possible that the lower water consumption of the pregnant animals is attributable to this fact. There appears to be no physiological basis for this finding.

(f) Respiration rate.

The average respiration rate for the last three weeks of pregnancy has been computed. Pregnant animals show a slightly higher rate than the controls. Due to the high degree of variability no differences were found between protamone or thiouracil treated animals and the controls. Table 9 shows the average

respiration rate for the last three weeks before parturition.

Table 9. Average Respirations per Minute for Three Weeks Before Parturition.

Group	Lot	Protamone	Thio-uracil	No prot-amone. No thio-uracil.
	1	87		
	3	86		
Pregnant	5		85	
	7	97		
	9	88		
	11			79
	2	84		
	4	72		
Non-	6		83	
pregnant	8	82		
	10	69		
	12			69

II. Effect of protamone and thiouracil treatment of guinea pigs during lactation.

The fasting weight and oxygen consumption of all lactating animals was determined on the seventh day post partum. The average daily feed consumption was computed for the first week of the nursing period. This data is given in Table 10.

Table 10. Average Weight, Oxygen Consumption and Feed Intake During Lactation.

Lot number	Average weight	Oxygen consumption		Daily feed intake
		Observed values	per 100 grams body weight	
	grams	ml/min	ml/min	grams
1 T*p	539	8.60	1.6	33
3 T P	532	8.56	1.6	33
5 T	620	7.39	1.2	27
7 p	589	8.99	1.5	34
9 P	628	11.30	1.8	40
11	656	8.65	1.3	21

*T Thiouracil 40 mg/day.
P Protamone 1600 ug/day.
p protamone 800 ug/day.

(a) Body weight.

During pregnancy the protamone treated animals showed slower gains than the untreated controls. This was pointed out in Table 8. It is interesting for this reason to see that these animals also weighed less than the controls on the seventh day post partum. No practical importance is attached to the fact that thiouracil treated animals had a lower body weight than the untreated controls.

(b) Oxygen consumption.

The actual oxygen consumption figures do not give a very clear picture of the effect of protamone and thiouracil during lactation. These values were determined after one week of nursing. If they are compared with the values in Appendix Table 1 it will be seen that in every case (except Lot 9) there was a drop in oxygen consumption after the birth of the young. The metabolic rate (oxygen used per 100 grams body weight) in contrast to this, actually showed an increase over the values determined just before parturition. (See Figure 6). It appears that the B.M.R. returned to the level considered normal for the full-grown animal (about six weeks before term). Protamone raised the B.M.R.

above normal whereas thiouracil had no effect.

(c) Feed intake.

The average daily feed intake was greater for all the treated animals than for the controls, but these differences are not significant.

(d) Respiration rate.

The average respiration rate per minute for protamone and thiouracil treated animals is shown in Table 11. Because of the variation in this experiment, no differences can be attributed to treatment.

Table 11. Average Respirations per Minute During Lactation.

Lot number	Average respirations per minute
1 T*p	84
3 T P	68
5 T	78
7 p	108
9 P	74
11	77

*T Thiouracil 40 mg/day.
P Protamone 1600 ug/day.
p protamone 800 ug/day.

(e) Young pigs.

(1) Birth weight.

The average birth weight of young pigs has already been discussed. The differences were attributed to chance variation rather than treatment.

The birth weight of guinea pigs depends in part upon the number in the litter and the subsequent growth to 21 days is also influenced by the number of young nursing *. For this reason the litters were reduced in size on the fourth day of the nursing period by discarding all but the two heaviest animals. This day was chosen because weak pigs had usually died before this time and the rest had gained back the weight they had lost shortly after birth.

(2) Daily gains.

Appendix Figure 1 shows the average daily weights of these guinea pigs from birth to fourteen days, including only those animals which survived the test. The results are difficult to interpret because the average initial weights of the lots differ. A truer picture is given by Figure 7 in which daily gains are shown from the fourth to the fourteenth day. The average gains of 190 guinea pigs in the stock colony are plotted on the same graph for comparison.

* Unpublished data from this laboratory.

The conclusion based on these findings is that protamone had no stimulating effect and thiouracil no depressing action on milk secretion as measured by the growth of the young. All young born on test grew at a slower rate than normal because they were obtaining all their nourishment from their mother's milk. Animals in the stock colony usually begin to eat feed about the fifth day. Of the 25 animals on test the fourth day, 24 were still alive on the seventeenth day. From this work it is evident that guinea pigs can be carried (for test purposes) on their mother's milk as the only source of food for seventeen days. After this time the animals lose weight rapidly.

(3) Post mortem findings.

All the young pigs were killed between the 17th and 21st day. Post mortem examination showed nothing unusual in any of the animals. The lungs were congested in several of the animals but this is often seen in the stock colony. Two young pigs, whose mother had received thiouracil alone, showed enlarged thyroids. Since these were not weighed and since no changes were visible microscopically, no significance is attached to this finding. The animals which died

before the 21st day appeared to have died of starvation.

(4) Histological examination.

There was no evidence of histological changes in the gonads, ovaries or adrenals of young pigs nursed by mothers receiving protamone or thiouracil.

The general conclusion concerning the effect of protamone and thiouracil treatment of mothers on nursing guinea pigs (in the amounts used in this experiment) is that neither of these substances had any effect upon the growth or development of the nursing young.

III. Effect of protamone and thiouracil treatment on post mortem findings of adult guinea pigs.

Many of the abnormalities found in the guinea pigs on post mortem examination have already been mentioned under the discussion of the outcome of pregnancy. The other unusual findings were distributed throughout the experiment without regard to treatment, from which the conclusion is drawn that the protamone and thiouracil brought about no grossly visible changes in the internal organs. This is in marked contrast to the findings of Carlson, Rooks and McKie (1912). They fed 0.3 grams of desiccated sheep's thyroid per day to fifteen guinea pigs. Post

mortem examination of these animals showed hyperemia and hemorrhagic conditions of the viscera, particularly the intestines. There was also congestion of the lungs. Since their work was done before much was known of the vitamin requirements of guinea pigs, it is quite possible that the diet used was grossly inadequate.

The fresh weight of adrenals was obtained by weighing two adrenals together from each animal. Some of the animals did not complete the test in time to be used in this study. The comparisons shown in Table 12 are based on twenty-seven observations. The pregnant animals include only the primiparas.

Table 12. Weight of Adrenals Including Primiparas Only.

Treatment	Average weight in grams.	Average weight per 100 grams body weight
Lactating	.5624	.0937
Non-lactating	.5712	.0962
High protamone	.6031	.1058
Low protamone	.5586	.1002
No protamone	.5310	.0796
Thiouracil	.5593	.0743
No thiouracil	.5738	.0834

The average weight of the adrenals of the lactating animals was less than that of the non-lactating controls. This is in contrast to the findings of Hewitt and Van Liere (1941). Their values were expressed as grams of adrenal per kilogram body weight. They found a ratio of 0.6176 for their controls and 0.9763 for their post partum animals. This latter group of guinea pigs, however, was killed within eight hours of the birth of the young. The values reported in Table 12 were determined on the 18th - 21st day post partum. It is quite conceivable that if the adrenals had increased in weight during pregnancy, they might have regressed to their original size during the lactation period.

The striking difference between the two levels of protamone is the only indication of a greater response of any kind to the higher dose level. For all other results, the two levels have been considered equal in effect. The marked increase in the weight of the adrenals of protamone treated animals corroborates the observation of Koger and Turner (1943) that thyroprotein feeding of rats caused a marked hypertrophy of the adrenals. The extent of hypertrophy paralleled roughly the level

of thyroprotein fed.

The weight of the adrenals of the thiouracil treated guinea pigs was less than that of the controls. Although the weight of adrenals follows a pattern expected from a review of the literature, further observations would be required to establish the significance of these results.

IV. Effect of protamone and thiouracil treatment on histological changes in adult guinea pigs.

(a) Thyroids.

When thyroprotein was fed to rats, the thyroids were found to be slightly smaller, inactive and filled with colloid (Koger and Turner, 1943).

When thiouracil was given to rats, hyperplasia occured in the thyroid gland within 24 hours; increased to a maximum at 10 to 15 days and then decreased (Paschkis et al 1945). The same condition developed in monkeys given 0.8 grams thiouracil daily for 73 days. The hyperplastic gland returned to normal by the 49th day after cessation of thiouracil treatment (Engle and Aranow 1946). In 1946, Andrews and Schnetzler fed thiouracil to chickens and found an effect on both the weight and the histology of the thyroid gland. Moreover the weight of the thyroid

gland of steers fed thiouracil for 100 days was also significantly increased (Andrews and Bullard, 1940).

Friedgood (1934) has described the hyperplastic appearance of the guinea pig thyroid, brought about by overstimulation with anterior pituitary extract. Microscopic examination of the thyroids of all treated animals revealed no such changes. No record was kept of the weight of the thyroid glands. Since thiouracil causes hyperplasia in rats, monkeys, chickens and steers, it must be concluded that guinea pigs, as a species, react differently to thiouracil or that 40 mg. per day was insufficient to bring about the hyperplastic changes.

(b) Adrenals.

In spite of the difference in adrenal weights of protamone and thiouracil treated animals, there was no histological evidence of damage to either the cortex or the medulla.

(c) Ovaries.

Several investigators have found differences in the weight of the ovaries in hypothyroidism or hyperthyroidism. Hammett (1926), on the other hand, stated that the ovary follows the body weight in its changes in growth retardation after thyroid removal.

Williams, Phelps and Burch (1941) suggested that hypothyroidism might give rise to disturbances of ovarian function in guinea pigs. Six of their thirty animals showed evidence of temporary or prolonged failure of corpus luteum formation and six others showed features suggestive of subnormal corpus luteum formation. However, they found no marked abnormalities of the follicles.

Much conflicting evidence has been reported concerning the effect of hyperthyroidism on ovarian function. Koger and Turner (1943) fed thyroprotein to rats and noted an increased weight of the ovaries with histological evidence of intense luteinization. Ershoff (1945) on the other hand, fed thyroid to rats and found a marked inhibition of ovarian development, both grossly and histologically. No mature follicles or corpora lutea were present, although there were numerous immature follicles and a normally appearing epithelium. These results might be explained by the work of Drill et al (1943). They found that the ovaries of hyperthyroid rats receiving a small yeast supplement showed a loss in weight. The histological picture of these ovaries suggested insufficient gonadotropic hormone secretion. The hyperthyroid

rats receiving the high B vitamin diet maintained normal ovarian weight and showed a normal ovarian histology.

Since no attempt was made to kill the guinea pigs in the present experiment at any particular stage of the estrous cycle, the ovaries showed a high degree of variability with regard to the number of corpora lutea and tertiary follicles present. No effect of either protamone or thiouracil could be detected.

(d) Mammary gland.

No differences were observed in the mammary glands of thiouracil or protamone treated guinea pigs in this experiment.

GENERAL DISCUSSION OF RESULTS

Thiouracil, in the amounts fed in this experiment, did not produce a hyperplasia of the thyroid gland. Neither did the drug lower the metabolic rate. It is concluded from these observations that thiouracil was without effect on the pregnant or lactating guinea pig. This agrees with the evidence formerly discussed that guinea pigs do not respond in the same manner as rats to ingested thiouracil.

Protamone, by contrast, produced marked effects on pregnant and subsequently on lactating animals. The effect of feeding 800 ug. per day was considered equal to that of feeding 1600 ug. per day.

The animals used in this experiment were sexually mature but not fully grown. When protamone was administered to them, the total oxygen consumed remained the same but the B.M.R. (millilitres of oxygen per minute per 100 grams body weight) was raised.

Moreover the rate of gain was subnormal. A possible explanation of this is given in a

statement by Brody (1945). "The acceleration of bodily oxidation is only one of the catalytic manifestations of the thyroid ..... In fact all anabolic processes are retarded by thyroidectomy ..... There is one physiologically optimal thyroid-dosage level ..... Above the optimal level the catabolic effect of the thyroid hormone over-balances the anabolic effect; indeed the extra hormone often becomes toxic". Undoubtedly catabolism was increased to such an extent in the protamone treated animals (because of the increased metabolic rate) that the anabolic processes which normally result in growth did not show their maximum effect.

It is interesting to observe, however, that although the protamone treated animals were fed ad libitum, they could not or would not eat enough food to meet the maximum growth requirement. This may be an indirect rather than a direct effect of protamone administration. It is possible that there is an adrenal involvement. This is suggested by the fact that the adrenals were hypertrophied in the treated animals and also by the fact that when the protamone treated animals were nursing,

they were more easily disturbed than the controls. This may have been due to a higher excitability brought on by an increased secretion of epinephrine. When the latter substance is injected into the body, it stimulates the sympathetic nervous system, which results in a relaxation of the muscles of the stomach and intestine and a closing of the sphincters (Dukes 1942). This would lead to a decreased capacity to eat and finally to the decreased rate of gain already noted.

SUMMARY AND CONCLUSIONS.

Guinea pigs were used in an experiment designed to study the effect of feeding protamone and thiouracil throughout pregnancy and lactation.

The basal diet consisted of the Macdonald College guinea pig mixture number six. Supplements of vitamin A, alpha tocopherol, vitamin D_2, ascorbic acid and either grass or cabbage and hay were fed directly to the animals.

Forty milligrams of thiouracil was fed daily as a dry powder. Protamone (800 ug. or 1600 ug./day) was given in an alkaline, aqueous solution.

The oxygen consumption of all guinea pigs was determined weekly, using a modified Teitelbaum and Harne apparatus.

The following conclusions were drawn from the results of this experiment:-

No difference was shown between the two levels of protamone fed. Wherever protamone and thiouracil was fed together, the guinea pigs reacted as if protamone had been fed alone.

Protamone had no effect upon conception, abortion, birth of dead pigs among living ones, size

of litter, birth weight of young pigs or their subsequent gain to fourteen days of age.

Autopsy was performed on all animals at the end of the test. The abnormal conditions seen were not attributed to protamone feeding. No histological changes were evident in the thyroids, adrenals or ovaries.

The efficiency of feed utilization was less for protamone treated and greater for thiouracil treated animals than for the controls. Thiouracil brought about no other changes under the conditions discussed above.

The conclusion is drawn that neither protamone nor thiouracil had any detrimental effect upon the adult guinea pig or her offspring.

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APPENDIX

I. Histological Procedure.

At the end of the nursing period all the guinea pigs were killed with chloroform. After post mortem examination the thyroids, adrenals and gonads were removed. A section was also taken from the mammary gland of lactating animals.

(a) Fixation.

The tissues were fixed in Bouin's solution for three hours.

(b) Dehydration.

Following this treatment, they were put in 70% alcohol over night. The next morning, the alcohol was poured off and replaced by 80% alcohol. This latter was changed twice daily until most of the yellow color had disappeared. The tissues were then placed in absolute alcohol over night.

(c) Embedding.

The absolute alcohol was changed the next morning and the tissues prepared for embedding in wax in the following manner:-

Absolute alcohol ..... 1.5 hours

Cedarwood oil ..... 1.5 hours

Wax (m.p. 52°C) ..... 2.5 hours

Wax (m.p. 62°C) ..... 2 hours

The tissues were then embedded in Fisher's Tissuemat Wax (m.p. 60 - 62° C).

(d) Sectioning.

The embedded tissues were mounted on wooden blocks and then sectioned with a microtome. The sections were cut ten microns in thickness and mounted on slides by the use of Meyer's solution. After drying in an oven at 40°C, they were ready for staining.

(e) Staining.

The slides were then placed in xylol to remove the wax. They were hydrated by putting them through alcohol in the following order:-
95%, 80%, 70%, 60%, 50%, 40%, 30%, 15% and finally into tap water. They were then stained with Ehrlich's hematoxylin solution for ten minutes. Following this, they were placed in water for one minute and then run through the series of alcohols in the reverse order up to 95%. From here they were put in

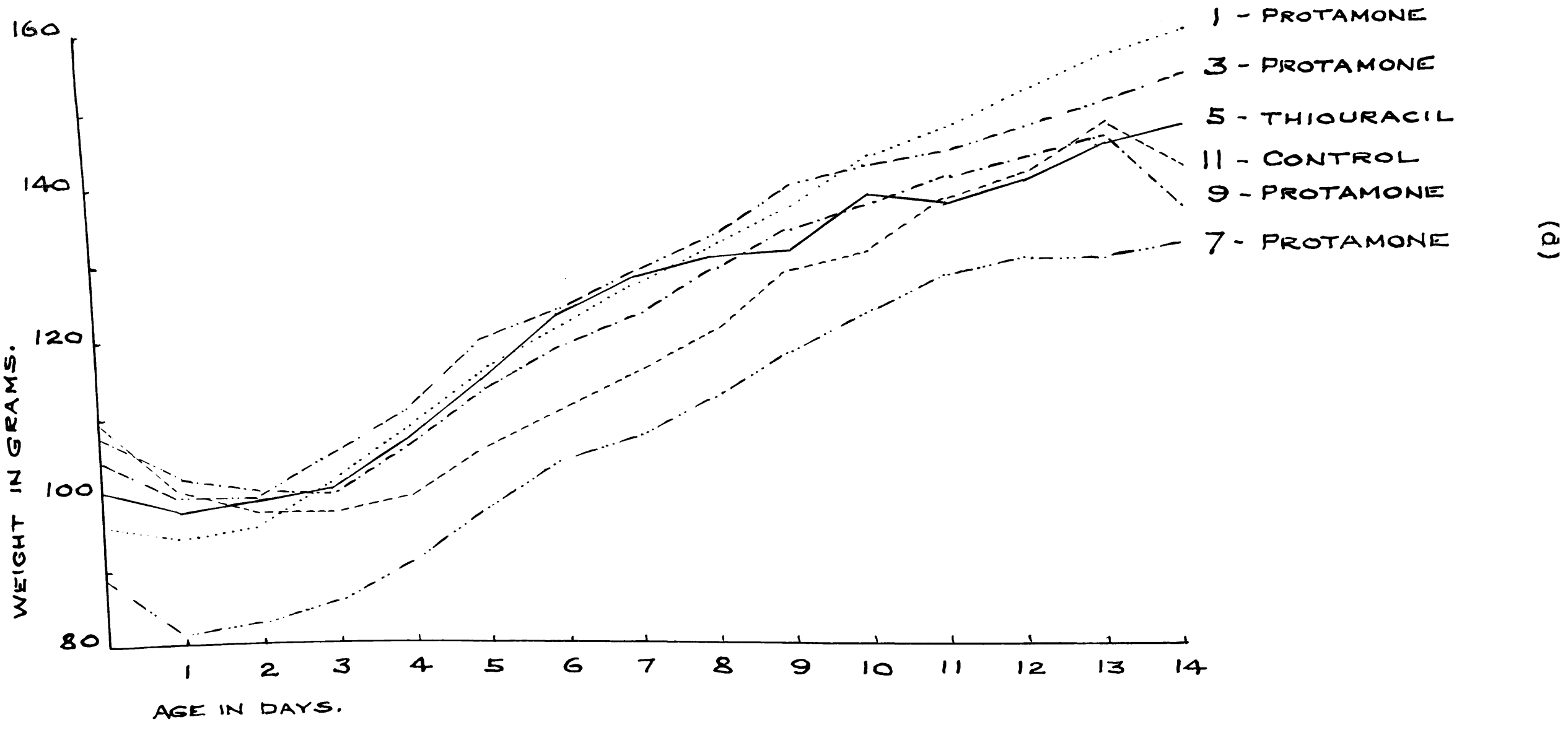
absolute alcohol and then into eosin in alcohol solution for 90 seconds. After staining, they were cleared in xylol and cleaned. Canada balsam was used to secure the cover slip in place.

II. Microscopic Examination.

The slides were then examined under oil immersion on the microscope.

APPENDIX-FIGURE 1.

DAILY WEIGHTS OF YOUNG PIGS BORN TO MOTHERS RECEIVING PROTAMONE AND THIOURACIL.



(a)

Appendix
Table 1.

Observed Oxygen Consumption in ml. per minute For Ten Weeks Before Parturition.

Lot No.	10	9	8	7	6	5	4	3	2	1
1	7.63	7.86	8.01	7.64	7.42	8.44	9.24	10.23	11.29	11.17
2	6.40	6.28	7.14	7.05	7.65	8.85	7.66	7.20	7.39	8.46
3	7.15	8.44	7.85	7.29	7.31	7.81	8.55	9.40	9.85	10.26
4	5.99	6.56	7.21	6.75	7.64	7.49	7.16	7.88	7.50	7.47
5	7.02	6.05	6.40	6.40	6.71	6.71	9.21	8.26	9.52	10.33
6	7.23	7.07	6.77	7.32	8.17	8.00	7.97	8.05	8.04	8.90
7	6.74	7.55	7.96	7.96	7.42	9.21	8.87	9.58	10.51	12.57
8	6.37	6.82	6.97	7.20	7.49	7.64	7.81	7.84	7.48	7.58
9	6.27	8.45	9.08	8.70	8.54	9.18	9.30	10.51	11.22	11.13
10	6.98	7.39	7.42	8.13	8.36	8.23	8.27	7.68	8.54	8.61
11	6.75	6.10	6.77	7.12	7.35	7.70	8.08	8.82	9.80	11.19
12	5.96	6.03	6.07	6.98	7.07	7.31	6.91	6.82	6.53	7.18

Appendix Table 2. Analysis of Variance for Total Gains During Pregnancy.

Sources of variation	D/F	Variance	F Value	
			Obs.	Req'd.
All causes	35			p=.05
Treatments	11	80,829	18.0	
Between levels of protamone	2	27,714	6.2	3.44
thiouracil	1	246		
pregnancy	1	793,288	176.5	4.30
interaction	7	5,742	1.3	
Error (2 calculated) values	22	4,495		

Appendix Table 3. Analysis of Variance for Total Feed Intake During Last Three Weeks of Pregnancy.

Sources of variation	D/F	Variance	F Value	
			Obs.	Req'd
All causes	35			p=.05
Treatments	11	37,059	2.63	
Between levels of protamone	2	34,938	2.48	3.44
thiouracil	1	25,494	1.81	
pregnancy	1	159,201	11.32	4.30
interaction	7	21,868	1.55	
Error (2 calculated) values	22	14,065		

Appendix Table 4. Analysis of Variance for Gain per 100 Gms. Feed Intake During Pregnancy.

Sources of variation	D/F	Variance	F Value	
			Obs.	Req'd.
All causes	35			p=.05
Treatments	11	540	18.6	
Between levels of protamone	2	112	3.9	3.44
thiouracil	1	148	5.1	4.30
pregnancy	1	5,018	173.0	4.30
interaction	7	79	2.7	2.47
Error (2 calculated) values	22	29		

Appendix Table 5. Analysis of Variance For Water Consumption During Last Three Weeks of Pregnancy.

Sources of variation	D/F	Variance	F Value	
			Obs.	Req'd.
All causes	35			p=.05
Treatments	11	805,983	2.59	
Between levels of protamone	2	2,069,762	6.66	3.44
thiouracil	1	42,504		
pregnancy	1	3,382,534	10.89	4.30
interaction	7	185,893		
Error (2 calculated) values	22	310,734		

Appendix Table 6. Analysis of Variance For Respiration Rate During Last Three Weeks of Pregnancy.

Sources of Variation	D/F	Variance	F Value	
			Obs.	Req'd.
All causes	35			p=.05
Treatments	11	1,890	1.70	2.22
Between levels of protamone	2	651		
thiouracil	1	427		
pregnancy	1	8,962	7.99	4.26
interaction	7	1,442	1.29	2.43
Error (2 calculated) values	22	1,121		

Appendix Table 7. Analysis of Variance For Body Weight During Lactation.

Sources of variation	D/F	Variance	F Value	
			Obs.	Req'd.
All causes	17			p=.05
Treatments	5	7,746	3.6	3.33
Between levels of protamone	2	9,000	4.2	4.10
thiouracil	1	17,236	8.1	4.96
interaction	2	1,747		
Error (2 calculated) values	10	2,134		

Appendix Table 8. Analysis of Variance For Oxygen Consumption During Lactation.

Sources of variation	D/F	Variance	F Value	
			Obs.	Req'd.
All causes	17			p=.05
Treatments	5	49,849	5.4	3.33
Between levels of protamone	2	36,181	3.9	4.10
thiouracil	1	95,922	10.5	4.96
interaction	2	40,481	4.4	4.10
Error (2 calculated) values	10	9,178		

Appendix
Table 9. Analysis of Variance For Feed Consumption During Lactation.

Sources of Variation	D/F	Variance	F Value	
			Obs.	Req'd.
All causes	17			p=.05
Treatments	5	6,372	1.1	3.33
Between levels of protamone	2	11,910	2.1	4.10
thiouracil	1	27		
interaction	2	4,008		
Error (2 calculated) values	10	5,620		

Appendix Table 10. Analysis of Variance For Respiration Rate During Lactation.

Sources of variation	D/F	Variance	F Value	
			Obs.	Req'd.
All causes	17			p=.05
Treatments	5	588	1.4	3.33
Between levels of protamone	2	72		
thiouracil	1	420		
interaction	2	1,187	2.7	4.10
Error (2 calculated) values	10	433		

Appendix Table 11. Analysis of Variance For Weight of Adrenals.

Sources of variation	D/F	Variance	F Value	
			Obs.	Req'd.
All causes	26			
Treatments	11	572,395	less	
Between levels of protamone	2	686,953		
thiouracil	1	301,016	than	
lactation	1	309		
interaction	7	660,159	one	
Error	15	833,220		