THE RECIPROCAL EFFECTS OF DIETHYLSTILBESTROL AND THYROPROTEINS ON FOOD AND WATER INTAKE OF FEMALE MICE

THESIS FOR THE DEGREE OF M. S.
MICHIGAN STATE COLLEGE

JULIAN K. MILLER
1948

This is to certify that the

thesis entitled

"The Reciprocal Effects of Diethylstilbestrol and Thyroproteins on Food and Water Intake of Female Mice"

presented by

Julian K. Miller

has been accepted towards fulfillment
of the requirements for

M. S. degree in Physiology

E. P. Reineke
Major professor

Date August 27, 1948

M-796

Julian K. Miller

THESIS ABSTRACT

THE RECIPROCAL EFFECTS OF DIETHYLSTILBESTROL AND THYROPROTEINS ON FOOD AND WATER INTAKE OF FEMALE MICE

As the literature indicates, diethylstilbestrol produced a decrease in the amount of food consumed by the experimental animals. A corresponding effect was produced on the water consumption, except in the range of the minimal effective doses.

Furthermore, the animals exhibited loss of body weight at all dosage levels, while hair and integumentary development showed definite deterioration in direct response to dosage.

The activity of the experimental animals was retarded; while the general appearance was indicative of marked malnutrition.

On the other hand, the administration of the thyroprotein, Protamone, produced an increase in the activity of the experimental animals and an increase in both food and water consumption. However, the quantity of these increases was directly proportional to dosage levels only within a prescribed range. Beyond this optimally effective range, responses were affected by the Laws of Diminishing Returns.

The Protamone-treated animals exhibited fine, sleek

coats in contrast to the estrogen-treated mice. Also, the body weights of these mice were increased and the general appearance and condition of these animals ranged from normal to enhanced.

From the apparent diametrically opposite effects produced by diethylstilbestrol and Protamone, each administered separately, it was presumed that given <u>together</u> there should be some measure of antagonism produced which would reflect in the responses of the experimental animals.

At first, it was gratifying to find that such was the case. Where diethylstilbestrol had formerly produced emaciation, those mice "fortified" with Protamone withstood the deleterious effects of the diethylstilbestrol. However, and this is significant, <u>in every case the animals so treated died within, or shortly after, the experimental period</u>; while the animals given the estrogen only, had few fatalities and showed a high percentage of recovery.

The seeming paradox of healthy-appearing animals being consistently survived by emaciated animals, is explained on the following basis:

1. The Protamone achieves its effect via the thyroid-endocrine system relationships.

2. The diethylstilbestrol achieves its effect via the ovary-endocrine system relationships.

3. Therefore, it does not follow that there need be a <u>direct</u> antagonism, since each drug operates via its own avenues. However, this does not preclude the possibility of an <u>indirect</u> antagonism.

4. If we assume an indirect antagonism, then how can we explain the fact that animals given both drugs show a radical decrease in survival ability?

5. The fact is, that the animals given both drugs show a significant loss of body weight when dehydrated; as compared to the controls. In other words, much of the apparent "weight" was simply due to high water retention.

6. In addition to the preceding circumstances, the Protamone produced an increase in metabolic activity which tended to further consume an already starved animal.

7. The combination of the foregoing factors explains the high mortality in the groups treated with both drugs. Furthermore, it indicates that there is no essential antagonism between the two drugs, though in some respects either drug may have had a dominant effect. For example, the Protamone seemed to have a dominant effect in regard to hair growth, while the diethylstilbestrol seemed to have a dominant effect in regard to water retention.

This study indicates that the behavior exhibited as a result of thyroid-estrogen balances is a vector of a great many inter- and intra-related responses which must be viewed not en toto; but rather from the standpoint of the artist who examines the leaves and grass before sketching the panorama of the forest.

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By

JULIAN K. MILLER

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

Department of Physiology and Pharmacology

Year 1948

ACKNOWLEDGMENTS

The author wishes to express his gratitude and acknowledge the assistance which he received in this work from Dr. E. Paul Reineke, scholar, scientist and inspiring teacher; Dr. Joseph Meites under whose conscientious guidance and upon whose suggestion this work was carried out; and to Professor B. B. Roseboom, administrator, scientist, teacher and scholar who will always be remembered for the nobleness of character which endeared him to all his associates.

Also, I wish to acknowledge the many writers listed in the bibliography, who in the tradition of science and scholasticism, made the results of their labors universal domain.

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CHAPTER I

THE PHILOSOPHICAL BASIS OF ENDOCRINE BEHAVIOR

A correct interpretation of endocrine behavior requires an awareness of a dynamic balance of interrelating forces. The study of endocrinology does not consist of the observation of morbid anatomy, since it deals directly with physiological function. Therefore a proper understanding requires constant orientation of the variant to the organism as a whole.

It is well established, in the physical as well as the natural sciences, that individual changes within a system produce a logarithmic rather than a linear change in the interrelationships within the system.

As a simple illustration we might cite the relationships of points A B C. The relationship as now

constituted produces a given plane. However, changing the position of any point on this plane produces a series of relationship changes in addition to the changed position of the individual point.

If we change just point C, thus: A B

C

not only position C has been changed but the relationship between A - B constitutes one plane as opposed to the new relationship of B - C which constitutes another plane. Also the new relationship of C - A constitutes still a third plane.

In addition, if point C had been moved not only downward but off the paper as well; the new relationships of C to the system A-B-C would be increased to the third power.

Now when we consider the multiplicity of endocrine factors; each interrelated, and the individual relationships of each hormone to the endocrine system and the organism as a whole, it becomes apparent that observing endocrine activity from a purely behavioristic standpoint is entirely inadequate, since it is obvious that the change observed is not a linear entity but a <u>resultant</u> of a series of interrelated re-orientations.

It is with this dynamic philosophy in mind that the behavior of hormone activity should be studied; since it takes into account the many and varied balanced forces and systems which we recognize as life processes. It is true that many, if not most, of these inter- and intra-related systems are still unknown; but it is better "science" to recognize ignorance than to ignore it.

It is therefore with a feeling of great humility that the study is approached; being aware of the myriad possible avenues through which the hormone may act, and

of the great number of system accommodations which might take place to produce the resultant observable behavior.

CHAPTER II

THE PURPOSE OF THE EXPERIMENT

From the preceding, it is apparent that we can not hope, with this short work, to untangle all, if any, of the mechanisms by which our results were obtained. However, since our repeated experiments produced consistent results; we may be permitted to conclude that certain changes in the hormone balance under specified conditions, will produce an accurately predictable result.

Considering all that might take place within the organism as a whole, from the initiation of the changed relationship until the final re-orientation; it is obvious then that our purpose is a very modest one.

By this experiment we hope to demonstrate certain antagonisms between the synthetic estrogen, diethylstilbestrol; and the synthetic thyroactive protein, Protamone.

From the review of the literature which follows, it is apparent that there are constant physiological effects produced by both drugs; some of which, acting individually, produce antagonistic phenomena.

The question which we hope to answer, is: "Will the drugs, acting together, maintain their previous charac-

teristics -- or, will they create a new relationship wherein together they will act as a whole -- or, might it not be a resultant of both possibilities?"

Also, this experiment should shed some light on whether the diethylstilbestrol exerts its effect, at least in part, via depression of the thyroid gland. If we assume that the hypothyroid-like effects of stilbestrol are due to an inhibitory effect upon the thyroid gland, then the administration of thyroxine should re-establish normalcy. Unfortunately, such re-establishment of normalcy still does not confirm the assumption since it does not eliminate the possibility that the stilbestrol might not have reacted directly upon one or more intermediate agents; and that the intermediate agents might have acted upon the thyroid. Furthermore, the possibility is still not ruled out that the stilbestrol has no effect upon the thyroid, but that it acts directly upon the organism as a whole to produce a hypothyroid-like condition.

It is hoped that the review of the literature and the data of our experimental work may yield some observations which will contribute toward a more rational hypothesis upon which the answers to these questions might be based.

CHAPTER III

THE EFFECTS OF ESTROGENS WITH PARTICULAR REFERENCE TO DIETHYLSTILBESTROL (STILBESTROL)

General Considerations

1. Definition.--The term "estrogen" denotes any substance which will induce cornification in the vagina of the adult mouse like that of natural estrus.

2. History.--Apart from knowing that the sexual functions of the female depend on the ovary and are not controlled through its nervous connections, our recognition of the hormonal basis of ovarian activity began with the observation by Marshall and Jolly (1905) that estrum could be induced in spayed dogs either by the injection of extracts of ovary removed from another dog during estrus or by implanting estrual ovaries into the peritoneum. These workers recognized that the ovary produces two different hormones, and that the secretion which causes estrus is different from that formed later by the corpora lutea. A few years later Adler (1912) reported that estrus could be brought on in guinea pigs by intravenous or subcutaneous injections of extracts obtained from whole ovaries or from corpora lutea. The next great advance was that of Allen

and Doisy (1923, 1924), who discovered that liquor folliculi from the sow's ovary caused estrus-like changes in the rat's vagina. The hormone thought to induce these changes, thereafter came to be known as theelin, estrin or folliculin. The active principle was at that time a hypothetical substance which could be recognized only by its biological effects. Later, the isolation of the hormone in crystalline form by Doisy, Veler and Thayer (1930) and also by Butenandt (1929), working independently, narrowed the meaning of the term estrin to the single well-defined chemical compound now known as estrone. However, with the isolation of other naturally produced estrogenic compounds such as estradiol, estriol, equilin and equilenin; the term "estrin" was again attributed to the original reference so that it might not be confused with the hormones found subsequently (Burrows, 1945). However, to date the terms are still used interchangeably. Altogether excluded from the term "estrin" are several artificially produced chemical compounds which, though producing biological effects like those of estrin, have not been found in the living organism and in some instances are different in molecular conformation from the naturally occurring estrogens. The particular synthetic estrogen with which this paper is concerned, is known as diethylstilbestrol.

3. Chemistry.--The discovery that estrogenic activity could be exhibited by substances not belonging to the

steroid group is due to the work of Dodds and Goldberg (1939) and their collaborators. They first investigated the simple compounds of phenanthrene and found that, though phenanthrene itself was inactive, the compound 1:2:3:4: - tetrahydrophenanthrene possessed definite estrogenic activity. Other substances were then investigated and it was found that estrogenic effects could be produced by a number of derivatives of 1:2:5:6: - dibenzanthracene. A number of compounds were prepared and three of them have proved of outstanding interest, viz. diethylstilbestrol, hexestrol and dienestrol. These compounds are more active than the natural estrogens and have added advantage of being very active when given by mouth (Dodds et al., 1939).

The ester of diethylstilbestrol, which in many instances, indicates an even greater potency; is quite simply prepared as dihydrodiethylstilbestrol. This was prepared by Docken and Spielman (1940) through a short and direct method from the oily, unstable hydrogen bromide addition product of anethole "described long ago by Orndorff and Morton". By coupling anethole hydrobromide by means of magnesium in dry ether there was formed an oil from which a high-melting ether was obtained in 15 to 20 per cent yield. The ether was smoothly demethylated with alcoholic potassium hydroxide to give dihydrodiethylstilbestrol in a nearly quantitative yield. The potency of this substance was found to be of the same order as that of estrone and estradiol.

4. Sources.--The natural estrogens may be derived from several sources. There is no need to quote the early work done which established these sources since at present there is no longer any question as to their authenticity. The principal organs from which estrogens have been extracted are the ovary, placenta, testis and adrenals. Estrogens have been found in other animal tissues and even in some plant substances used as food. There is still some argument amongst histologists as to just which cells are responsible for their production and the intracellular chemistry of their formation, but the locale of their origins is well established.

5. Distribution.--There is evidence that even before birth and even before the differentiation of gonadal tissue in the fetus, parental hormones permeate the placenta, and are carried to the fetus. In support of this contention Courrier (1930) gave between 80 and 100 r.u. of an aqueous preparation of estrin to guinea pigs during the last six days of pregnancy. At birth the newborn females had cornification of the vagina and vulval swelling, their mammae secreted a little colostrum and the uterine horns were distended.

It is known that finely particulate or colloid matter present in the blood will become concentrated in inflamed tissues. Brunelli (1935) showed that estrin becomes localized in this way after its introduction into the bloodstream.

He shaved the abdomens of twelve male rabbits and treated each of them as follows. Gauze wet with chloroform was applied for some minutes to one part of the shaved area to cause local inflamation, and to another part of the shaved area gauze wet with normal saline solution was applied. A watery solution containing 1,000 m.u. of estrin was then injected into an ear vein. One hour later each rabbit was killed, and the two treated areas of skin were separately excised with the underlying tissues, dried and extracted. When tested for estrogenic activity the extracts prepared from the inflamed tissues gave positive results in every instance, whereas those prepared from the non-inflamed saline-treated skin were all negative. This phenomenon may be of particular significance at a later point of discussion.

Effects upon Metabolism

1. Respiration.--In determining oxygen consumption in estrogen-treated rats, T. C. Sherwood (1940, 1941) indicates a consistent decrease in the oxygen consumption rate. This is explained by Brobeck et al. (1947), as a result of lesions produced in the hypothalamus caused by the treatment. He advances the hypothesis that the hypothalamus is the level of the central nervous system most intimately concerned with control of oxygen consumption as well as other functions effected at this level. Another explanation offered by Biskind (1946) is that the decrease in oxygen consumption may be the expression of a safety mechanism; since the rise of body estrogen, due to the inability of the liver to inactivate the increment, causes depression of pituitary activity with regard to the secretion of the thyrotrophic principle and that in turn accounts for the decrease in oxygen consumption. Still another theory offered (Gordon and Elliott, 1947), is that estrogens inhibit the action of the estrogen-inactivating enzymes. These authors also claim to have produced anesthesia in rats by inhibiting the succinoxidase system of the brain with diethylstilbestrol. This effect finds a few corroborators amongst other workers in the same field.

2. Circulation.--Clinical experience suggests that estrogens might tend to lower the blood pressure. A raised blood pressure is apt to accompany virilism, and severe

hyperpiesis is more frequent in men than in women, and in the latter is thought to be associated with the menopause. Liebhart (1934) made tests on more than three hundred individuals, including men, girls, and normal, spayed and postmenopausal women, giving doses of estrone ranging from 100 to 5,000 m.u. and determining the blood pressure. He concluded that estrone has a capacity for lowering the blood pressure, and that this action is more pronounced in women than in men, and in adult women than in girls before puberty. In some instances a fall of the systolic pressure equivalent to 30 mm. Hg. was obtained within half an hour of giving estrone. Guirdham (1941) has found that estradiol lowers the blood pressure in cases of menopausal hyperpiesis, arterial sclerosis, and renal disease, as well as in normal conditions.

Reynolds (1941) examined the effects of estrogen on the blood vessels of the ear in the spayed rabbit, and also, by means of a plethysmograph, on the blood vessels of the human finger. The usual results of both tests was a dilation of the small blood vessels which followed the injection of estradiol within a few minutes and persisted for at least two hours. The human tests were performed on twenty men and on twenty-five women who had passed the menopause. Other experiments bearing on this matter have been done by McGrath (1935) and Thomas (1940). Knowlton (1947) reports that administration of desoxycorticosterone produced both renal and cardiac enlargement. It would be interesting

to know whether this is a direct effect or a physiological compensation due to the drop in blood pressure described before.

An effect of estrogens on the permeability of the capillary blood vessels in certain regions of the body has been reported by Hechter, Krohn and Harris (1942), who treated rats with estradiol, estrone or estriol, and after an interval gave each of them an intravenous injection of 1 cc. of a 1 per cent solution of trypan blue per 100 g. of body weight. In every instance there followed an abnormally large concentration of dye in the uterus and vagina. This was not the consequence of increased affinity of the genital tissues for the dye, because such tissues when minced and tested in vitro took up no more dye than was accumulated by the tissues from other parts of the body. Of course in evaluating this activity one can only postulate upon the physico-chemical effect which the dye itself might have had upon the permeability of the capillaries.

3. General growth and body weight.--In most mammals the male is larger than the female. Stotsenburg (1909, 1913) and others have shown that this difference is caused by ovarian action. Stotsenburg found that castration of male rats early in life had little or no effect on the subsequent growth curve, whereas the female spayed at the same period grows to a larger size than her intact sister. These

observations have been supplemented by Steinbach and Holzknecht (1916), who interchanged the gonads of young male and female littermate guinea pigs, implanting testes into spayed females and ovaries into castrate males. The males bearing ovaries failed to attain the general dimensions or weight of normal size males or females and the females grafted with testes grew to an unusual size -- larger in fact than normal untreated males.

Bugbee and Simond (1926) showed that repeated injections of a follicular extract retarded growth in male and female rats, whether their gonads had been removed previously or not. Riddle and Tange (1928) observed a similar effect of estrogen prepared from sow's follicular fluid on the growth of pigeons. Wade and Doisy (1931) obtained the same results with estrin in both male and female rats. The results of their work clearly indicated that the rats treated with estrogen showed a marked decrease in general body weight.

Korenchevsky and Dennison (1934) gave to three groups of immature male rats 20, 60 or 100 i.u. of estrin daily for a period of forty-three days. At the end of this time the ratios of weight increase in the estrin-treated rats compared with controls which received no estrin were 88:127, 83:114, and 86:127 in the three groups respectively.

An inhibitory influence on body weight is displayed by synthetic estrogens, for example diethylstilbestrol (Gaarenstrom and de Jongh, 1939). They implanted into

rats various synthetic estrogens in dry form and noticed that they checked the rate of body growth to a greater degree in females than in males and this effect was not pronounced until a weight of between 100 and 120 g. had been reached, after which the body growth became much retarded.

It seems that the growth-checking action of estrogen is to some extent at least the result of an inhibition of hormone production by the pituitary. Reece and Leonard (1939) removed the ovaries and pituitary from immature rats and five days later gave them eleven daily pituitary implants. The results indicated a reduction of the growth promoting influence of the pituitaries of rats under treatment with estradiol.

Gaarenstrom and Levie (1939) found that the general growth of immature rats could be arrested almost completely by daily doses of 200 or 250 mcg. of stilbestrol. This inhibition of growth was overcome by giving pituitary growth hormone at the same time as the stilbestrol. Gaarenstrom and Levie do not think, however, that these results must mean that the inhibition of body growth by estrogen is the direct consequence of a suppression of the supply of growth hormone from the pituitary; the inhibition of growth might be due, they suggest, to an interference by estrogen with the growth of the bone at the epiphysis. Freud, Levie and Kroon (1939) point out that after hypophysectomy the membrane bones grow normally, whereas long bones do not, and they

believe that the terms "growth hormone" and "chondrotrophic hormone" are synonymous.

Griffiths and Young (1942) arrested the growth of rats by hypophysectomy and by the subcutaneous implantation of 15 mg. of diethylstilbestrol. Thereafter pituitary extracts were given and records were kept of the body weight and rate of growth of the tail. It was found that whereas after hypophysectomy and the administration of pituitary extracts the increase in body weight and tail growth were in constant proportion, this was not the case in rats which were treated by diethylstilbestrol and given the same pituitary extracts, a result which suggests that the arrest of growth cannot be explained by the lack of pituitary hormone only, nor attributed entirely to a deficiency of chondrotrophin.

Whether the result is brought about by an arrest of the supply of growth hormone or of chondrotrophin, or by some other cause, the fact remains that estrogens restrain the general growth of the body.

Bogart et al. (1939) present substantial data to indicate that breeding stimulates growth in female rats. The mechanism is not explained but one might infer that this was due to the decrease in estrogenic hormones prior to parturition. Another possibility might be that the estrogens inhibit growth through an inhibiting effect upon the pituitary and the corpus luteum removes this inhibition.

Korenchevsky and Dennison (1934) note that though estrone decreases the body weight, it does so without any change in fat deposition.

R. Bogart et al. (1941) indicate that progesterone increased body weight. Similarly, Deanesly and Parkes (1941) contend that the anti-growth and anti-gonadotrophic effect of stilbestrol in male rats can be inhibited partially by the simultaneous administration of testosterone.

Noble (1939a) produced evidence that body growth may be directly affected by estrogens and not via the pituitary. Noble (1938) concluded that synthetic estrogens produced a marked decrease in fluid intake.

Richards and Kueter (1941) confirm that the effect of stilbestrol upon growth and weight can be offset by administration of growth hormones.

Marx and Evans (1941) report that estrogen does not reduce growth by inhibiting secretion of growth hormone.

It has been known for some time that estrogens, when administered at relatively high levels, inhibit the growth of normal animals. This was originally believed to be due to effects upon the hypophysis. Simultaneous administration of anterior pituitary extracts and estrogens to hypophysectomized rats indicated an inhibition of the former's growth promoting action by the estrogens. (Reece and Leonard, 1941.) The possibility can therefore not be excluded that an extra-hypophyseal mechanism is responsible for the observed antagonism.

4. Inactivation.--The ephemeral nature of the responses to gonadal hormones when given artificially, shows that these hormones are not stored in an active form in the body and that they must be continually produced in order to sustain a biological effect. Fee, Marrian and Parkes (1929) conducted an experiment which seemed to indicate that the inactivation of estrin in the bloodstream might be caused by oxidation in the lungs. Zondek (1934) conducted some experimental work which led him to believe that the inactivation probably took place in the liver. Subsequent work by Israel, Meranze and Johnston (1937) as well as a host of others confirm Zondek's contention that the liver is the site of inactivation. The fact bears special significance in that it related liver injury to the inability to inactivate these hormones. Heller (1940) attributes the inactivation of estrogens to specific enzymes in the liver.

Apparently the thyroid may take part in the inactivation of estrogen. Van Horn (1933) gave large doses of desiccated thyroid daily to rats. This treatment caused a loss of body weight and persistent anestrus.

Golden and Severinghaus (1938) made homotransplants of ovaries to the mesenteries of rats so that the ovarian hormones, including estrogen, would mostly pass through the liver before entering the general circulation. In other rats their ovaries were transplanted into the axillae. The transplants became established at both sites. The rats

with ovaries attached to the mesentery remained anestrus throughout; those with ovaries in the axillae showed normal estrus cycles. Furthermore, when the pituitaries were assayed for gonadotrophic potency, those of the rats with mesenteric ovaries showed an enhanced potency equal to that of the spayed rats. In some of the rats with mesenteric ovaries these were removed from the mesentery and implanted into the axilla. In all but one of these animals estrus cycles were resumed within the next eight to twenty days. The results seem to indicate that ovarian estrogens are inactivated in the liver.

Other experiments have shown inactivation of estrogen by the liver. Biskind (1941) implanted pellets of estrone into the spleen in normal adult male rats and displaced the spleen with its contained pellets of estrone into the subcutaneous tissues of the loin. In some of these rats the splenic vessels were tied sixteen days later. The animals were killed forty-two days after the insertion of the pellets. In the rats with intact splenic vessels which had allowed immediate access to the liver of dissolved hormone the testes were normal and there was no evidence of any reaction to estrogen in other organs. In the rats with ligated splenic vessels the testes and accessory generative organs were atrophic, showing that the dissolved estrogen which had been prevented from passing directly through the liver had not been so quickly inactivated.

From later experiments, Biskind and Biskind (1942)

have concluded that vitamin B complex is needed to enable the liver to inactivate estrogen. They implanted pellets of estrogen into the spleens of spayed rats. As long as the animals were kept on a normal diet they remained anestrus. If, however, they were placed on a diet deficient in vitamin B complex protracted estrum ensued. The addition of the necessary vitamins to the food caused the rats to become anestrus again.

In some of the earliest work with estrogens it had been noticed that these hormones were relatively ineffective when given by intraperitoneal injection; probably this was because they had early encountered the influence of the liver.

The influence of the liver on the inactivation of estrogen in man is illustrated by the fact that hepatic cirrhosis may be accompanied by an excess of free estrogen in the urine. Glass, Edmundson and Soll (1940) examined fourteen men who were suffering from chronic disease of the liver. Signs of excessive estrogenic action were noticed, atrophy of the testicles being present in all. Assays of the urine showed a diminished output of androgen and a raised excretion of estrogen; moreover, the urinary estrogen was in a free form, showing that the liver had failed to conjugate it. To discover in what part of the liver estrogen becomes inactivated Zondek and Sklow (1941) blocked the reticulo-endothelial system of immature rats by the intracardiac injection of 0.4 cc. of a colloidal solution of

copper. Half an hour later 0.25 mg. of estrone was given subcutaneously. Control rats, untreated with copper, were given similar injections of estrone. Four hours later the animals were killed and macerated. Extraction of the tissue showed that in both groups 98 per cent of the estrone had been inactivated, from which the experimenters conclude that the hepatic cells and not the Kupfer cells contain the inactivating factor.

Heller (1940) found that slices of liver inactivate estradiol in vitro, and that this inactivation is prevented by sodium cyanide. Estrone, also, is rendered biologically inert by the liver, but all the other tissues examined enhanced the potency of estrone, probably, Heller thinks, by enzymatic reduction to estradiol. The endometrium showed this enhancing ability in the greatest degree among the tissues examined. The inactivation of estrone by liver, Heller ascribes to enzymatic oxidation. Estriol was less affected by the liver than estrone. Heller states that the kidney, though to a less degree than the liver, has some capacity for inactivating estradiol.

Westerfeld (1940) has stated that tyrosinase, on incubation, inactivates estrone, estradiol and diethylstilbestrol. Zondek and Sklow (1942) believe that the inactivation is caused by an enzyme (estrinase) which, though having properties closely resembling those of tyrosinase, is not identical with it.

It has been found by Smith and Smith (1938) that

the inactivation of estrogen is interfered with by progesterone, under the influence of which a larger amount of active estrogen will be available for use in the body and the increased quantity will be found in the urine.

Another side of the hormone-enzyme relationship is reported by McShan and Meyer (1936) who have observed that stilbestrol, hexestrol and dienestrol are effective inhibitors of the succinoxidase system (carbohydrate metabolism), cytochrome oxidase (respiratory enzyme). Their work was done with mixtures of enzyme extracts with hormones.

Hertz (1938) indicates that of the B complex vitamin factors necessary for estrogen inactivation, folic acid is the specific agent which acts upon the estrogens. His work shows that folic acid deficiencies are directly related to a failure of estrogen inactivation. Prior to this discovery Hertz (1936) had already established a definite relationship between the role which the B complex vitamins play in the endocrinological aspects of reproduction.

Heller, Rollin and Thayer (1938) corroborated the work done by Hertz, by maintaining chicks on a diet deficient in folic acid. This produced a very slight oviduct response when estrogen was administered. As folic acid was increased, a ten-fold increase in size of the oviduct was observed. Thus a highly specific dietary factor may be shown to be involved both quantitatively and qualitatively in a hormonal response resulting in new tissue formation.

Hormones affecting the somatic system for the most

part stimulate either new tissue formation or qualitative alteration in the constitution of pre-existing tissue. These effects naturally create a demand for the essential constituents of such tissue substance; consequently their absence impairs the hormonal response.

5. Excretion.--The primary estrogen elaborated in the ovaries appears to be estradiol. According to Callow (1938) estradiol is converted by oxidation into estriol and estrone, in which forms they are excreted by the kidneys. Estrone has been isolated only from the urine; it may be regarded therefore as a waste product. Estradiol is not normally present in the urine except perhaps in small quantities. Smith and Smith (1938) noted the presence in some samples of urine of an estrogen which was neither estrone nor estriol; they suspected it to be estradiol. Later Huffman et al. (1940) identified estradiol in urine collected from women during labor. They had chosen this urine for the investigation because it was known to contain the largest amount of an estrogen which, until then, had not been identified in the urine.

In their combined forms estrone and estriol are relatively inert, and in the earlier estimations of estrogen in the urine only the amounts of free estrone and estriol were recognized. In spite of this drawback useful information was obtained.

The site of the change from estradiol to estrone

seems uncertain. Fish and Dorfman (1941) gave 50 mg. of alpha-estradiol dipropionate by mouth on two successive days to each of four female guinea pigs, the first dose being given a few hours after the beginning of estrum. The urine was collected during the next six days, in the luteal phase of the cycle. They found that the administered estrogen was excreted as estrone. The same observation was made with spayed females and normal males, from which it appears that transformation of estradiol into estrone does not necessarily take place in the ovary or the uterus.

Loewe and Lange (1926) discovered that the amount of estrin in the urine of normal women varies at different times, the highest yield being obtained about the middle of the menstrual cycle. Siebke (1930, 1934) made periodic assays of urine throughout the menstrual cycle in normal women, and found the highest concentration of estrogen, namely about 200 i.u. per diem, twelve to ten days before menstruation, and the lowest during menstruation. Gustavson and Green (1934) and Gustavson, Wood and Hays (1936) carried out similar investigations and noted a sudden rise in the output of estrogen between the ninth and twelfth days of the cycle, about the time of ovulation, followed by a rapid fall to zero. A second rise occurred between the fourteenth and twenty-first days of the cycle and was followed by a gradual fall. Frank, Goldberger and Spielman (1934) observed an increased excretion of estrin

on about the tenth day of the cycle followed by a fall and a second rise about three days before menstrual bleeding. Smith and Smith (1935) found in normal women the highest output of estrogen on the fifteenth day of the cycle and the minimum on the first day of menstruation. Palmer (1937) obtained corresponding results. He says that on about the fourteenth day of the cycle there is a sudden large output of free estrone, that is to say estrone not combined with glycuronic acid. Yerby (1937) found two high peaks of estrin excretion during the menstrual cycle, namely at about the middle of the cycle and just before menstruation.

Spurrell and Ucko (1938) tested the estrogen excretion of two normal women throughout the menstrual cycle. The early morning specimens of urine during each successive period of four days was bulked and assayed. In one of the women the maximum output of estrogen took place between the eleventh and fifteenth days, and in the other between the sixteenth and twentieth days. In both, the lowest level occurred at menstruation.

Allen et al. (1936) made similar assays of urine during the menstrual cycle in the chimpanzee. Estrin was present in the urine throughout the cycle. The highest yield being at about the time of greatest genital swelling, midway between two menstruations and corresponding approximately with the time of ovulation; the lowest yield was obtained during the menstrual flow. Palmer (1940) states that menstrual bleeding is accompanied by the excretion of

uncombined estrogen in the urine, and this is found also to accompany normal labor, abortion and menorrhagia.

Others have confirmed these observations (Smith, Smith and Pincus, 1938; Von Haam and Rothermich, 1940). It seems possible that the second high level of estrogen excretion during the estrus cycle may be attributed to the action of progesterone in preventing the inactivation of estrogen or in assisting its conversion into estriol, which is more readily excreted (Smith, Smith and Pincus, 1938).

Reference has been made already to the formation and excretion of estrogen by males, and to the fact that the artificial administration of androsterone or testosterone to men is followed by an increased concentration of estrone in the urine. The results show that the output of gonadal hormones in the urine is not widely divergent in the two sexes in adult life; in normal conditions men, regarded collectively, excrete more androgen and less estrogen than women, but the average amount, in each case, lies within the limits of individual variation in the other sex. This is shown by the results of assays carried out by Gallagher, et al. (1937).

Kochakian (1946) showed that the state of gonadal function of the individual modifies the effectiveness of the protein anabolic properties of the steroid hormones.

Qualitative as well as quantitative differences have been recognized in the excretion of estrogens during

the menstrual cycle. Smith and Smith (1931) noticed that in eight women who were being treated with chorionic gonadotrophin the concentration of estrin in the blood and urine decreased after four or five days of treatment. Following up this observation they experimented on rabbits and found that by the administration of either a luteinizing gonadotrophic extract or progesterone to normal female rabbits the urinary output of estrin could be increased tenfold. No increase occurred in spayed rabbits under the influence of gonadotrophin. If a dose between 600 and 700 r.u. of estrin was given intravenously into spayed rabbits, about 30 r.u. were excreted in the urine during the next four days. The same dose of estrin, if progesterone also was given, was followed by a recovery of 500 r.u. of estrin from the urine. From these results Smith and Smith concluded that progesterone facilitates the excretion of estrin. In a later paper (1938) they give the results of urine assays made in a twenty-seven year old woman at different stages of the menstrual cycle. Throughout the cycle the output of estrone was greater than that of estriol but the amount of estriol excreted was increased during the luteal phase. They believe that progesterone facilitates the formation of estriol, which is a less active estrogen and is more readily excreted than estradiol or estrone. Their views are supported by observations on the output of estrogens during pregnancy and pseudopregnancy.

Apparently the liver not only inactivates estrogen

but takes part also in its excretion. Cantarow et al. (1942) made biliary fistulae in dogs and examined the bile for estrogen. In twenty-four hour specimens of bile from untreated dogs no estrogen was detected. After a single intravenous injection of 250,000 i.u. of estrone, 120,000 i.u. of total estrogen, of which 100,000 i.u. were free, were detected in the bile excreted in the twenty-four hours succeeding the injection, and 120,000 i.u., of which 88,000 i.u. were free, were excreted in the next twenty-four hours. These workers believe there may be an 'enterohepatic circulation of estrogens' like that of bile. It is clear that further experiments will have to be done before any estimate can be made of how much active estrogen is excreted by the kidneys and liver respectively, and how much is inactivated in the body.

An effect similar to testosterone is produced by estrogen in that they both cause retention of salt and water (Meites, 1947), which is generally true of all hormonally active steroids. Of course, as already noted, estrogen has an opposite effect of testosterone in regard to growth and metabolism.

Ingle (1941) has shown that stilbestrol is capable of inducing a prolonged hyperglyceria and glycosuria in the normal rat, though he was unable to explain the mechanism of the diabetogenic activity.

Janes and Dawson (1946), in studying the effect of stilbestrol upon Alloxan diabetes, observed a marked decrease

in food intake but an increase in liver glycogen. They concluded that it is not apparent that stilbestrol is a diabetogenic agent or that it ameliorates diabetic symptoms; but that the decrease in glycosuria may simply be associated with the reduced food intake. The same conclusions were drawn from a similar experiment by Ingle et al. (1947).

Noble (1939b) reports a marked decrease in fluid intake as a result of estrogen administration; and at the same time an increase in fluid retention. This work was done on rats. Confirmation of the same results was reported on work with monkeys, by Zuckerman (1939), on humans, by Thorn and Emerson (1940) and on frogs by Dow (1939).

In Addison's disease the administration of estrogens produced a retention of urine after large amounts of water were ingested (Reforzo-Membrives, 1946).

Adolph (1947) sums up part of the estrogen effect by noting that patterns of ingestion and excretion were manifested that showed the coordinations among the several factors of turnover. Not only was water ingestion tempered to excretory capacities, but also water ingestion to absorptive and roughage-handling capacities. These patterns are items in a large complex of regulatory mechanisms concerned in bodily maintenance.

Another explanation of the cause of water retention is offered by Shipley (1945). He suggests that the greatest portion of the administered water is retained in the extra-

cellular compartment and probably remains unexcreted because of failure of elimination by the kidney. Under such conditions the plasma volume is slightly decreased and as a result of this there is leakage of protein through the capillaries.

An interesting observation was made by Gordon, Li and Bennett (1946), who noted that though urine volume decreased, as a result of the estrogen, urinary nitrogen excretion increased in direct proportion with loss of body weight.

Thorn and Harrup (1937) also noted a marked decrease in sodium excretion in the urine of dogs treated with estrogens.

Reduction of both urinary nitrogen and salt were further confirmed by Zuckerman et al. (1939); Gaunt and Hays (1938); Berdnickoff and Champy (1934); and Selze and Friedman (1940).

Kenyon et al. (1940) believe that body weight would decrease much more were it not for the large retention from the urine of nitrogen, sodium, potassium, chlorine, creatine, inorganic phosphorus and water. Selye (1940a) however, reports that in cows estrogen administration produced diuresis. Whether this is a chance observation or a real species difference will require further study. In another study by Selye (1939) he notes that in many instances of hydronephrosis, supposedly induced by stilbestrol, the urinary passage was obstructed by concrements.

An interesting point is made by Ingle et al. (1947)

showing the relationship between the varying effects produced by administration of estrogens with changes of diet. Generally, they noted increased excretion of non-protein nitrogen in the urine, also inhibition of growth, loss of body weight and glycosuria; but animals on a high protein diet showed the least response, while those on a high fat diet showed the greatest response. This is interesting in the light of a previous study made by Korenchevsky and Dennison (1934) who noted that despite the many changes produced as a result of estrogenic administration, there was no change in fat deposition.

Effects upon the Endocrine System

1. Anterior pituitary.--Evans and Simpson (1929a) noticed that in the female rat the pituitary is larger than in the male. In ninety-two male rats the average weight of the pituitary was 8.8 mg., and in eighty-four females it was 11.6 mg. Hohlweg (1934) found that in the female rat repeated doses of estrin caused enlargement of the anterior pituitary lobe with characteristic histological changes. Selye, Collip and Thomson (1935) noted that 800 mcg. of estrone given to lactating rats daily for ten days caused enlargement of the pituitary to an average weight of 18.5 mg. Zondek (1936a) treated male rats by injecting large doses of estrin, 5,000 or 10,000 m.u. being given twice a week. Zondek says that whereas the pituitary of the male rat is always enlarged after prolonged treatment with estrin, that of the female treated in the same way is usually macroscopically unaltered. The pituitary of the female rabbit also, Zondek says, fails to enlarge under the influence of estrone. Other workers have found that the pituitary becomes enlarged in both sexes under the influence of estrogen.

Noble (1938), Deanesly (1939) and others have observed an increase in the size of the pituitary in rats and mice under the influence of estrogen. Noble found that stilbestrol caused enlargement of the pituitary in male and female rats, the two sexes reacting alike in this respect. Brooksby (1938) has recorded enlargement of the pituitary in spayed

rats under treatment with estrogen, the increase in size being accompanied by a considerable increase of mitotic activity. Deanesly has noted that, like most of the effects of estrogen in the adult individual, the enlargement of the pituitary is a reversible change, the organ returning to its normal size after cessation of treatment. Her experiments consisted of the subcutaneous implantation of tablets of estrone (2.25 to 16 mg.) or estradiol (11.8 to 16.5 mg.), which were removed after varying periods. Cramer and Horning (1936) reported that of twelve mice treated by them for prolonged periods with estrin, eleven had enlarged pituitaries, eight of which were normal in shape while three were adenomatous.

Nelson (1941a) made the interesting observation that enlargement of the pituitary in rats under the influence of estrogen does not progress steadily with time, but shows a pronounced acceleration after the treatment has continued during a considerable period. He gave 50 mcg. of diethylstilbestrol daily to twenty-eight normal male and female rats for eight months or longer, and noticed that the enlargement of the pituitary increased rapidly after the eighth month. The increased size of the pituitaries was due to the presence of chromophobic encapsulated adenomata.

Although, as Zondek remarked, the pituitary which has become enlarged under the influence of estrin as a rule has a decreased gonadotrophic function, this perhaps may not be so always. Burrows (1936a) has examined a male mouse whose

pituitary, after prolonged subjection to estrone, was much enlarged, being 5x5 mm. in its two accessible diameters; it consisted mainly of chromophobes. In this mouse features were present which suggest that the pituitary was still producing trophins in spite of the continued application of estrone; among these features were a thyroid adenoma, extensive mammary development with secretion, and testes of normal size showing spermatogenesis.

There can be no doubt that the pituitary tumors occurring in estrogen-treated mice are attributable to the estrogen, for the spontaneous occurrence of such tumors in mice is a rarity. Selye, Holmes and Wells (1931) state that in 11,188 mice only one instance was seen. Gardner, Strong and Smith (1936) have reported an example which is of particular interest. The mouse was an untreated breeder, 695 days old. The pituitary was 4x3x3 mm. and the pars anterior consisted of chromophobe cells with a few scattered eosinophiles. Both ovaries were much enlarged. They contained no follicles or corpora lutea and consisted of tumor tissue resembling that of the so-called granulosa cell tumors of human ovaries. Cystic endometrial hyperplasia was present, and there were six mammary cancers. In this case it seems, as with similar ovarian tumors in man, there must have been an excessive output of estrogen to which the other abnormalities, including the pituitary enlargement, may be attributed.

Oberling, Guerin and Guerin (1936) have made some

curious observations on the occurrence of pituitary tumors in rats. They castrated immature male rats and grafted them with ovaries from littermates. Among seven of these which lived for more than a year after the operation pituitary tumors were present in four. In one of these the ovarian graft had disappeared, in the other three the grafted ovaries were present and functionally active. In another series of ten rats a crystal of 3:4-benzpyrene was placed under the pia mater in contact with the brain. Three of these rats survived for more than ten months and all of them had pituitary tumors.

An instance which may perhaps exemplify enlargement of the pituitary in man under the influence of estrogen has been recorded by Zondek (1940a). The patient was a twenty-six year old woman whose breasts had been removed for cancer. Metastases appeared, and on this account she was given daily doses of 0.6 g. of estradiol benzoate during the sixty remaining days of her life. After death her uterus showed advanced cystic endometrial hyperplasia and her pituitary weighed 710 mg. as compared with the normal of 595 mg. and microscopical examination revealed an adenoma or localized hyperplasia of eosinophil cells.

Richards and Kueter (1941) determined that inhibition of growth and loss of body weight produced by stilbestrol, could be fully compensated for by adequate administration of pituitary growth hormone. This, they suggest, indicates that the mechanism of chronic suppression is at least partly

due to inhibition of production of pituitary growth hormone by the stilbestrol treated animals. Support for this contention is offered by Reece and Leonard (1941a) who administered both estrogen and growth hormone to hypophysectomized rats. The estrogen, they believed, inhibited the normal effect of the growth hormone, resulting in a decrease in body weight. In a subsequent paper Reece and Leonard (1941b) come to a somewhat different conclusion. They note that though estrogens, when administered at relatively high levels, inhibit the hypophysis, simultaneous administration of anterior pituitary extracts and estrogens to hypophysectomized rats indicated an inhibition of the former's growth promoting action by the estrogens. The possibility can therefore not be excluded that an extra-hypophyseal mechanism is responsible for the observed antagonism.

Buchwald and Hudson (1947) report that estrogens administered to hypophysectomized rats results in marked changes of the blood chemistry. The mechanism is not explained but the writer notes that absence of the pituitary might well affect bone formation which in turn would be reflected in the blood picture.

Mixner, Meites and Turner (1944) report ~~an increase~~ the initiation of in lactation in response to limited doses of stilbestrol. This they explain on the basis of an increase of lactogenic hormone from the pituitary.

Meites in a later work (1947) reports that while stilbestrol may decrease milk production and food and water

intake; the combination of thyroxine and stilbestrol produced an increase in these factors. The paradoxical effects of stilbestrol upon lactation are explained on the basis of dosage. It seems that a specified limited dose stimulates secretion of pituitary lactogenic hormone, while an excessive dose decreases lactation as a result of increased adrenal-cortical activity which increases the rate of deaminization of the nitrogenous precursors of milk protein.

Korenchevsky, Burbank and Hall (1939) report that the administration of estrogens to ovariectomized animals resulted in hyperplasia of the hypophysis.

Samuels, R. M. Reinecke and Petersen (1941) found that well nourished hypophysectomized rats did not show mammary development when stimulated by large doses of estradiol. They concluded that some pituitary factor plus estrogen is needed to produce mammary development.

Von Haam et al. (1941) reports that stilbestrol produced an increase in weight of the pituitary but a decrease in the number of acidophils and basophil cells.

Bradbury (1947) also notes an increase in pituitary weight as a result of estrogen treatment; concomitantly he also finds a decrease in gonadotrophic content of the glands.

Baker and Everett (1947) injected stilbestrol daily into immature thyroidectomized rats for four days; or into adult ovariectomized-thyroidectomized rats for ten days. They noted an increase in weight of the hypophysis, cellular enlargement, an accelerated mitotic activity among the acido-

phils and an increase in their number. Thus, it was demonstrated that the stimulating action of estrogen on the hypophysis is not mediated by the thyroid gland and that it may be elicited in adult as well as in immature rats.

Biskind (1946) believes that estrogen depresses the pituitary with a resulting diminution in secretion of the thyrotrophic principle. He found that administration of estrogen to hyperthyroid cases resulted in reduction of the basal metabolic rate.

There seem to be two schools of thought on the effect of estrogen upon the hypophysis. Bogart and Sperling (1939) support the contention that estrogen inhibits growth hormone secretion. Griffiths and Young (1941) observed that when young rats, whose normal increase in body weight has been inhibited by the implantation of stilbestrol tablets, are used for the assay of growth-hormone preparations, the dose per unit body weight response is satisfactory, but the resultant changes in body weight and tail length are not proportional.

Schilling and Laqueur (1941) administered both estrone and progesterone and observed that as far as the pituitary was concerned the two hormones appeared to nullify one another.

Samuels (1947) contends that the effects upon the body of malnutrition may not be due to any direct cause but that the resultant effects are produced on the whole body

due to the principal effect of malnutrition upon the pituitary. It appears to this writer that Samuels will have to produce considerably more evidence to prove that malnutrition manifests itself only via the pituitary; granted that the pituitary certainly plays a very important role.

2. Thyroid.--Refer to: Chapter V "Thyroid-ovarian relationships".

3. Adrenals.--Leiby (1933) gave daily doses of 4 mg. of estriol to adult spayed mice for six days. At the end of this period the adrenals were found to be considerably enlarged. Andersen (1935) spayed rats at about eighty days of age and six weeks later gave them 5 r.u. of estrin three times a day for two days. She killed the rats at intervals of 24 to 120 hours after the first injection, and found an increased weight of the adrenals with an increase of lipoid in the cortical cells accompanied by congestion of the reticular zone. These changes were present even in the rats killed twenty-four hours after the first injection of estrin.

Korenchevsky and Dennison (1934, 1935) have shown that injections of estrone cause enlargement of the adrenals in rats, and Ellison and Burch (1936) obtained similar results by giving estrone, estradiol or estriol to rats whether castrated or not. The effects on the adrenal depended on the presence of the pituitary. Selye, Collip and Thomson (1935) and Selye and Collip (1936) found that daily doses of 100 gamma of estrin did not cause enlargement of the adrenals in immature rats nor did the continued giving of 200 gamma of estrin daily prevent involution of the adrenal after hypophysectomy. Further, they state that with adult rats the adrenals enlarged by the influence of

estrin contain but little lipoid, whereas abundant lipoid is present in the adrenal cortex within forty-eight hours of hypophysectomy even if the doses of estrin are continued. They believe that enlargement of the adrenal in female rats treated with estrin is an indirect action mediated by the pituitary, and they suggest that the presence of corpora lutea may be necessary for the result.

Noble (1939b) reports that the synthetic estrogen, triphenylethylene, like the natural estrogens, does not cause enlargement of the adrenal in hypophysectomized rats.

The x zone was first described by Elliott and Armour (1911) and by Thomas (1911) as occurring in man, and since then has been investigated in mice by many inquirers. The x zone is present in the adrenal cortex of infantile mice of both sexes and is composed apparently of the cells of the zona reticularis and the innermost parts of the zona fasciculata. These cells in early life are especially numerous and cause a relative increase in thickness of the cortex. The cells forming the x zone are smaller and stain more densely and evenly with eosin than those of the outer part of the zona fasciculata, the cells of which contain a varying amount of material which is not stained by eosin.

Martin (1930) studied in mice the effects of prolonged treatment with estrone and observed that a total degeneration of the x zone is caused in immature castrated males and in normal and spayed females. In immature non-castrated males estrone caused a persistence and in non-

castrated adult males a reappearance of the x zone. It seems that castration and the administration of estrone have a similar influence on the presence of an x zone in the male mouse. However, the effects of estrone on this zone depend largely upon the size of the doses, the length of time during which they are given and perhaps on the age of the animal. Waring (1942) gave 0.01 mg. of estrone during fourteen days to spayed and non-spayed female mice which were forty days old when the experiment began. These doses had no effect on the x zone, which remained intact.

In old untreated female mice there is often to be seen a peculiar degenerative change in the adrenals (Burrows, 1936b). The chief feature is an accumulation of lipoid-like material in the cells of the zona reticularis and the innermost cells of the fasciculate zone, that is to say in the same situation as the x zone. As the condition advances the accumulation of lipoid increases and eventually adjacent cells coalesce to form rounded masses lying in the vascular stroma between the cortex and medulla. These masses are composed of (1) droplets of lipoid-like material which are not stained by the usual dyes, (2) a small but variable amount of cytoplasm stained pink by eosin and (3) scattered, often pyknotic, nuclei stained with hematoxylin. The vascular stroma occasionally appears to be increased in amount and a pronounced hyperemia may be present. An identical change can be induced in the adrenals of male mice, whether castrated or not, by giving continued doses of estrogen. This form of

degeneration in the adrenal cortex may often be seen in untreated old female mice and occasionally to a slight extent in untreated old males.

4. Ovary.--It has been established that estrogens inhibit the production of gonadotrophin by the pituitary and it seems that this is why estrogens, when given to an immature animal, arrest the development of the ovaries and testes.

The arrest of ovarian activity caused by estrogen is not permanent; the ovaries soon resume their normal appearance and function if the excessive supply of estrogen is stopped.

In spite of the atrophic effect of estrogens on the ovary which is so obvious when they are supplied persistently and in large enough amount, several observers have noticed that estrogens in certain circumstances exercise an effect comparable with that caused by gonadotrophin. As already mentioned, androgens in suitable conditions exert a similar influence on the ovary and testicle.

Williams (1940) implanted tablets of stilbestrol into immature hypophysectomized rats weighing between 40 and 50 gm. with the result that ovarian atrophy was prevented or retarded and the response of the ovarian follicles to gonadotrophin from pregnant mare's serum was greatly increased. The rats were killed and examined fifteen days after the operation and implantation. The same doses of

stilbestrol did not cause any increased weight of the ovaries in normal rats of the same age and weight. Pencharz (1940) performed the same kind of experiment on rats with the same results. Hypophysectomy was done when the animals were between twenty-one and twenty-three days old and was followed at once in some instances, and seven days later in others, by the implantation of tablets of diethylstilbestrol, estradiol dipropionate, or testosterone propionate. To some of the rats chorionic gonadotrophin ('Antuitrin S') was given. Both of the estrogens used caused enlargement of the ovaries, which contained numerous medium-sized, closely packed, follicles, with a reduction in volume of the interstitial tissue. The largest ovaries were found after the combined administration of estrogen and gonadotrophin. Testosterone propionate had no effect in preventing ovarian atrophy after hypophysectomy. It is curious that the addition of 'Antuitrin S' did not cause enlargement of the ovaries in the rats treated with testosterone.

At all ages an excessive supply of estrogen will prevent the maturation of follicles by checking the supply of gonadotrophin (FSH) from the pituitary. In consequence of this action no fresh corpora lutea will form, and if none are already present when the administration of estrogen is begun the ovaries will become diminutive compared with those of untreated controls. Even if corpora lutea are present at first, when they eventually degenerate the ovary will become infantile in type and remain so as long as the treatment

with estrogen is continued (Bialet-Laprida, 1933).

Estrogens, while preventing the output of FSH from the pituitary, enhance the output of LH; consequently they will cause any corpora lutea already present to hypertrophy and to remain in an active condition. Because of this abnormal development of the corpora lutea the ovaries will be enlarged as compared with those of untreated control animals (Hohlweg, 1934). This enlargement of the ovaries caused by the maintenance of luteinization led to some confusion as to the influence of estrogens on the ovary in some of the earlier experimental work in which changes in ovarian weight alone were used as criteria of effect.

Several facts suggest that estrogens cooperate with FSH to enhance the output of LH. Selye, Collip and Thomson (1935) found that if rats were given pituitary extract alone the heaviest ovaries obtained weighed 64 mg., whereas when estrin in daily doses of 100 gamma was administered in conjunction with the same pituitary extract the ovaries weighed 165 mg., the increase being due entirely to the enhanced size of the corpora lutea.

Champy (1937) states that in mammals an overgrowth of the ovarian rete is invariably induced by persistent doses of estrone, and that small adenomata are apt to appear in this region. Nearly always, he says with a prolonged period of estrin injections invaginations of the germinal epithelium occur and cause the formation of mucoid cysts within the ovary.

When considering the various effects of estrogen on the ovary just mentioned it is to be remembered that the result is mainly indirect, being the consequence of estrogen acting on the pituitary so as to alter the supply of gonadotrophin. In addition to these indirect actions, it seems that estrogen may affect the ovary directly. Robson (1937a) induced pseudopregnancy in rabbits by injecting gonadotrophin intravenously. Later he ascertained the presence of corpora lutea by direct inspection. He then removed the pituitaries and thereafter gave daily doses of 10 gamma of estrone or 5 gamma of estradiol. As a result of these injections and in spite of the absence of the pituitary the corpora lutea were maintained in an active condition. Merckel and Nelson (1941) found that the corpora lutea in the rat's ovary could be maintained by estrogen after hypophysectomy.

5. Pancreas.--Goetsch, Cushing and Jacobson (1911) noticed that dogs with pituitary insufficiency withstand the effects of partial excision of the pancreas better than normal dogs. Some time later Houssay and Biasotti (1931) discovered that hypophysectomy reduces the glycosuria and prolongs the life of dogs after pancreatectomy, and they say that when deprived of the pituitary dogs become very susceptible to insulin coma, from which they recover if given injections of sugar. Barnes, Regan and Nelson (1933) found that, so far as the reactions just mentioned are concerned, effects comparable with those of hypophysectomy can be produced by

giving estrogen. Daily subcutaneous injections of 200 r.u. of estrogen ('Amniotin') were given to four pancreatectomized female dogs, three of which received this treatment during three weeks before pancreatectomy; the other dog's pancreas was removed so as to cause copious glycosuria, and not till then were the daily injections of estrogen begun. Glycosuria was checked by the injections and, though losing some weight, the dogs remained lively. At the end of three weeks the treatment with estrogen was stopped and within three days the dogs suffered from severe glycosuria. Nelson and Overholser (1934) made the same sort of experiment on rhesus monkeys. Daily doses of 100 r.u. of estrin were given for a fortnight, at the end of which the pancreas was excised, the injections of estrin being continued for six days afterward; during this time no appreciable glycosuria occurred. Cessation of the injections was followed by glycosuria, which disappeared under the daily administration of 200 r.u. of estrin.

Vasquez-Lopez (1940) noticed, in mice which had been treated with estrogen for several months, in every cell of all the islets of Langerhans a hypertrophic Golgi apparatus larger than ever seen by him in the normal mouse. He suggests that this histological change indicates a functional hyperactivity of the cells resulting from the influence of estrogen on the pituitary. Fraenkel-Conrat et al. (1941) support this suggestion. They implanted 10 mg. of estradiol into normal rats at weekly intervals. The rats were killed

on the twenty-first day. Control rats were hypophysectomized and treated in the same way, receiving pituitaries of untreated rats. The insulin content of the pancreas was then estimated for the donors and recipients of the pituitaries. In the pituitary donors the administration of estradiol dipropionate had caused a rise of the pancreatic content of insulin by 54 per cent in one batch and 90 per cent in another. The implantation of pituitaries from these estrogen-treated rats into hypophysectomized rats caused a rise in the pancreatic insulin of the recipients of 39 per cent as compared with hypophysectomized controls which had received pituitaries from untreated rats. Griffiths and Young (1940) implanted tablets of estrone or stilbestrol subcutaneously in Wistar rats and found that a rise of the insulin content of the pancreas was thereby caused. One month after the implantation of 12 mg. of estrone the pancreatic content of insulin amounted to 1.12 units per 100 g. of body weight, and a comparable result was obtained with stilbestrol.

Effects upon other Physiological Functions

1. Appetite and nutrition.--Most investigators agree that stilbestrol consistently produces a decrease in appetite and water intake. This is corroborated by Korenchevsky and Dennison (1934); and by Biskind (1946), who relate the phenomenon to deficient inactivation of the estrogen by the liver. Brobeck et al. (1947) relate the phenomenon to the estrus cycle. They find a decrease in food and water intake during the estrus phase and a compensating intake of nutriment during the luteal phase. Hertz (1946) explains the mechanism as an inhibition of certain vitamins of the B complex.

Reduction of food and water intake is further substantiated by Cameron, Guthrie and Carmichael (1946); Meites (1947); Mixner, Meites and Turner (1944); Noble (1939b) who report that though estrogen decreased water intake it also caused an increase in water retention. This is further corroborated by Zuckerman (1939); Thorn and Emerson (1940); and Dow and Zuckerman (1939).

Kenyon et al. (1940) report an increase in weight of women though their appetites decreased, as a result of retention of urine, nitrogen, sodium, potassium, chlorine, creatine, inorganic phosphorus and water. This is substantiated by Thorn and Harrop (1937) who got similar results on the dog. Samuels (1947) explains this phenomenon as a pituitary response which shifts the balance between synthesis of protein from, and deamination of, amino acid in the direction of the latter.

2. Mammae and lactation.--Heape (1906) noticed a close association between the primary development of the mammae with proestrum. This observation led him to attribute the early stages of mammary growth to some substance secreted by the ovary at this stage of the estrus cycle. Marshall (1910) extended our knowledge by noting that the formation of corpora lutea is accompanied by events which differ from those occurring at proestrum; he was thus able to infer that the ovary forms two different kinds of secretion each of which corresponds with a particular stage of follicular evolution.

It is now generally recognized that during each estrus cycle changes occur in the mammae in response to the output of hormones from the ovaries. In women there is apt to be some fullness of the mammae, perhaps with discomfort or even pain, in the second half of the estrus cycle. These happenings may be attributed largely to hyperaemia of the breast and distension of the mammary ducts by secretion, such effects being due to the combined actions of estrogen and progestin derived from a young corpus luteum. Astwood, Geschickter and Rausch (1937) observed in the rat that during proestrus and estrus the mammary ducts were narrow and empty whereas at metestrus the ducts were distended with fluid. Doubtless progesterone shares in the production of these changes. The general effect of ovarian secretion on the breast was tested experimentally by Steinach and Holzknecht (1916), who grafted the ovaries of guinea pigs into

the kidneys of castrated male littermates; after this operation the nipples and mammae became enlarged, milk formed and the guinea pigs, though male, were able to suckle young and showed an inclination to do so. Lipschutz and Tutso (1925) carried out similar experiments on guinea pigs and rats and obtained results like those of Steinach and Holzknecht. Vintemberger (1925) gave repeated injections of follicular fluid obtained from cows into male and female rabbits and so induced an increased size of the mammae in both sexes. Hartman, Dupre and Allen (1926) observed a similar result in the opossum, and Loeb and Kountz (1928) in the guinea pig. Allen (1927) reported that injections of estrin caused extensive development of the mammary ducts in ovariectomized rhesus monkeys.

Mixner, Meites and Turner (1944) report that small optimal injections of stilbestrol increased lactation; larger doses had an inhibitory effect. They explain the lactation-stimulating effects of small dosages of stilbestrol as being due to its ability to stimulate the secretion of the lactogenic hormone by the anterior pituitary gland, while the lactation-inhibiting effects are believed to be correlated with increased adrenal-cortical activity resulting in an increased rate of deaminization of the nitrogenous precursors of milk protein.

Samuels, R. M. Reinecke and Peterson (1941) report that hypophysectomized rats given estrogens, show no mammary

development, leading them to believe that estrogens and some pituitary factor must both act directly on the mammary gland to produce normal development.

Gardner (1941) reports that estradiol benzoate and propionate, given in large doses, inhibited mammary development in both male and female animals. Spielman and Ludwick (1941) record an increase in butterfat of cows' milk with no effect on total production; after administration of stilbestrol.

The first step toward milk production is the anatomical development of the mammae. When this development has proceeded far enough the onset of lactation and its continuance seem to depend mainly on the pituitary. Heape (1906) showed that lactation does not depend on any influence derived from the fetus or placenta for it occurs in pseudo-pregnancy.

Before considering what part estrogens play in this process, attention may be given to the influence on lactation of the pituitary.

The dependence of lactation on pituitary hormones is proved by the facts (1) that it can be induced by pituitary extracts, and (2) that it is prevented by hypophysectomy.

Stricker and Grueter (1928) gave repeated injections

of an aqueous extract of pituitary to immature female rabbits between one and one-half and two and one-half months old. Under this treatment the follicles matured and ovulation took place with follicular hemorrhage and luteinization, but no appreciable change occurred in the mammae. Nine mature rabbits were submitted to infertile coitus and eight to ten days later pituitary extract was given; lactation followed in all. Four rabbits were spayed ten days after sterile coitus and a course of injections of pituitary extract was begun on the next day. Two or three days after the first injection lactation ensued and became abundant. In addition to these experiments Stricker and Grueter gave pituitary extract to a rabbit fifteen days after she had weaned her young, with the result that lactation was resumed. The same result was obtained after a single injection of pituitary extract into a dog ten days after she had weaned her puppies. Stricker and Grueter (1929a) and Grueter (1930) also found that, provided the mammae were fully developed, lactation could be induced by pituitary extracts though the gonads were absent. Houssay (1935) injected an alkaline extract of bovine pituitaries into male and female dogs. These injections caused lactation in adult females whose mammary glands were already well developed, but did not have this effect in males or immature females. In the latter, after preliminary treatment with estrone, lactation could be induced by pituitary extract.

3. Liver.--Korenchevsky and his colleagues (Korenchevsky and Dennison, 1934; Korenchevsky, Hall and Ross, 1939) reported a decrease in the weight of the castrated rat's liver under the influence of estrogen; this result was less obvious in non-castrated rats. Selye (1940b) noticed a reduction in the weight of the liver in rats which had been given estradiol. Such an effect of estrogen does not appear to be a pronounced or constant reaction. Griffiths, Marks and Young (1941) noted an increase in the weight of the liver in rats which had been treated with estrogen.

Histological changes in the bile ducts have been observed by Gardner, Allen and Smith (1941) in mice after the administration of estrogen. The compounds used were estradiol dipropionate or benzoate (16.6 to 50 gamma weekly), estrone (250 gamma weekly) and stilbestrol (250 gamma weekly). This treatment caused the bile ducts to become thickened, rigid, white and somewhat nodular. The main duct was less affected towards the duodenal end. The cystic duct was thickened up to the neck of the gall-bladder. Microscopically the enlarged ducts showed an increase of the epithelial folds and of the glands, which sometimes reached as far as the serosa. The epithelium was hyperplastic and the ducts increased in extent.

Battaceano and Vasiliu (1936) say that when given to dogs estrone causes at first a diminution in the volume of the liver which is followed by an increase. In a dog

with a biliary fistula doses ranging from 1,000 to 5,000 r.u. arrested the flow of bile.

MacBryde et al. (1940) report liver degeneration as a result of both synthetic and natural estrogen administration. Russell et al. (1941) also report liver damage but only after sustained high dosage.

Castrodale et al. (1941) report liver degeneration which they attribute to a series of indirect causes. They believe that the liver is only ultimately affected due to the principal degenerative effects upon bone and blood formation.

Richards and Kueter (1941) failed to find any appreciable effects upon the liver after sustained high dosages of estrogens. There are many investigators who corroborate this. The writer will discuss this point further in the section relating to toxicity. Since there are two definite and opposing opinions on this point the writer has taken pains to note that neither school of thought opposes the other since in each instance corroboration occurred where the investigators worked on the same species of animal.

4. Bone and blood.--There is little doubt that the effect of estrogen on the body weight is largely the result of bony changes. Students of human anatomy have noticed a precocity of skeletal development in the female compared with the male. This precocity appears in two forms, namely (1) an earlier establishment of some of the centres of ossifi-

cation, and (2) an earlier union of epiphysis and metaphysis. Frazer (1920) in his textbook of human anatomy states that the centres of ossification in the carpus appear from a few months to a year earlier in the female than in the male. The secondary centres of ossification in the human vertebrae also appear earlier and reach full development sooner in girls than in boys. As the growth in length of a bone ceases when the epiphysis unites with the shaft, the fact that such union occurs earlier in the female than in the male accounts largely, and perhaps entirely, for the greater stature of the latter.

Steinach and Holzknecht (1916) demonstrated that the difference between the two sexes in skeletal growth is due to ovarian action. They grafted ovaries obtained from female guinea pigs into castrated male littermates and after the animals had attained full growth various elements of the skeleton were measured.

The effect produced on the bones by the ovary growing in the castrated male is greater than that of the ovary growing naturally in the female. The counterpart of this effect was noticed, inasmuch as the spayed females carrying grafted testes grew to greater dimensions than normal males. Spencer, D'Amour and Gustavson (1932a) obtained the same effects on the length of bones in rats by injections of estrin.

Zondek (1936b) has reported similar results in rats treated with estrin, their long bones being shorter than

those of untreated controls. The length of other long bones and of the skull showed similar changes.

That the reduced length of bones under the influence of estrogens is due to a premature union of the epiphyses with the shafts has been demonstrated in dogs by Tausk and de Fremery (1935) and by Gardner and Pfeiffer (1938a) in mice. This effect was observed also in the vertebrae of the rat's tail by Levie (1938), who treated two groups of castrated male rats, forty-two days old and weighing 80 gm. at the start, with daily doses as follows: Group I, 500 gamma of estradiol; Group II, 500 gamma of testosterone. Other rats of the same age were used as untreated controls. At the end of fourteen days the tails of the rats treated with estradiol had grown less than those of the control or the testosterone-treated animals. Premature union of the epiphyses was found to be the cause of the diminished tail-growth.

Estrin has other effects on bones than the epiphyseal changes mentioned above. Zondek (1937) observed in rats and cocks which had been under continued treatment with estrogen a supercalcification of the skeleton with partial obliteration of the marrow cavities. These changes were readily detected by X-rays, and on splitting a femur longitudinally it could be seen that the medulla was largely occupied by finely porous, easily crumbling, osseous tissue. In addition to the change of texture, the bones were shorter than normal because the epiphyses had united earlier than

usual with the shafts. Gardner and Pfeiffer (1936b) also reported alterations in the structure of bones brought about by estrogens. They treated thirty-four male and female mice with estrone benzoate or equilin benzoate for periods extending to 3½8 days. After this treatment the femurs were white, opaque and very hard and brittle, and the marrow cavities were almost completely replaced by compact bone. These changes, which it seems are reversible, were shown by radiographs to be present throughout the skeleton except in the bones of the symphysis pubis. Testosterone propionate prevented the osseous changes induced by estrogen; weekly doses of 1.25 mg. inhibiting the action of weekly doses of 1,000 i.u. of estradiol benzoate. Sutro (1940) reports similar osseous changes in mice following weekly doses of estradiol benzoate ranging from 150 to 1,000 r.u. He says that proliferation of new bone in the medullary cavities is especially pronounced in the femur and tibia, and that the calvarium is affected in the same way. Miller, Orr and Pybus (1943) have made a detailed study of the osseous changes induced by estrogen in mice. They have observed, like Gardner and Pfeiffer, that the changes are reversible.

Lorenz, Chaikoff and Entenman (1938) found a great rise in the fat content of the blood of fowls within forty-eight hours of an intramuscular injection of 3,000 r.u. of estrone.

Entenman, Lorenz and Chaikoff (1938) obtained the same kind of effect by injecting a pituitary gonadotrophin

into immature hens. They say that all the blood lipids were increased, including cholesterol, phospholipid and free and combined fatty acids. Even more striking results were obtained by Zondek and Marx (1939) by giving a cock 4 mg. of diethylstilbestrol on each of six consecutive days. H. G. Loeb (1942) has noticed a similar, but less pronounced, effect of estrogen in rats. He kept male rats on a diet which was rich in fat and treated them with estradiol for a period of twenty-four days, at the end of which their blood showed between 531 and 566 mg. of lipoid per 100 cc. as compared with 351 mg. per 100 cc. in the controls fed in the same way but not given estrogen.

In connection with the changes already mentioned as occurring in bone under the influence of estrogen, it may be noted that estrogens cause a rise of the blood calcium. This was first observed in birds, and though in them it occurs in a pronounced form and probably assists the formation of egg-shell, it is surmised that it very likely also happens in mammals.

Riddle and Reinhart (1926) noticed that each ovulation in the pigeon is accompanied by a large rise in the blood calcium. The rise begins about 108 hours before ovulation and 123 hours before the beginning of the formation of the shell. At the time of ovulation the concentration of calcium in the blood may rise to 19 mg. per 100 cc. of serum. During sexual quiescence no difference was found in the blood content of calcium in the two sexes. As a result

of further studies Riddle and Dotti (1936) state that gonadotrophin causes an increase of serum calcium in normal or hypophysectomized pigeons but not after gonadectomy. The reaction is obtained more quickly in males than in females, and is not caused by corticosterone or testosterone. Estrone, they say, caused an increase of blood calcium in normal, castrated or hypophysectomized pigeons and rats, and in normal dogs, fowls and doves, but the reaction was not obtained in rabbits. Progesterone, they found, had the same effect in a less degree.

A mobilization of calcium by estrogen in some instances may cause urinary calculi. Burns and Schenken (1939) gave estradiol dibenzoate in weekly doses which ranged from 100 to 1,500 r.u. to mice of the C_3H strain. The mice were fed on Purina fox chow with lettuce once a week. Among the mice so treated calculi were found with considerable frequency in the bladders, ureters and kidneys.

It seems probable, if the formation of erythrocytes is to some extent regulated by a pituitary hemapoietic hormone as Flaks, Himmel and Zotnik (1938) believe, that estrogens might, in view of their depressing activity on the pituitary, hinder to some extent the supply of erythrocytes. Several workers have reported that red cells are normally more numerous in the male than in the female. Such observations have been made on many different species. Steinglass, Gordon and Charipper (1941) have investigated the matter, using an inbred strain of rats. They found that after gonad-

ectomy both the red cells and the hemoglobin increased in the females and fell in the males. In spayed females estrogen reduced the erythrocyte count from 8.9 to 6.7 millions per mm^3 and in castrated males the same treatment caused hypoplasia of the marrow.

MacBryde, et al. (1942) found that in dogs daily doses of 10 mg. of estrone, 1.66 mg. of estradiol benzoate or 5 mg. of diethylstilbestrol caused changes in the marrow with a diminution of erythrocytes and hemoglobin in the circulating blood, and Tyslowitz and Dingemanse (1941) obtained similar results in dogs with daily doses of 1 to 5 mg. of estrone.

Plum (1942) found that in women both the reticulocyte count and the amount of erythrocyte-ripening substances in the blood are at their maximum during menstruation and at a minimum in the mid-menstrual period. The rise in both is rapid and begins about one week before menstruation, falling rapidly after this phase. The observation accords with the supposition that estrogen checks the formation of the red blood cells.

There is little doubt that estrogens affect the production of white cells. Tyslowitz (1939); and Tyslowitz and Dingemanse (1941) found that daily injections of 5 mg. of diethylstilbestrol to dogs brought about an agranulocytic anemia within the next twenty-five to fifty days. In untreated mice various abnormalities of the white blood cells are frequently seen, and they comprise an unbroken series

from a general leucemia to lymphosarcoma. These conditions are more frequent in female than in male mice. Mercier (1938), basing his observations on 465 mice of a single strain, recorded lymphosarcoma in 64.9 per cent among the 254 females, and in 34.1 per cent among the 211 males. Others have called attention to the special proclivity of female mice to leucemia and lymphosarcoma (Gorer, 1940; Pybus and Miller, 1941; Miller and Pybus, 1942). Cole and Furth (1941) point out that leucemia occurs not only more frequently in female mice than in males but appears earlier in the females. It seems probable that this tendency to the development of leucocytic neoplasia among females is in part at least dependent on the action of estrogen. Lacassagne (1936a) noticed the occurrence of lymphosarcoma and leucemia among mice which had been under treatment with estrogen.

Heller, Rollin and Thayer (1948) report that stilbestrol produces an increase in cholesterol but no increase in the fatty acid content of the blood. Buchwald and Hudson (1945) gave daily injections of .1 mg. of stilbestrol and .2 mg. of testosterone propionate to castrated male and female rats respectively, for twenty-eight day period. There was no change in the serum calcium or phosphorus nor in the output of calcium and phosphorus in the feces. As in the intact males, stilbestrol produced a decrease in the acid phosphatase activity of the blood serum. Castration did not magnify the effects of the hormone injection. The level of the acid phosphatase activity of the blood serum was raised by castration.

5. Integument and hair growth.--There are areas of the body surface where the specific effects of estrogen are well recognized. These areas are not the same for all species; the reactions include those cutaneous peculiarities by which the two sexes can be distinguished, for example the hen-feathering of birds. Apart from these widespread, though regional, responses to estrogen there are certain local responses which occur in most, and perhaps in all, mammals, namely a thickening of the epidermis of the nipples. (Burrows, 1945).

Apart from the special effects to which reference has just been made, estrogens seem to exert a general inhibitory action on the growth of epidermis. It seems a fact that, in many species at least, the male possesses a thicker hide than the female. Wilson and Morris (1932) compared the pelts of male and female angora rabbits which had been kept on the same food and were of the same age when killed. When eleven months old the average weight in ounces of the pelt in males was 9.08 as compared to 7.58 in females; in rabbits at twenty-four months the ratio was 8.92:8.25.

Gardner and De Vita (1940) gave estradiol dibenzoate in weekly doses ranging from 133.3 gamma to 333.3 gamma to five male and five female fox terriers. Under this treatment the hair failed to regrow after shaving or clipping. The arrest of hair growth continued if testosterone propionate in weekly doses of 5 or 10 mg. were given also. Mulligan (1943) performed a similar experiment on dogs,

using stilbestrol, with the same results, i.e. the hair did not become restored after clipping; moreover it became thinned over the abdomen, perineum and around the base of the tail.

Emmens (1942) studied the growth of hair in rats and concluded that in normal males the hair grows faster than in females, whereas in castrated males it grows at the same pace; he noticed further that by giving estrogen the regrowth of hair in epilated regions of the skin was retarded. Hooker and Pfeiffer (1943) found that 83 gamma of estradiol dibenzoate given subcutaneously twice a week to rats caused some loss of hair, retarded the regrowth of hair after shaving and caused a diminution in the size of the sebaceous glands. These effects could be prevented by giving testosterone.

The loss of hair and "saddle-nose" appearance of mice treated with stilbestrol has generally been observed by most workers in the field.

Toxicity in Relation to Dosage and Species

There have been conflicting reports as to the toxicity of various estrogenic substances. Stilbestrol, in particular, has been reported as being anywhere from extremely toxic to completely non-toxic. As will be observed however, the response to the drug seems to indicate a species difference.

Selye (1939) reports many fatalities in mice, given stilbestrol, that resulted from liver damage; or death resulting from hydronephrosis due to obstruction of the urinary passage by concrements.

Ingle (1941) notes an induction of prolonged hyperglycemia and glycosuria in rats given stilbestrol.

Page, et al. (1941) report that stilbestrol in excessive doses produced toxic changes in one-third of their experimental animals. MacBryde et al. (1940) report a high degree of toxicity in studying the effects of stilbestrol upon dogs. This is corroborated by Arnold (1939) who also noted a decrease in the hemoglobin and red blood cell count of the dog, with a corresponding increase in the number of leukocytes.

In women however, no toxic effects of stilbestrol have been observed even after prolonged administration of very excessive dosages. This is corroborated by Ratschow (1941); Karnaky (1941); and Russell et al. (1941) who did note some adrenal hemorrhage. Morrell and Hart (1941) did extensive work in this field and were able to autopsy several

women and a few men thus treated. The autopsies showed no degeneration attributable to the prolonged application of stilbestrol.

Summary - Stilbestrol

The review of the literature indicates substantial agreement upon the following effects produced by the administration of estrogens. In general, it is agreed that these hormones may cause:

1. Enlargement of the pituitary.
2. A decrease in the gonadotrophic function of the pituitary.
3. Tumors of the pituitary.
4. A decrease in thyrotrophic function of the pituitary.
5. Adrenal hypertrophy.
6. Atrophy of the ovaries.
7. A decrease in growth hormone formation.
8. A retardation of growth.
9. A decrease in body weight.
10. A degeneration of bone.
11. An inhibition of hair and skin growth.
12. A decrease in oxygen consumption.
13. An inhibition of the succinoxidose system (carbohydrate metabolism); cytochrome oxidose (respiratory enzyme).
14. A decrease in food and water intake.
15. A decrease in milk production, except in minute doses.
16. An inhibition of estrogen-inactivating enzymes.
17. A vitamin B deficiency, especially significant in the liver.
18. An inability of the liver to excrete estrogen into the bile.

19. Usually a decrease followed by a marked increase in weight and size of the liver.

20. Liver degeneration, except in man.

21. Vasodilation and decrease in blood pressure.

22. An increase in capillary permeability.

23. A retention of water and salts in the extracellular compartment.

24. The plasma volume to decrease as a result of leakage of protein through the capillaries.

25. A decrease in hemoglobin and red cell count with a corresponding increase in leukocytes.

26. An increase in blood calcium. in birds

27. Interference with estrogen excretion in the urine.

28. Hyperglycemia and glycosuria.

29. Renal insufficiency and urine retention.

30. A decrease of urine volume and an increase of urine nitrogen.

31. Urinary obstruction due to concrement formations.

32. An increase of pancreatic activity and insulin production.

33. Renal and cardiac enlargement.

One must bear in mind that the foregoing effects were accomplished over a wide range of dosage and under varying experimental conditions.

CHAPTER IV

THE EFFECTS OF THYROACTIVE SUBSTANCES, WITH PARTICULAR REFERENCE TO THE THYROPROTEINS

General Considerations

The effects which the thyroid produces were amongst the earliest studies undertaken in the endocrine field. This gland and an explanation of its functions were described as early as the fifteenth and sixteenth centuries. In 1855, Claude Bernard published his epic,, "Lecons de Physiology Experimentale", in which he differentiated between glands of external and those of internal secretion. He correctly described the thyroid as a gland of internal secretion. Since then, subsequent workers have amassed a good deal of corroborative data pertaining to the functions of this gland. Of course there is still much that is in the realm of controversy. However, since it is not the purpose of this paper to discuss the highly theoretical aspects of thyroid behavior, this writer considers that it shall be sufficient for the purpose to offer in condensed form the works of a few authoritative observers whose studies represent the classic contributions in this field.

1. Effects of thyroidectomy.--The lethal effect of surgical removal of the gland from animals in early attempts was a handicap to further progress since there was no known way to experimentally produce a hypothyroid condition. As a result, it remained for the next real progress to be made as a result of surgical removal of the gland from humans. Kocher (1878) was the first to practice surgical removal of the thyroid for treatment of goiter. A few years later, Kocher (1883) and Reverdin and Reverdin (1883) published papers dealing with pathological conditions arising a few months after total thyroidectomy. Kocher termed the condition "cachexia strumiprivia" and described a condition similar to myxedema. Reverdin and Reverdin described the condition as "operative myxedema". Neither of the authors apparently recognized the parallelism of myxedema and cretinism. During the same year Lombard (1883) reviewed the previous literature on the thyroid indicating that the gland was essential for proper development of children and that its integrity was essential for normal mentality and appearance of adults.

In 1884 Horsley reported that thyroidectomy of young monkeys led to myxedema and pointed out the similarity of the condition to that described by Schiff arising from thyroidectomy of dogs, the "cachexia strumiprivia" of Kocher and "operative myxedema" of Reverdin and Reverdin (Paget, 1919). He concluded further that the conditions arising from thyroid removal closely paralleled the condition found in clinical myxedema.

2. Effects of thyroid replacement.--The pathological features appearing after the removal of the thyroid from human patients naturally led to attempts at replacement therapy. The first reported, however, was made by the physiologist Schiff (1884). In that year he published an important paper reporting that part of the harmful effects of thyroidectomy could be relieved by transplanting a gland from another dog. He even ventured the suggestion that similar results might have been secured by injection of an extract or by ingestion of thyroid glands. Murray (1891), following the suggestion of Schiff, found that myxedema in humans was relieved by injection of an extract of sheep thyroids. The following year, Fox (1892) successfully treated a case of myxedema by giving thyroid by mouth. The technique of studying the physiology of a gland by removal, followed by replacement therapy, was firmly established.

About that time Magnus-Levy (1897) added another method of approach. He reported upon the feeding of thyroid to normal subjects and studied the effect on oxygen and nitrogen metabolism. He also studied the metabolism of myxedematous patients and those who had been treated successfully by thyroid therapy. Moussu (1899) reasoned that if a small amount of thyroid is necessary for growth, would not a little extra result in more rapid growth? Acting upon this hypothesis, he fed fresh thyroid to dogs and observed rapid skeletal growth with early maturity.

Thus, knowledge of the physiology of the thyroid

gland was beginning to expand rapidly. By 1900 publications on the gland were numerous and investigations extended into many branches of biology and medicine. The thyroid was beginning to attract the attention of chemists, also. As early as 1820 iodine was shown to relieve certain types of goiter (Harington, 1933), and Baumann (1896) had shown that iodine was a normal constituent of the thyroid.

The developments along the different types of investigation have given rise to a multitude of reports, a review of which is quite beyond the scope of a dissertation such as this. This review will consequently be confined to subjects with which this investigation has been concerned.

Effects upon Metabolism

1. Carbohydrates, fats and proteins.--Kendall (1929), Harington (1933) and Lerman (1941) have reviewed the literature on the effects of hypothyroidism on carbohydrate metabolism which indicates that the results have been quite variable. There is a tendency for blood sugar to be low (Bodansky, 1942) and liver glycogen is frequently low. Increased sugar tolerance is usually associated with hypothyroidism, but Harington (1933) has suggested this may be due to decreased absorption from the gut. This suggestion is supported by the report of Althausen and Stockholm (1938) that dextrose absorption from the intestines is low after thyroidectomy. They have suggested that the rate of sugar absorption may be used as a criterion of thyroid function.

Fat metabolism is altered also in hypothyroidism. There is a tendency for fat to be stored in the tissues (Harington, 1933) and apparently the fat cannot be oxidized for energy as in normal animals (Kommerell, 1929). Blood lipids are high (Schmidt and Hughes, 1938; Lawson, Fleischmann and Block, 1941a; Entenman, Chaikoff and Reichert, 1942) and vitamin A is poorly utilized (Patek and Haid, 1941; Lerman, 1941). The ability to transform carotene into vitamin A is also impaired (Salter, 1940).

2. Growth.--If thyroid deficiency develops early in life the skeleton is also affected in a striking manner. The long bones are shorter and frequently more dense than

normal (Aub et al. 1927). There is a marked delay in appearance of the ossification centers and in epiphyseal unions, resulting in a subnormal "bone age". If the deficiency develops after growth has been attained, skeletal derangements are not necessarily noticeable but myxedema, along with mental derangement, rough, dry skin, and course hair, are soon manifest (Kocher, 1883; Reverdin and Reverdin, 1883).

The features of hypothyroidism in animals are analogous to those observed in humans. Schiff (1856) first thyroidectomized dogs and a few lived long enough to develop conditions resembling myxedema in humans. Dott (1923) thyroidectomized dogs, taking precautions to leave the parathyroids intact, and kept the animals alive for long periods of time. They grew subnormally, assumed a dull, lethargic appearance, and skeletal development was markedly reduced as shown by radiograms. Similar results with dogs were reported by Binswanger (1936) from an extensive study of thyroidectomy of young puppies.

In vitro studies of Maeda (1927) indicated that thyroidectomy resulted in a lowered oxygen consumption of all tissue. Hammett (1926) expressed the belief that thyroid deficiency resulted in decreased size of the cells, but had no good evidence in support of his theory. The work of Von Haam and Cappel (1940) with heart tissue in vitro showed that the rate of cell division of heart tissue is accelerated by thyroid treatment.

Activity of the growth zones of the bones is clearly

depressed by hypothyroidism. Dott (1923) concluded that epiphyseal activity in the thyroidectomized dog was reduced by 81 per cent as judged by radiograms of the ends of the long bones. He reported that there were no degenerative changes which took place and that the result was simply an arrest of cell activity. To all appearances the bones remained in much the same state as those of younger dogs. Boettiger and Osborn (1938) likewise found the epiphyseal picture of seventy day old dwarf mice to be much the same as twenty-five to thirty day old normals. Thyroid treatment restored the picture to normal. Todd, Wharton and Todd (1938) made an extensive study of skeletal maturation of sheep thyroidectomized at one to five months of age. They found deficient growth and modelling of the epiphysis and when ossification was as completed as would be possible in operated animals, the epiphysis was abnormal in character and was inadequate to cap the growing end of the shaft. The long bones were short due to decreased velocity of growth, and duration of growth was not extended to compensate for this deficiency. The authors stated that the skeletal proportions were similar to those of primitive wild sheep. Laqueur, Dingemanse and Freud (1941) observed similar derangements in bones of young rats thyroidectomized at an early age. The detailed histological studies of Silberberg and Silberberg (1940) on guinea pigs and of Becks et al. (1942) on rats have shown that thyroidectomy results in virtually a cessation of proliferation of the

epiphyseal cartilage. Thus the evidence is clear that hypothyroidism suppresses bone growth.

Topper and Cohen (1928) fed thyroid to children who showed no gross signs of hypothyroidism. They observed no increase in metabolic rate but a spurt in growth occurred. Dorff (1935) administered small amounts of thyroid to growing children and obtained results similar to those of Topper and Cohen. To explain the response of children with apparently normal thyroid function, he postulated the condition of "masked hypothyroidism". Molitch and Poliakoff (1938) treated forty-three boys of subnormal stature, but with no other signs of hypothyroidism, with thyroid alone or in combination with pituitary extract. One grain of thyroid per day resulted in an average growth of 1.25 inches in six months as compared to 0.75 inches for untreated boys. Variation within the groups cast doubt on the significance of the results, but they were suggestive. Wilkins (1940) has stated in a general discussion of thyroid medication of children that growth is more rapid when hypothyroid cases are made slightly hyperthyroid than when metabolism is merely raised to normal.

The above reports have all come from treating children with some evidence of retarded growth and no results have been reported with strictly normal children. Hertz and Galli-Mainini (1941) and Reilly (1942), however, have presented evidence that a cardinal symptom of hyperthyroidism of children is rapid skeletal growth during childhood and

adolescence but that maximum stature is no greater than that of normal individuals. Body weight was not mentioned but would presumably be somewhat below normal due to thin condition. Rapid skeletal growth of dogs fed fresh thyroid has been observed by Moussu (1899).

Johnston and Maroney (1939a) and Johnston (1941) have studied the effect of mild thyroid treatment on nitrogen and calcium retention of children. In certain cases they have observed greater retention of both elements when small amounts of thyroid were administered to children with normal metabolic rates. Calcium retention was influenced to a greater extent than nitrogen. They have stressed the point, however, that if the metabolic rate is raised much above normal, decreased retention, especially of nitrogen, will occur. Nitrogen excretion was usually increased by thyroid medication but was compensated for by increased food intake.

Moussu (1899) reasoned that if small amounts of thyroid were essential for normal growth, would not a little extra thyroid result in growth above normal? He used paired littermate dogs and cats and fed small amounts of fresh horse thyroid to one of each pair. He concluded that thyroid in small amounts always resulted in faster growth in young dogs. They became less fat, however, and never reached more than normal stature. He used height as an index of growth and stressed the finding of long limbs. He did not mention the cat in his results. Dott (1923) confirmed Moussu's report of rapid growth of dogs following thyroid medication. He

reported that growth stopped prematurely and that the dogs never reached the maximum stature of the controls.

Bircher (1910) fed rats small amounts of thyroid and reported accelerated skeletal growth but body weight was slightly below normal. Schafer (1912) fed small amounts of thyroid to growing rats and observed increased rate of growth and feed intake of females. The males were apparently unaffected. Herring (1917) fed 0.1 to 0.2 gm. of fresh sheep thyroid to female rats. On the average, growth rate was not greatly affected. Some animals grew rapidly and to a large size while others were retarded. Hoskins (1916) fed 10 mg. daily of dry thyroid to growing rats and from his observations stated that "the treated animals averaged slightly heavier than controls but the difference is perhaps too slight to be significant. If the loss in fat of the thyroid fed animals be taken into account, an increased weight of the remainder of the body appears." Dulzetto (1928) injected twenty to twenty-five day old animals with a thyroid extract (not described) and obtained slight, but probably insignificant, gain above controls. Evans, Simpson and Pencharz (1939) injected small quantities of thyroxine into rats and showed growth curves of treated rats that were slightly above those of controls, but the differences were insignificant.

The effect of mild thyroid treatment on growth of mice was studied by Robertson (1928). In a series of trials he found that both males and females responded to feeding of

1.9 mg. fresh thyroid per day by increased rate of growth early in life. The animals reached the same maximum weight as controls but obtained this weight in a shorter period of time. Mean life duration was reduced by about fifteen weeks or to about 85 per cent the life span of controls.

Evidence has been presented that the growth rate of chicks during early life can be increased to a limited extent by feeding small amounts of thyroid while feathering is markedly improved (Parker, 1943; Irwin, Reineke and Turner, 1943).

There are several theoretical considerations suggesting that an active thyroid state is associated with rapid growth: (a) The thyrotrophic potency of the anterior pituitary is highest during the period of rapid growth of rats (Turner and Cupps, 1940), rabbits (Bergman and Turner, 1941), cattle (Reece and Turner, 1939) and swine (Elijah and Turner, 1942). (b) In rats, a species wherein the male grows more rapidly than the female, the thyrotrophic potency of the anterior pituitary is higher in the male than in the female (Turner and Cupps, 1940), whereas the thyrotrophic potency of the two sexes is similar in rabbits, a species in which the growth rate of the two sexes is similar (Bergman and Turner, 1941). (c) The pituitaries of slow-growing strains of swine have been reported to be lower in thyrotrophic potency than those of faster growing strains (Elijah and Turner, 1942).

3. Appetite and nutrition.--Feed intake is low in hypothyroid animals including man (Kojimi, 1917; Kunde, 1926; Johnston and Maroney, 1939b; Richter, 1933), and as would be expected, nitrogen retention is decreased below that of normal. The efficiency of utilization of nitrogen by hypothyroid animals is not established. Protein requirement is low in hypothyroidism and nitrogen excretion is usually lower than in normal individuals (Kendall, 1929). Nitrogen retention was increased in others but was accounted for by increased nitrogen ingestion.

Zanasi (1934) first reported that mild thyroid treatment stimulated gastric motility in laboratory animals. He also found that several other hormones caused a similar response and attached little significance to this property of thyroid materials. Increased peristalsis of stomach and intestines has been reported also by Rossiiskii (1937), Althausen (1939), Morrison, Samuel and Feldman (1939, 1940) and Castleton and Alvarez (1941).

Eidinova (1936) fed small amounts of thyroid to dogs and by means of Pavlov and Heidenhain pouches determined that the flow of gastric juices was increased above normal and that the "digestive power" of the juices was also increased. Overdoses of thyroid inhibited gastric flow.

Most investigators have reported increased nitrogen excretion upon thyroid administration. This finding has been universal in treatment of myxedema patients and represents nitrogen lost in connection with diuresis. At the

same time the fluids in the tissues are reduced and the urine nitrogen is thought to indicate a loss of "deposit protein" (Lorman, 1941; Harington, 1933). This increased excretion of nitrogen disappears upon continued treatment. Similarly, a smaller amount of nitrogen may be lost upon administration of thyroid to normal individuals. Rudinger (1908) stressed the point that increased nitrogen excretion did not occur in states of moderate hyperthyroidism provided sufficient carbohydrate is available to meet the energy requirements of the increased metabolism. Thus, Weymuller, Wyatt and Levine (1932) administered thyroid to well-fed infants and observed no increase in nitrogen excretion and in some cases excretion was actually decreased. Johnston and Maroney (1939b) have shown that small amounts of thyroid administered to children lead to increased nitrogen retention so long as metabolism is not raised much above normal. The above reports are not evidence of beneficial effects of mild hyperthyroidism, but do emphasize the fact that ample thyroid activity is essential for optimal nitrogen retention.

Terroine and Babad (1939) reported increased retention of nitrogen by rats even though thyroxine was given in amounts to cause an actual loss in weight. Marx et al. (1942) have presented evidence that rats receiving "purified thyrotrophic" preparations stored more nitrogen than controls but failed to note a similar retention after short periods of thyroxin injection. It is of interest that

Zitowskaya (1939) found that livers of thyroid-treated dogs had the ability to synthesize amino acid from pyruvic acid and ammonia *in vitro* at a markedly faster rate than livers of control dogs.

There have been no reports of increased calcium and phosphorus balance due to thyroid treatment of normal humans or experimental animals. However, thyroid medication of hospital patients with apparently normal thyroid function has led to improved calcium balances (Silvestri and Tossati, 1907; Johnston, 1941). Again Johnston has emphasized the point of keeping the metabolic rate near to normal if increased balances are to be expected. The finding of increased skeletal growth of dogs, mice and children in states of mild hyperthyroidism would suggest that the condition is not unfavorable for calcium and phosphorus retention.

4. Vitamin relationships.--The literature has been reviewed recently citing numerous reports of an "antagonism" between thyroid hormone and vitamins A, C and the B complex (Korenchevsky, Hall and Clapham, 1943). This supposed "antagonism" has been assumed to explain the finding that hyperthyroidism is relieved to a limited extent by vitamin therapy and that thyroid treatment exaggerates vitamin deficiencies. Korenchevsky et al. (1943) interpreted these findings, logically it seems, to indicate that vitamin requirements are increased by thyroid treatment due to increased metabolism. This suggestion is in keeping with the well-established fact

that the vitamin B_1 requirement is proportional to the rate of metabolism of the tissues (Drill, 1938).

Korenchevsky, Hall and Clapham (1943) fed rats on a normal ration and found that mild thyroid treatment did not greatly affect the animals while larger doses of thyroid were toxic. Fortifying the ration with cod liver oil, crystalline vitamins C and B and yeast extract relieved the toxic effects. With a vitamin deficient ration which barely supported control animals, mild thyroid treatment was toxic. Addition of crystalline vitamins relieved the toxic symptoms completely.

Thus, it seems well established that thyroid treatment increases the requirements of most vitamins. It would appear logical to assume that many of the contradictory reports on the effects of small amounts of thyroid can be explained on the basis of failure to recognize this fact.

Effects upon the Endocrine System

1. Pituitary.--The available evidence indicates that the weight, and to a greater extent, the activity, of the thyroid gland is depressed by thyroid administration (Cameron and Carmichael, 1920; Hewitt, 1920; Kamiovski, 1938; Azerad et al., 1939; Irwin, Reineke and Turner, 1943; Korenchevsky, Hall and Clapham, 1943).

A few reports concerning the effects of thyroid feeding on the pituitary are not in complete agreement. It seems well established that the basophilic cells are increased while the relative number of acidophils are reduced (Severinghaus et al., 1934; Campbell and Wolfe, 1934). These observations were made following large doses of thyroid.

Most reports indicate that the weight of the pituitary is decreased by thyroid feeding (Herring, 1917; Campbell and Wolfe, 1934; Evans and Simpson, 1930). However, Hoskins, (1916), Herring, (1917) and Cohen (1935), working with small numbers of animals, reported increased weight of pituitaries of male rats following thyroid feeding. Irwin, Reineke and Turner (1943) observed a slight reduction in pituitary weight of male chicks due to feeding small amounts of thyroid while the effect in females was inconclusive. Koger and Turner (1943) report that the size of the pituitaries of male rats was not affected by the levels of thyroprotein used in their trials. There was a slight indication of depression of the size of the pituitary in females due to

thyroprotein feeding.

Reineke, Bergman and Turner (1941) and, Stein and Lisle (1942) have reviewed the available evidence concerning the effects of thyroidectomy on the pituitary hormones. The reports concerning thyrotrophin and lactogen are inconclusive and no very marked departure from normal concentrations has been reported. Reports agree that gonadotrophin is reduced in hypothyroidism. Reineke et al. (1941) reported that the pituitaries from thyroidectomized goats showed a marked decrease in the factor which elevates blood sugar and in gonadotrophin, while lactogen and thyrotrophin were unchanged.

It would be expected that the thyrotrophic potency of the pituitary would be reduced following thyroid treatment and Kuchinsky (1933) and Kohlweg and Junkmann (1933) have reported this to be the case. There is indirect evidence also that hyperthyroidism results in decreased thyrotrophin secretion. The stimulation of thyroid glands of test animals by injection of thyrotrophic hormone is reduced by simultaneous injection of thyroxine or feeding of thyroid (Aron et al., 1931; Loeser and Thompson, 1941). Loeb and Seibert (1930) fed thyroid to partially thyroidectomized guinea pigs and reported that the thyroid remnant failed to hypertrophy and that the acinar epithelium was low and the acini filled with hard colloid. A decrease in thyrotrophic hormone content of blood and urine has been reported in thyrotoxicosis (Cope, 1938), although the evidence

is not conclusive. Reforzo-Membrives (1943) has reported a thyroid-inhibiting principle in pituitaries from animals receiving thyroid treatment. He injected normal and hypophysectomized guinea pigs with pituitaries from thyroid-fed rats and reported that the weight of the thyroid glands and metabolism of these guinea pigs were reduced below those of control animals.

Increased gonadotrophic potency of the pituitary due to thyroid feeding has been reported by Evans and Simpson (1930), Van Horn (1933) and Cohen (1935). Smelser (1939), reported that experimental hyperthyroidism did not affect the gonadotrophic hormone in the blood of castrate rats.

Reece and Turner (1937) found that injection of 0.01 mg. thyroxine daily into rats produced no significant change in pituitary lactogen, while larger doses which were toxic caused a marked depression. Hurst et al. (1941) injected thyroxine into goats in amounts which increased milk flow, but observed no change in the lactogenic hormone excreted in the urine.

2. Thyroid.--Koger and Turner (1943) report that differences in size of the thyroids of different groups of animals were within limits of experimental error due to the great variability of size of the gland within groups of similar treatment. However, there was a tendency for the thyroids of treated animals to be slightly lighter than those of controls, especially in females. Histological

examination of the glands showed the thyroids of all treated animals to be inactive and filled with colloid.

3. Adrenals.--Marked hypertrophy of the adrenals occurs in both sexes of rats due to thyroid treatment. The extent of hypertrophy parallels roughly the level of thyroprotein fed. This response confirms numerous reports in the literature (Koger and Turner, 1943).

4. Ovary.--Ovarian function is abnormal in both hypo- and severe hyperthyroidism. Kraatz (1939) reported that brief treatment of virgin female rats with thyroid previous to mating, resulted in increased litter size if thyroid treatment was carried out in a cool environment. Treatment during hot weather reduced the litter size. Herring (1917) and Korenchevsky, Hall and Clapham (1943) reported hypertrophy of the ovaries of female rats due to thyroid treatment. Luteinization was pronounced in most cases. Weichert and Boyd (1933) have reported that typical pseudo-pregnancy was produced in rats by feeding dried thyroid. Kamlovsky (1938) fed large amounts of thyroid to rats and reported a depression of ovarian weight.

Koger and Turner (1943) report that feeding thyroprotein at the levels of 0.01, 0.02 and 0.04 per cent of the ration to female rats did not apparently affect the weight of the ovaries. Feeding at the levels of 0.08 and 0.16 caused an increased weight of the ovaries with histological evidence of intense luteinization. This observation agrees with

certain reports in the literature. Feeding thyroprotein at the level of 0.32 per cent of the ration, an extremely toxic dosage, resulted in ovaries somewhat lighter in weight than in the controls. The ovaries were depressed on all levels of treatment. Sexual immaturity may possibly account for this result since the treated animals were smaller in size.

5. Pancreas.--The pancreas has been reported also to undergo hypertrophy following thyroid treatment. Kojimi (1917) reported that the islet tissue of the pancreas underwent hypertrophy as judged by increased numbers of mitotic figures in pancreas from animals which had undergone thyroid treatment. Florentin and Wolff (1910) confirmed the report of Kojimi. Cameron and Carmichel (1920) reported that the weight of rabbit pancreas was increased by thyroid feeding and cited several earlier reports confirming this response. Fraenkel-Conrat et al. (1942) reported an increased insulin content of normal rat pancreas following thyroxine injection. The insulin content of pancreas of hypophysectomized rats was decreased by the same treatment.

Effects upon other Physiological Functions

1. Heart, liver and kidney.--Kojimi (1917) thyroidectomized growing rats and observed retarded growth, reduced calcium and nitrogen retention, and decreased feed intake. Hammett (1927) observed like effects and also reported that both relative and absolute weights of the heart, liver, lungs, kidneys, and spleen were below those of normal controls. Salmon (1938a) reviewed the literature indicating that thyroidectomy of young animals results in more acute symptoms of hypothyroidism than if the operation is performed on older animals. She thyroidectomized rats at birth and observed early growth stasis with such animals reaching a maximum weight of about 30 gm. Replacement therapy started at time of operation resulted in practically normal growth, whereas therapy begun later was ineffective.

Simpson (1924) thyroidectomized one each of seventeen pairs of twin lambs and left their mates as controls. When the operation was performed two or three weeks after birth, marked stunting resulted with the controls reaching a weight three times that of the operated animals. The experimental animals showed the usual short legs, short dished face, slipped wool, and "pot belly". If operation was delayed until the animal was three or four months of age, retardation of growth was only slight.

Similar symptoms of hypothyroidism have been recorded for a great number of animals including the mouse (Davenport and Swingle, 1927), the guinea pig (Silberberg and Silberberg,

1940; Williams et al., 1941), the cat (Dott, 1923), the rabbit (Kurde, 1926; Basinger, 1916), the goat (Simpson, 1928; Reineke and Turner, 1941a), the cow (Brody and Frankenbach, 1942), and the monkey (Fleischmann, Schumacker and Straus, 1943).

Hypertrophy of the heart, liver, spleen, kidneys and adrenals following administration of thyroid to experimental animals is well known (Hoskins, 1916; Herring, 1917; Hewitt, 1920; Cameron and Carmichael, 1920; Addis et al., 1938; Sternheimer, 1939; Korenchevsky, Hall and Clapham, 1943). This finding has been almost universal among experimenters and apparently, large amounts of thyroid material are not required to elicit this response. The increase in weight of these organs appears to be due to true growth since it is accompanied by increased nitrogen content of the tissues and increased mitotic figures (Addis et al., 1938; Sternheimer, 1938).

Both absolute and relative weight of the heart, liver and kidneys was increased due to thyroprotein feeding of male and female rats, according to Koger and Turner (1943). The degree of enlargement paralleled the amount of thyroprotein fed. With the smallest dosages in males, no noticeable effect occurred.

It is well known that thyroid produces enlargement of the heart, liver and kidneys, and the data from these experiments bear out the fact. On the other hand, the lowest dosages given to male rats did not bring about this response. (Koger and Turner, 1943).

The lowest levels of thyroprotein treatment given male rats apparently did not affect the weight of any of the glands or organs weighed. Larger amounts in either sex resulted in hypertrophy of the heart, liver and kidneys. The adrenals were enlarged in most cases, but males failed to show this response. The pituitary weight of females was depressed by thyroprotein treatment, while in males, pituitary size was unaffected. The weight of the thyroid was not significantly altered, but there was a tendency for thyroids of treated animals to be reduced in size and they showed histological evidence of inactivity. The ovaries were enlarged by moderate levels of thyroprotein while larger dosages caused a depression. Moderate amounts of thyroprotein did not affect testes weight, while large amounts caused a reduced weight. The thymus was enlarged in thyroprotein-fed females, but was unaffected in the other animals.

The effects of thyroprotein feeding on organ weights are variable with dosage, sex and strain of animals.

2. Circulation.--It is well known that oxygen uptake is low in hypothyroidism. Blood flow is reduced and anemia is usually present (Kendall, 1929; Abramson and Sidney, 1942).

The effect on endogenous metabolism of nitrogen has not been studied extensively. Palladin and Savrow (1927) reported that thyroidectomy does not change the excretion

of creatinine. Allison and Leonard (1911), working with thyroidectomized rats, observed a decrease in creatine excretion with no change in creatinine. Glaser (1912) thyroidectomized rats with like results and found further that the operated animals had a greater tolerance for injected creatine. Since the injected creatine could not be accounted for by changes in creatinine excretion or by storage in tissues they suggested that the thyroid exerts some unknown influence on creatine metabolism.

Silvestri and Tossati (1907) also reported reduced excretion of calcium following administration of thyroid to patients with low metabolic rates. It is clear that total calcium retention is low in hypothyroidism and the available evidence indicates that utilization of available minerals is lower than normal. Thus, Breitbarth (1910) has reported that calcium excretion of thyroidectomized dogs was lowered by administration of small amounts of thyroid. At the same time calcium intake was increased.

3. Water, salt and colloids.--When the organism is deprived of the thyroid, there takes place storage of water, salts and protein. In 1925, Boothby and associates showed that in human myxedema a large amount of extra protein -- so-called "deposit protein" -- is stored in the body. It is contained in the body fluids and not in the cell protoplasm. When thyroid is administered, this extra deposit protein is quickly oxidized and eliminated in the urine along with the

extra salts and water held in combination with the protein. Consequently, an appreciable diuresis is a characteristic finding when active hormone is administered to myxedematous patients for the first time. In fact, the diuretic action of thyroid may also be striking in normal persons, and is made use of in the treatment of nephrosis. The work of Byrom (1934) indicates that the diuresis produced in patients with myxedema is accompanied by a loss chiefly of sodium salts, whereas in normal persons the loss is chiefly of potassium salts. Consequently, in the former the fluids are derived largely from extracellular sources whereas in the latter the fluids are derived largely from intracellular sources. Byrom has suggested that the abnormally collected protein in myxedema is in the nature of a mucoprotein derived from the ground substance of the cell. Since fetal tissue, like myxedematous tissue, contains an excess of mucin, it seems that one function of the thyroid is to provide the cells with a "mature" type of environment.

Soon after Boothby's work became known, Thompson (1926) described a significant reduction in plasma volume in myxedema and a return to normal on treatment with thyroid. These results have been confirmed by Gibson and Harris (1939), who made the additional finding that the blood volume in thyrotoxicosis tends to be above normal. Along with the reduced plasma volume there is an increased concentration of plasma protein, with a corresponding increase in spinal fluid protein (Thompson et al., 1929).

On administration of thyroid both the plasma and the spinal fluid revert to normal. In thyroidectomized animals the changes in the blood are similar to those in myxedema.

The metabolism of various inorganic salts is bound up with the state of thyroid activity. Aub and collaborators (1927) showed that in hypothyroidism there is a diminished rate of exchange of calcium and phosphorus, the amounts eliminated in the urine and stool being less than in the normal person. The actual concentrations in the blood are not greatly altered. The reverse holds when thyroid is administered and in spontaneous hyperthyroidism. These effects, according to Low, Wilson and Aub (1934), are not brought about by changes in phosphatase activity in the bones. Talbot (1939), however, described low phosphatase in children with untreated hypothyroidism, associated with delayed osseous development. Thyroid treatment repairs these abnormalities. In the growth period the effect of thyroid on calcium metabolism may be different from that in later life. Thus Maroney and Johnston (1938) found that retention of calcium and nitrogen was increased by the administration of thyroid to a cretin and to an adolescent after thyroidectomy. When administered in large doses thyroid may actually lead to premature cessation or retardation of growth (Smith and McLean, 1938).

h. Integument and hair growth.--The literature is in agreement that hypothyroidism, whether induced or spon-

taneous, is detrimental to growth. Curling (1850) first associated the absence of the thyroid with the characteristic condition known as cretinism in humans. In such individuals body growth in general is subnormal with characteristic disproportions of the body. The individual is extremely short with coarse features, dry, rough skin and hair, and usually a typical "pot belly". There is an accumulation of fluid in the intercellular spaces of the body giving the characteristic myxedema of hypothyroidism (Lerman, 1941).

In addition to subnormal growth, hypothyroidism causes a depression of other processes such as lactation (Graham, 1934; Preheim, 1940), egg production (Winchester, 1939) and growth of hair (Chang, 1926). Thus, hypothyroidism appears to be unfavorable to all the "anabolic" processes.

Hair and feather growth have also been shown to be affected. Chang (1926) reported that undernourished rats showed a retardation of hair growth. Thyroid fed to such animals kept on a semi-starvation diet improved hair growth in spite of further decreased body weight due to treatment of the originally deficient animals.

5. Lactation.--It has been well established that treatment of cows or goats with moderate amounts of thyroid will increase milk and fat production for short periods of time (Graham, 1934; Ralston et al., 1940; Reineke and Turner, 1942).

Toxicity in Relation to Dosage and Species

The effects of severe hyperthyroidism are well known and will be mentioned only briefly here. Thyroid in large amounts is toxic, resulting in loss of nitrogen, extreme emaciation and hyperirritability. Given in large enough quantities, the unusual finding of reduced oxygen consumption and feed intake may be encountered (Kojimi, 1917), followed quickly by death. Usually, however, food intake and gaseous metabolism are increased.

Work with experimental animals indicates that uncomplicated hyperthyroidism does not lead to decalcification of bone. Calcium retention, however, is low or nil (Drill, 1941; Smith and McLean, 1938). Since excessive hyperthyroidism is toxic, the reactions of an organism to the conditions are nonspecific and a study of the severe hyperthyroid state actually contributes little to the understanding of normal thyroid physiology.

Most of the experiments that have been reported have dealt with relatively large doses of thyroid material. Parhon (1912) early emphasized the point that dosage largely determines the effect to be expected and suggested that a small amount is "anabolic", whereas a larger amount is "katabolic". Still most workers failed to appreciate thyroid potency and in many cases worked with severe hyperthyroidism. Aub and coworkers (1927) actually criticized early work that had shown small amounts of thyroid to cause an increase in calcium retention (Silvestri and Tossati, 1907) because dosage was "too low".

Summary - Thyroactive Substances

The review of the literature indicates a concensus of opinion upon the following effects produced by the administration of thyroactive substances. In general, it is agreed that these substances may cause:

1. An increase in growth.
2. An increase in metabolism.
3. An increase in food and water intake.
4. A loss in fat.
5. An increase in pituitary growth hormone secretion.
6. An increase in peristalsis.
7. An increase in flow of gastric juice.
8. Diuresis and reduction of tissue fluids.
9. A loss or increase in body weight, depending on dosage.
10. An increased requirement of vitamins B and C.
11. A decrease in weight and activity of the thyroid gland.
12. A decrease in pituitary weight.
13. A decrease in thyrotrophic hormone secretion.
14. An increase in gonadotrophic hormone secretion. ?
15. An increase in milk output with no increase in lactogenic hormone secretion.
16. Hypertrophy of the ovaries, except with excessive doses. ?
17. Hypertrophy of the pancreas and increase of insulin secretion.

18. Hypertrophy of the heart, liver, spleen, kidneys and adrenals.

19. An increase in protein and carbohydrate oxidation.

20. An increase in salt and water excretion.

21. An increase in plasma volume.

22. An increase or decrease (depending upon dosage) in retention of calcium, phosphorus and nitrogen.

23. An improvement of skin, hair and feather growth.

24. "Anabolic" effects in small doses and "katabolic" effects in high doses.

One must bear in mind that the foregoing effects were accomplished over a wide range of dosage and under varying experimental conditions.

CHAPTER V

THYROID-OVARIAN RELATIONSHIPS, WITH PARTICULAR REFERENCE TO THE RECIPROCAL EFFECTS OF DIETHYLSTILBESTROL AND THYROPROTEIN

Chu and Yon (1945) present evidence that in the rabbit, the secretion of the follicle stimulating hormones are under the direct regulation of the thyroid which mediates the effects of estrogen. Nelson (1942) reports that estrin will correct the basophilism which occurs in the hypophysis of thyroidectomized rats.

Kochakian (1946) reports that steroid hormones produced greater sensitivity to thyroid treatment. Korenchevsky and Hall (1941) suggest that when both thyroid and estrogen are given, the two drugs produce a greater loss of body weight than would either administered individually.

Sherwood (1938) however, believes that estrogen reduces the duration of thyroid intoxication of rats.

Reece and Leonard (1941b) claim that estrogens do not affect the secretion of thyrotrophic hormone.

Biskind (1946) claims that excessive dosages of estrogen produced a low metabolic rate which is a reflection of a diminution of the secretion of the thyrotrophic principle. He substantiates this by pointing out that administration of estrogen to hyperthyroid individuals leads to diminution in the basal metabolic rate.

Brobeck et al. (1947) present some experimental

evidence to indicate that the regulatory mechanism for the thyroid-ovarian relationship might be found in the hypothalamus.

Sadhu (1947) suggests the possibility that thyroid activity decreases, after administration of estrogen, as a result of the accumulation of excess vitamin A, the inference being that the estrogen interferes with the normal oxidation of the vitamin. On this basis he suggests that there would be a decrease in metabolic rate and thyroid size because thyroxine iodine is taken up by the double bond of the vitamin A. The resulting iodinated vitamin A acts like thyroxine to depress pituitary thyrotrophic hormone secretion, thereby reducing the thyroid size. However, there is no experimental proof of this.

Lipsett and Winzer (1947) in a report on the effects of vitamin A deficiency on thyroid function studied with radio-active Iodine concluded that: a) in vitamin A deficiency, the thyroid glands of the rat were heavier; b) the thyroid showed distended follicles as well as some degeneration; c) that despite the increase in the size of the thyroid, the total I^{131} uptake was normal; and d) vitamin A deficiency decreased the rate of formation of thyroxine. These results indicate that iodine metabolism is abnormal in the vitamin A deficient rat.

Shilling and Laqueur (1941) report that neither castration nor subsequent replacement with estrone affected thyroid weights. Progesterone, however, when injected into

castrated animals, both alone and simultaneously with estrone resulted in a decrease in weight of a like order. Thus the effect was due to progesterone and not estrone, which is confirmed by the fact that the thyroids of castrated animals treated with progesterone alone showed a significant decrease in weight when compared to untreated castrates.

Blumenthal and Loeb (1942) believe that if nothing else would inhibit the thyroid, the mere effect of decrease in food intake resulting from estrogen administration would produce a marked diminution of mitotic activity in the thyroid gland.

Aron and Benoit (1924) believe that a direct inhibitory effect of estrogen upon the thyroid gland is implied in the observation that it prevents the thyroid hyperplasia and hyperactivity which would otherwise follow the administration of anterior pituitary thyrotrophic extract.

Meyer, Thewlis and Rusch (1940) contend that the effects upon erythropoiesis is conclusive evidence that the estrogens suppress thyroid activity.

Sherwood (1938) suggests that the effect of estrogens on the basal metabolic rate is at least partly independent of the thyroid gland, since thyroidectomized animals receiving thyroid substance followed by estrogen return more rapidly to their previous hypothyroid level than those given thyroid substance alone. This contention is supported by Laprida (1933) in an earlier work which indicated similar conclusions.

Sherwood (1936) concludes that in the lower animals, large doses of estrogen or prolonged treatment depresses the thyroid gland. In a previous experiment by Pincus and Werthessen (1933) they observed that injections of estrogens for five to ten days lead to thyroid enlargement; but when administered for longer periods, estrogen produced thyroid involution.

Fleischmann (1946) notes that the inhibitory effect of thyroxine is confined to metabolic changes, whereas the structural changes brought about by estrogen are not affected. This involved a study on the effect of thyroxine on estrogen-induced changes in fowl.

In a study of the effect of the level of thyroid activity upon the response of ovariectomized rats to estrone, Wright and Gustavson (1947) found that the weight loss and decrease in sensitivity to estrone appeared to be logarithmic functions of the thyroxine dose.

Emge and Laqueur (1941) report that increased metabolism incident to the feeding of thyroid substances, increased the speed of estrogen elimination. Also, that the demand for estrin rises with the increased supply of thyroid hormone and conversely, withdrawal of ovarian hormones produced a decrease in thyroid activity. They contend that pituitary gonadotrophin can be depressed when thyroid activity is increased, by the administration of thyroxine or desiccated thyroid.

Chouke and Blumenthal (1942) report that in female

guinea pigs during the sexual cycle, the proliferative activity of the thyroid gland is greater than in male animals of corresponding weight and age.

Brobeck, Wheatland and Strominger (1947) conclude that in estrus the rat spends more energy than it takes in; while in diestrus and pseudo-pregnancy it takes in more than it spends. They believe that this balanced mechanism is controlled by the thyroid and ovary via the pituitary.

McDonald, Riddle and Smith (1945) report that thyroxine prevented the marked increase of plasma neutral fat which follows the use of estrogen alone. Also, that thyroxine inhibited the estrogen-induced increase in plasma calcium, inorganic phosphorus, protein phosphorus, lipid phosphorus; and it did not inhibit the ability of estrogen to promote growth of the oviduct.

Astwood, Bissell and Hughes (1945) found that generally there are two types of chemical structures which inhibit thyroid activity. The more active class possessed a thiocarbonamide grouping; the less active an aminobenzene group.

Selle and Selle (1948) report that the administration of diethylstilbestrol to chicks that received thiouracil for forty-three days had no effect upon the weight of the thyroids per kilogram of body weight. Following the withdrawal of the thiouracil, either with or without stilbestrol, the males recovered completely. In the females there was a lag in the recovery from the effects of the thiouracil;

and those that had received stilbestrol in addition, showed a slightly greater recovery than those that had not.

Mahaux (1944) reports that administration of large doses of estrogen caused a rapid fall in the basal metabolism. The calorigenic action of thyroxine was decreased in intensity and duration. The mechanism of this behavior is not explained. However, these results are corroborated by Sherwood (1940) who reported similar results. Further credence for this behavior is given by Gessler (1936); Sherwood (1936); and Janes (1942).

Summary - Thyroid-Ovarian Relationships

The review of the literature indicates a concensus of opinion upon the following effects produced as a result of the reciprocal action of estrogenic and thyroactive substances. In general, it is agreed that the reciprocal effects of these hormones may be:

1. That steroids produce greater sensitivity to thyroid.
2. That estrogen reduces the duration of thyroid intoxication. (While this contradicts the first statement, there is sufficient support to retain this controversy.)
3. That estrogens do not affect the secretion of thyrotrophic hormone.
4. That estrogens reduce metabolism by reduction in food intake.
5. That thyroid activity decreases after administration of estrogen due to excess accumulation of vitamin A.
6. That estrogen may have a direct inhibiting effect upon the thyroid.
7. That the metabolism-decreasing effect of estrogens is at least partly independent of the thyroid gland.
8. That the weight loss and decrease in sensitivity to estrone appears to be a logarithmic function of the thyroxine dose.
9. That increase in thyroxine produces an increase in speed of estrogen elimination.
10. That the demand for estrin rises with the increased supply of thyroid hormone; the reverse also being true.
11. That increased thyroid suppresses pituitary gonadotrophin.

12. That thyroxine inhibits estrogen-induced increases in plasma calcium, inorganic phosphorus, protein phosphorus and lipid phosphorus.

13. That estrogen decreases the calorigenic intensity and duration of effects produced by thyroxine.

Again, one must bear in mind that these effects were obtained under specified conditions. Therefore, it follows that an intelligent understanding of these results requires a knowledge of the circumstances under which they were produced.

CHAPTER VI

EXPERIMENTAL PROCEDURE AND DATA

In the following experiments all possible conditions were standardized so that the only variables were the drugs and their concentrations. All the mice used throughout were of a genetically well-established commercial strain. They were all of similar age and size; recently mature females 23 to 25 grams in weight. Their food consisted of a commercially standardized laboratory product providing a balanced normal diet. The food (Purina Laboratory chow) was in the form of finely ground meal and fed in a specially constructed tray to prevent spilling.

Water was available at all times from an inverted bottle which allowed of no spilling. Food and water was provided ad libitum and consumption was noted with regularity. Temperature and humidity were charted every day. However, these data have been omitted since the laboratory conditions varied so slightly as to make such data insignificant. A temperature of 22-24°C. was maintained by automatic devices designed for this purpose, while the humidity varied from 45 to 54 per cent.

The hormonally-active materials, in powder form, were thoroughly mixed into the feed to the desired dosage and uniformity was assured by extensive use of a mechanical mixer.

Data were recorded every twenty-four hours on the

amount of food taken, the amount of water consumed and the appearance and behavior of the experimental animals. Control groups were run with each experiment and comparisons noted. Despite artificial lighting, conditions of light and darkness were similar to that prevailing in the normal day. So far as is known, there were no undue changes in the physical environment which may have had any significance.

Animal weights were recorded every other day. Dead animals were removed as soon as discovered, to prevent being consumed by those remaining.

All groups consisted of ten matched mice in each cage. Aside from the confinement imposed by the cages, all the animals were ad libitum in regard to their normal habits.

The drugs used were commercial products of standard quality and determined potency. To insure uniformity, all drugs used came from the same lot.

In all the experiments, the animals were put on the experimental diet for fifteen days after a preliminary run of five days to determine the normal levels of consumption. In some instances a fifteen day recovery period was recorded after conclusion of the experimental treatment.

The Effects of Varying Concentrations of Protamone

The control animals consumed approximately 4 gms. of food per day per animal throughout the recorded period of thirty days. Water consumption remained at approximately 7 cc. per day for each animal.

The experimental animals were kept on the experimental diet for fifteen days and a recovery period of fifteen days was recorded subsequently.

The animals receiving .3 per cent Protamone showed a rise in food consumption after the sixth day and this rise continued steadily until the fifteenth day, when total intake reached 7.5 gms. of food per animal (Figure 1). The fact that the peak was reached three days after the Protamone was withdrawn may be explained on the basis of time lag evident when the drug was first administered. This lag seems to indicate that physiological adjustment to the drug is not very immediate. This is also borne out by the fact that the rise in water consumption did not occur until the sixth day and also reached its peak of 14.5 cc. per animal on the eighteenth day. However, this delay in response may be more apparent than real, since the weights of these animals began to show a marked increase on the second day. Animals weighing 25 gms. began to gain weight and continued to rise to a level of 28 gms. from the twelfth to the eighteenth days, after which there was a steady decline. These animals survived the entire period without fatalities.

The animals receiving .2 per cent Protamone showed a rise in food consumption after the sixth day until the seventeenth day when a peak of 6.5 gms. was recorded. Water intake exhibited a similar curve to that shown by the .3 per cent group, except that the curve rose to about 0.5 cc. less.

The weights of this group showed a rise similar to that of the .3 per cent group, except that the rise continued for five days beyond the experimental period and exceeded the peak of the .3 per cent group by approximately 0.5 gm.

Those receiving .1 per cent Protamone followed a similar curve to the preceding two groups, except that the rise in food consumption was approximately .4 gm. less than that attained by the .2 per cent group. The same was true for water consumption, with an intake of 1.2 cc. less than the .2 per cent group. Weight showed a rising curve similar to the .2 per cent group, except that the peak was 1.5 gms. less than the former.

Those receiving .05 per cent showed a similar curve to the others for food intake, except that the peak attained was 5.4 gms., or an amount proportionately less to the concentration of the drug; the same could be said of the water consumption. However, though the curve of weight increase paralleled the previous doses it continued to rise until the twentieth day and rose to a level of 29.3 gms. to exceed all the previous trials.

In the .025 per cent group, the rise in food intake was more immediate but at its peak it rose slightly higher than with the preceding stronger dose. The water intake closely paralleled this, in that it also rose quickly but at its peak was approximately equal to that observed with the preceding dose. The interesting observation noted at this point, was that the weight of this group at its peak exceeded that of all the previously treated groups.

It appears from the preceding, that food and water intake are in themselves no criterion as to weight. We note that those animals with the highest consumption of both did not experience the maximum increase in weight. This suggests that there is a point of diminishing returns, wherein the increase in metabolism induced by the Protamone exhibits a predominately "katabolic" effect in relation to the relative "anabolism" shown. From the standpoint of weight increase, one might conclude from this study that the .025 per cent concentration was the most effective dose. Also, as is apparent from the curves drawn on all of these groups, the effect of Protamone in all the experimental concentrations produced a corresponding increase in food and water intake and an increase in body weight; though it is notable that in this last respect the increase did not necessarily correspond to the Protamone dosage.

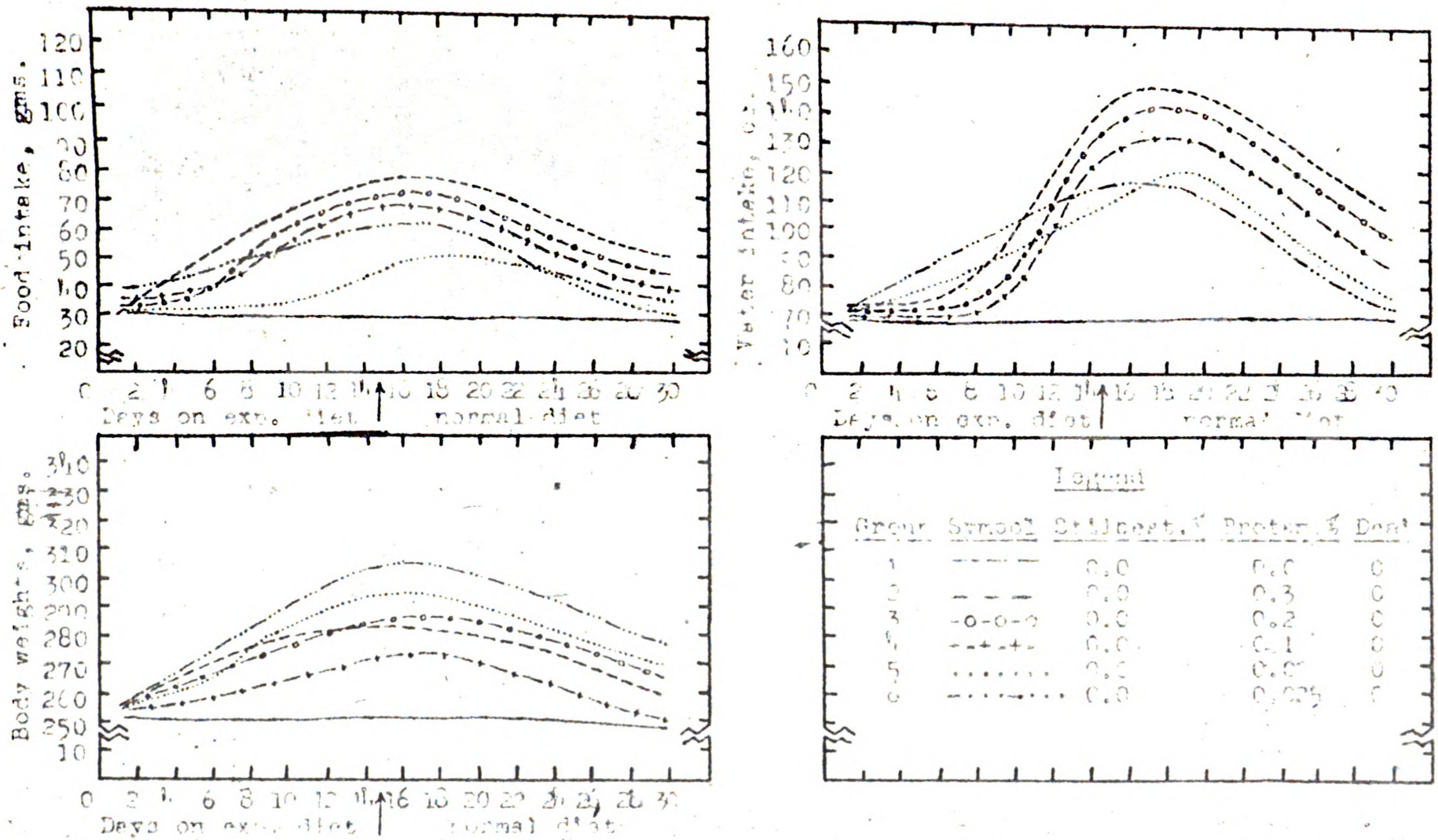

Figure 1. EFFECT OF VARYING CONCENTRATIONS OF PROTEINS

The Effects of Varying Concentrations of Stilbestrol

The control animals of this experiment indicated an average weight of slightly over 25 gms., an average food consumption of 40 gms., and an average water intake of 60 cc. daily (Figure 2).

Three groups of mice were used, each given .1 per cent, .50 per cent and .25 per cent stilbestrol respectively. In all instances the effect upon food intake was immediate and dropped in almost identical curves to a low of 1 gm. per animal daily at the fifteenth day. Recovery was not quite as rapid as the earlier decline but at the end of the fifteen day recovery period all the groups were fairly close to normal food consumption. However, it is notable that the group receiving .1 per cent recovered more rapidly and actually showed a slight increase in weight.

The water intake was somewhat different. While the food decrease was identical for all three doses, the water decrease was in indirect ratio to the concentration of hormone so that the .1 per cent group dropped to 5.1 cc. per animal, the .50 per cent dropped to 5.5 cc. and the .25 [illegible] cc. per animal daily. The body weights of these groups followed the same pattern with a drop to 17.5 gms. per animal for the .1 per cent group, 16.0 gms. for the .50 per cent group and 15.0 gms. for the .25 per cent group at the fifteenth day.

It is obvious from this study that stilbestrol has, in whole or in part, an inhibiting effect upon food and water intake resulting in a decrease in weight. The interesting point however is this seeming paradox -- that since food decrease was identical and since the drug obviously inhibits food and water intake, one would expect a direct relationship between dosage and decrease in both food and water consumption; yet the drop in food showed no relationship, direct or otherwise, to the dosages given. On the basis of this then, one would expect the water intake curves to respond similarly; yet oddly enough the drop in water intake is in inverse ratio to the concentration of the drug, as is also the decrease in body weight.

From the fact that there is no spread in the declining food consumption curve, one might surmise that this may be because the dosages used surpass the maximal range, therefore each concentration achieved maximal suppression.

The ratio between water and body weight loss can be explained logically as a direct one. Namely, the .1 per cent stilbestrol group suffered the least loss of water intake and consequently the least loss of body weight; the remaining groups following the same pattern. The question still remaining is why should this water-weight picture be in <u>inverse</u> relationship to the concentration of a

drug which is an established inhibitor? If we surmise that the drug has the ability to increase water retention, then this inverse ratio is logical; since the amount of water retention would be in direct proportion to drug concentration; it follows that the higher the concentration, the more water retained, and the more water retained, the less loss there will be in body weight.

This still leaves one dangling piece of illogic. If there is an increase in water retention, then why does the higher dose cause more water consumption? For lack of a more positive explanation one would like to postulate that, as a result of water retention there is also an increase in toxic accumulation and that this in turn may be the cause for the higher water intake with the higher drug dosage.

As in the experiments with Protamone no fatalities occurred throughout the recorded period. However, here the parallel ends, for while the Protemone treated mice remained active and healthy in appearance, those treated with stilbestrol soon became unkempt, inactive and emaciated. The degree of degeneration seemed to be in direct ratio to the dosage concentration. This may give further credence to the postulation expressed above.

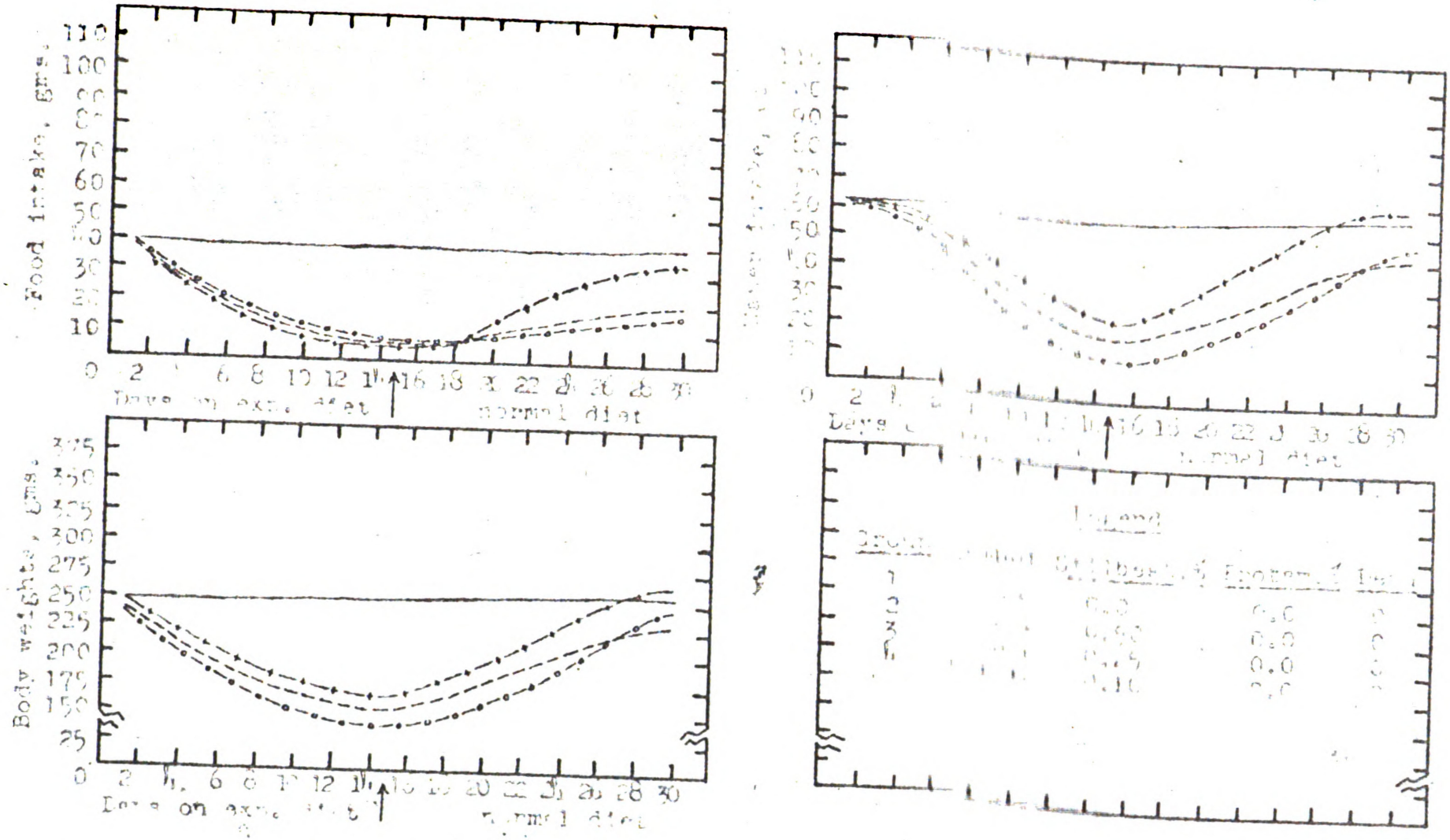

Figure 2. EFFECTS OF VARYING CONCENTRATIONS OF [illegible]

The Effects of Varying Concentrations of Protamone with a Fixed Concentration of Stilbestrol

In this experiment the animals of the three experimental groups were given .1 per cent stilbestrol in combination with .1 per cent, .2 per cent and .3 per cent Protamone respectively. This experiment was watched with particular interest since it was hoped that on the basis of the preceding experiments one might expect to see a reciprocal antagonism on the part of these two drugs.

From the standpoint of food intake all the groups showed similar curves to those produced earlier by stilbestrol alone, namely an immediate decrease (Figure 3). However, this continued until only the seventh day and then returned to normal. This behavior is similar to that observed in the study on Protamone alone; namely, that in regard to the food picture there is a six to seven day lapse before an increase is noted.

From the standpoint of water intake it is interesting to note that the inhibiting effect of stilbestrol was overcome, and that the effect of Protamone on water intake was the same as previously shown; namely, an immediate rise in intake. It is also worth noting that unlike stilbestrol, the relationship between Protamone concentration and water intake remains in direct proportion; the presence of stilbestrol notwithstanding.

From the effects already evidenced upon food intake, it was logical to expect all the groups to undergo

a decrease in body weight. This decrease continued until the ninth to tenth days when the Protamone effect observed at the seventh day in regard to food intake, began to be evidenced in body weight.

This situation poses several interesting questions. First, in regard to food, the curve seems to be a composite in that the stilbestrol effect was manifest first; followed as expected, by the opposite Protamone effect which seemed modified or to some extent "antagonized" by the stilbestrol. The body weight curves for all the groups seem to indicate this same relative effect. However, the curves for water intake show no stilbestrol modification. They indicate an immediate increase in water intake and that, in direct ratio to the concentration of Protamone. This poses the following question -- to what extent is the weight picture influenced by either the food and/or water curves?

In this instance it appears that the Protamone-water effect, as marked as it was, was unable to offset the parallel and opposite effects of the stilbestrol upon food and weight. If this is so, the question next arises as to what happened to this additional weight associated with the marked water increase? If we recall, this is consistent with the observation made on Protamone alone, where we found that the highest concentration caused the greatest water and food intake as against a relatively low weight increase.

Before we can answer these questions it is important to note that, though it was intended to run these animals

for a fifteen day recovery period after the fifteen day experimental period, so many animals had died by the seventeenth and eighteenth days that further data would have been of no statistical value. The number of dead varied directly with the concentration of Protamone. It is interesting that those same concentrations without the .1 per cent stilbestrol produced no fatal results. Most of the dead animals were approximately their normal weights at the time they died. The unkempt appearance seen in the .1 per cent stilbestrol group was modified to a fair degree and the emaciation associated with the .1 per cent stilbestrol treatment was totally absent. However, the animals were so inactive that they barely moved.

In the light of these observations we might explain the results as follows -- since the food decreased and the water intake markedly increased, the weight should not have changed drastically but since the weight loss was proportional to the food loss, the increase in water intake can only be explained on the basis of the carcass weight being actually much less than the apparent weight, since that already contained the weight of the increased water. If this is so, it explains the fatalities, the lack of activity and the seemingly normal weight at time of death. However, data merely on food and water intake, without excretion data, provide no definite evidence on the amount of each retained in the body. This still leaves the question of the possible mechanism for this behavior. We shall postulate upon that later.

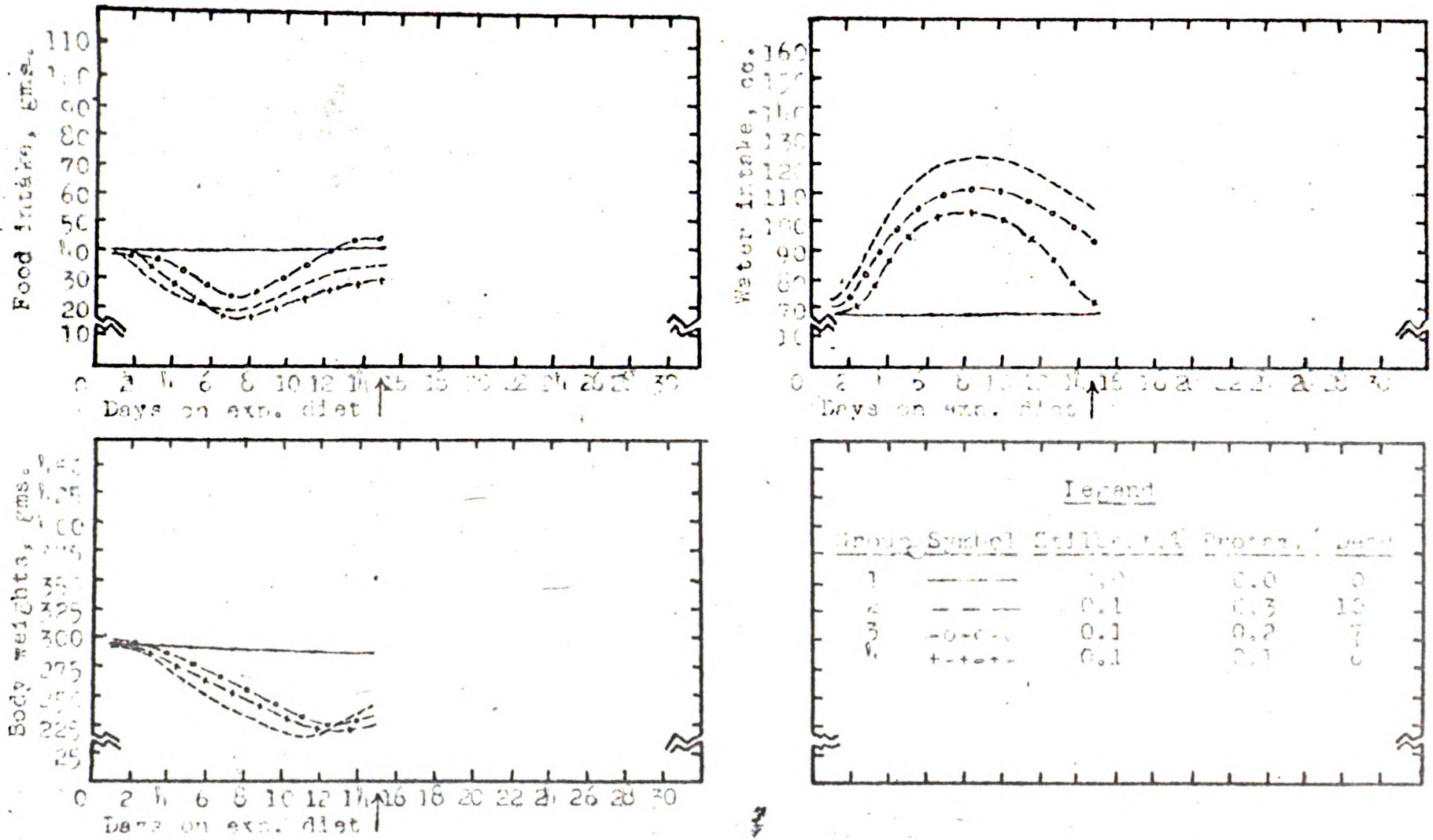

Figure 3. EFFECTS OF VARYING CONCENTRATIONS OF PROTAMONE WITH A FIXED CONCENTRATION OF STILBESTROL

The Effects of Varying Concentrations of Stilbestrol with a Fixed Concentration of Protamone -- Part I

In this experiment two groups of animals were given .1 per cent Protamone with .05 per cent and .025 per cent stilbestrol respectively. Two other groups were given the same doses of stilbestrol without Protamone and used as a control.

The controls performed as expected (Figure 4). The .05 per cent stilbestrol group suffered the greatest loss of food, water and weight. Next, in the same manner but to lesser degree was the .025 per cent stilbestrol group.

The experimental groups also performed as anticipated. Both Protamone-stilbestrol groups showed a decline in food intake until the sixth to eighth days, after which the Protamone restored the .05 per cent stilbestrol group to normal by the fifteenth day; during the latter period the .025 per cent stilbestrol group exceeded the norm.

The water intake record of the experimental groups confirmed our predictions. The Protamone effect was produced immediately; but unlike the previous experiment, the stilbestrol effect was not completely inhibited since by the eighth day the high Protamone-induced water level was reduced to normal, only to return to its previous high by the fifteenth day. However, since the water levels differed between the controls and not between the experimental groups, we might surmise that this "leveling" effect was due to the action of the Protamone in the experimental groups.

The rest of the observations of this experiment confirm those made in the preceding experiment. The body weights varied as follows: The least loss was noted in the Protamone-low stilbestrol group; the next group was the low stilbestrol group; the next group was the Protamone-high stilbestrol group and the lowest body weights were recorded for the high stilbestrol group.

Here again we encounter the low food-high water-low weight -- "what became of the water?" -- problem. This time the answer seems quite apparent and confirms our previous suppositions, for here again we encountered many fatalities.

Of particular interest is the fact that the deaths occurred only among the groups receiving both drugs despite the fact that the group which suffered the greatest weight loss was the high-stilbestrol group. There is no proof at this point that the two drugs in combination cause an excessive weight loss which is mediated by the increased water intake due to the Protamone. However, since we are dealing with only three variables, two of which show a twin relationship while the third shows a mirrored relationship, it is obvious that any changes which occur must come as a result in the change of the mirrored factor -- in this case, the water intake which is the opposite of the food-weight picture.

In the next experiment we shall attempt to lift this reasoning from the realm of postulation to something more substantiable.

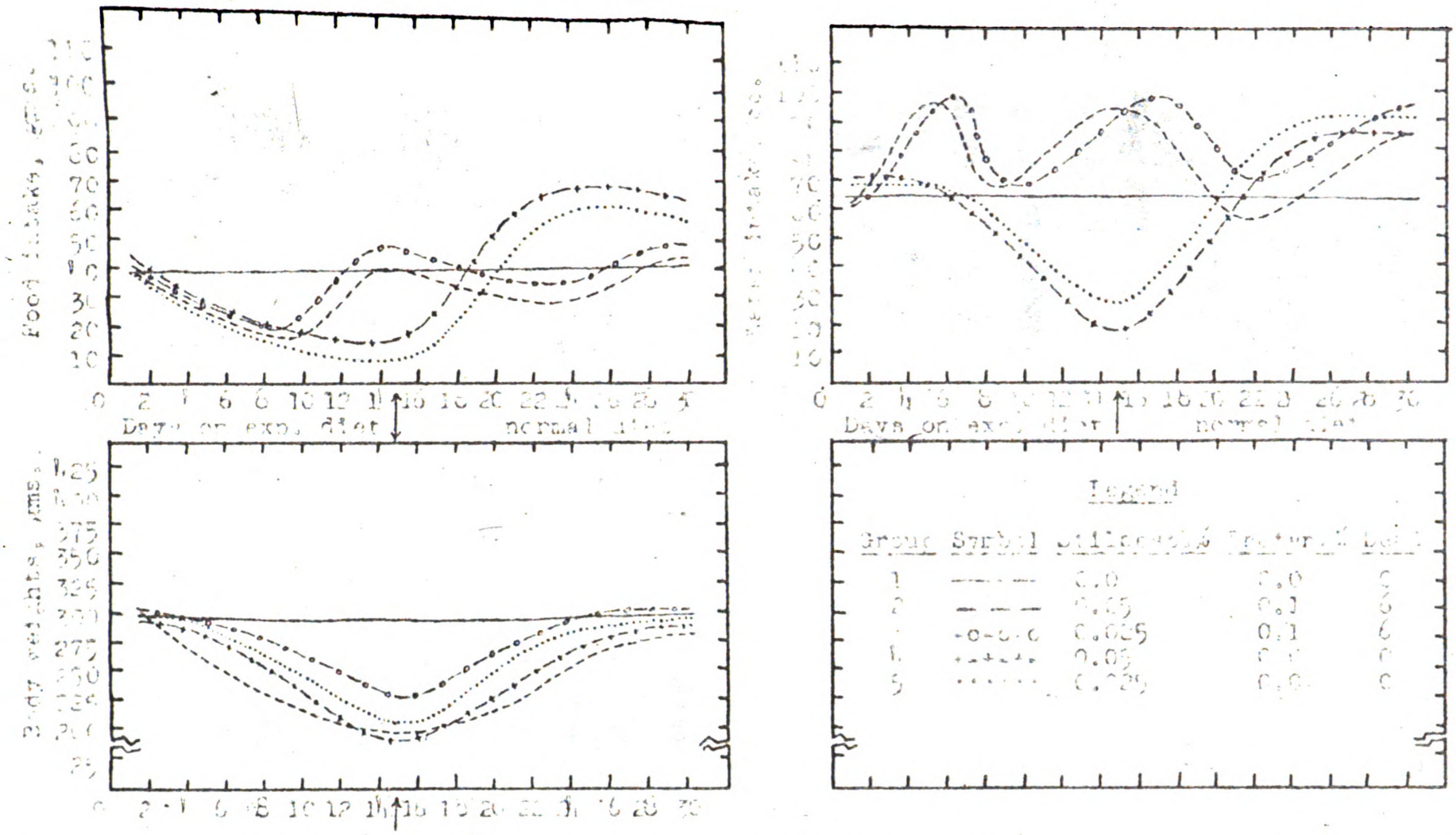

Figure 4. EFFECTS OF VARYING CONCENTRATIONS OF STRYCHNINE WITH A FIXED CONCENTRATION OF PENTOBARBITAL - PART I

The Effects of Varying Concentrations of Stilbestrol with a Fixed Concentration of Protamone -- Part II

This experiment involved seven groups of mice. Group I was used as a control. Group II received .1 per cent Protamone and .0125 per cent stilbestrol. Group III received .1 per cent Protamone and .00625 per cent stilbestrol. Group IV received .1 per cent Protamone and .003125 per cent stilbestrol. Groups V, VI and VII were used as stilbestrol controls, each received concentrations of .0125 per cent, .00625 per cent and .003125 per cent respectively. In each case the hormones were given as the indicated percentage of the food.

Group II, the Protamone-high-stilbestrol group, responded as anticipated by showing a decrease in food intake followed by a compensating increase (Figure 5). This was consistent with the previous behavior except that the subsequent increase was not as large. The water intake curve again showed the typical Protamone rise, while the body weight took the smallest drop. Allowing for slight variations, due to dosage, the pattern for the remaining Protamone-stilbestrol groups was identical. Namely, a drop and rise in food intake, a sustained rise in water consumption, and a steady decline in weight.

However, the stilbestrol controls at these doses behaved differently than before. Their food intake went down slightly and their weight went down markedly but their water intake was not adversely affected. In fact it even

showed a little rise. Since the water consumption remained static, it seems likely that the drop in weight was in direct response to the drop in food consumption.

Again, as in the previous experiments fatalities were significantly higher in the groups receiving the two drugs than in the controls. Also, as previously noted, cannabalism was rife amongst the groups treated with both drugs. As is known, mice often eat their dead, but in those groups undergoing both thyroid and estrogen treatment, the stronger devoured the weak without awaiting necropsy. This behavior was not noted amongst those groups that received high stilbestrol diets and whose weights indicated the greatest malnutrition.

It is apparent at this point, that body weight under these circumstances, is not necessarily the criterion for determining relative malnutrition. In order to confirm this, it was intended to withdraw food from all the cages for a limited time to allow the clearing of the intestinal tract and then sacrifice the mice and determine the dry matter content of the carcasses. However, within one hour after removal of the food trays, the mice in groups II, III and IV (particularly groups II and III) set upon each other with ferocity, necessitating immediate death for the entire population.

The body weights, after death, were recorded and compared to the original weights. All of the six experimental groups showed a weight considerably less than the control group, so apparently from the standpoint of weight

no "antagonism" of stilbestrol by Protamone could be demonstrated in this experiment.

The viscera were removed from all of the animals and weighed. It was noted that the livers in both groups receiving the higher stilbestrol doses were exceedingly enlarged. Those receiving the lowest stilbestrol were less so, but still much larger than the controls. To a lesser extent the same was true for the kidneys and adrenals.

The carcasses were then thoroughly dehydrated in one batch under identical conditions by drying in an oven for ten hours at 45°C. After dehydration the temperature was raised sufficiently to cause rendering of fat. It was noted that fat was rendered only by the control animals. This was collected and included in the weight of the dry matter.

After dehydration the carcasses were weighed again and the amount of water loss was determined in percentage of body weight. This was added to the percentage of body weight of the viscera and the total subtracted from the body weight at death. This left us the weight of the carcass dry matter.

It is recognized that the viscera constituted an inaccurate entity since a certain amount of body fluid flowed out of the peritoneal cavity upon excision and a certain amount of food was still contained within the gut. However, when we consider that the intake of food amounted to 2.0 to 3.6 gms. per day (Figure 5) we can estimate from

the food chart approximately how much food was contained in the tracts of each group. At any rate, the difference in this factor is so small compared to the total carcass weights that it is of no particular significance in the interpretation of the results.

This work was conducted under the supposition that Protamone enhanced growth, body weight, etc., while stilbestrol inhibited these same processes. Since this was not effected by equal dosages, it was assumed that the stilbestrol was dominant and therefore an attempt at a balance was made by favoring the relative Protamone concentrations. This still failed to establish the expected balance.

In retrospect it is now apparent that only the lowest dosage of Protamone produced an increase in body weight relative to food and water consumption. That is, in relation to quantity of food and water intake, the relative body gain was much higher. Also, where we used the smallest doses of stilbestrol a slight <u>increase</u> in water intake was noted, though food intake and body weight exhibited the usual effect.

It is suggested that on the dosage levels referred to, an apparent antagonism could be produced since neither drug at these levels has entered the "self-consuming" stage. In fact there is reason to believe that these dosages come closer to those produced <u>in vivo</u> and which exhibit what we recognize as normal endocrine adjustment.

It is suggested that when excessive doses are administered the delicate balance of the whole endocrine system is upset to the point where the usual checks and adjustments are unable to operate. An extension of this study into the dosage realms indicated might well be highly rewarding.

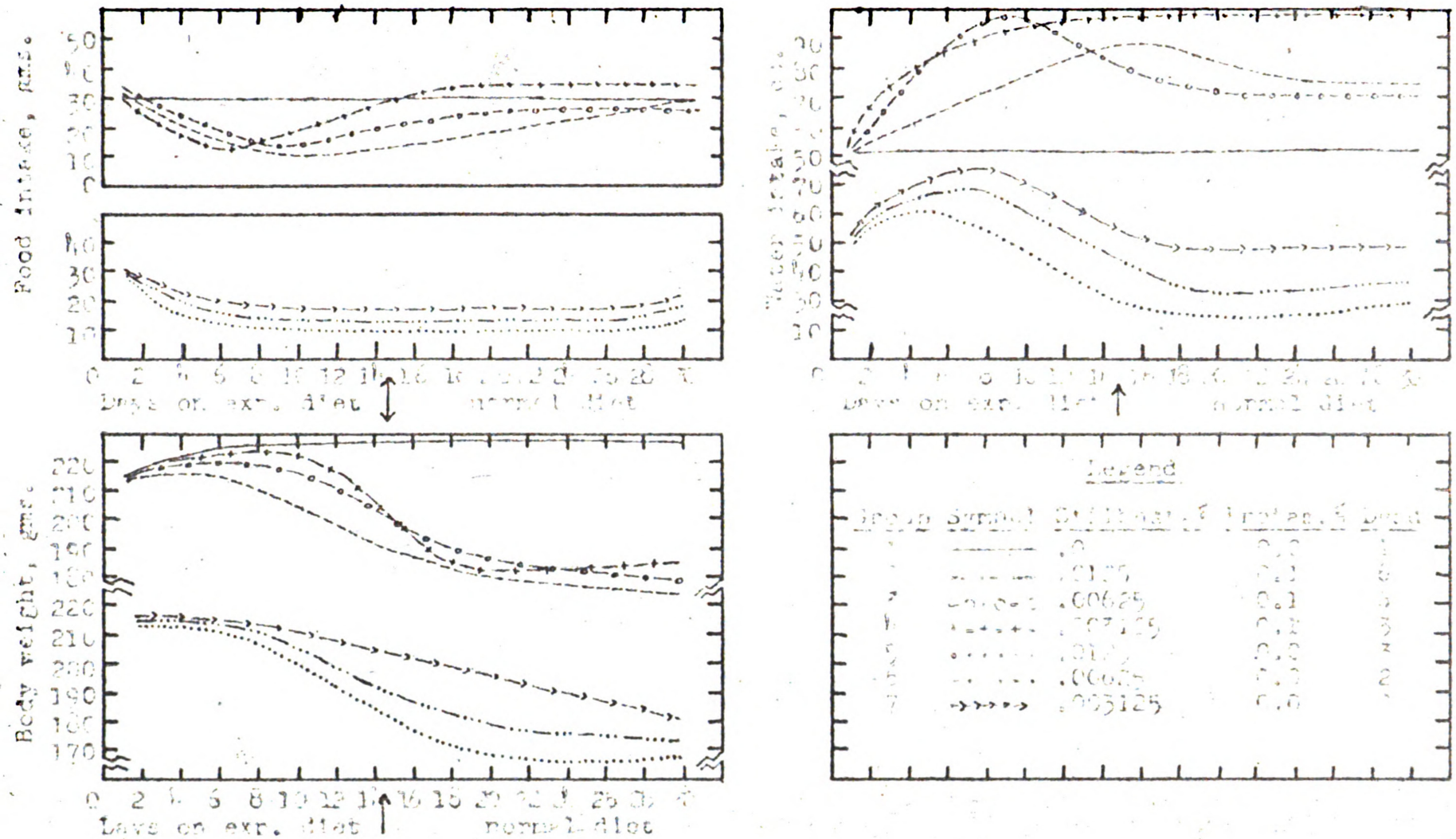

Figure 5. EFFECTS OF VARYING CONCENTRATIONS OF ETIRIDERET WITH A FIXED CONCENTRATION OF PROPAZOLE -- PART II

CHAPTER VII

DISCUSSION

In (Table I) we note that the control group showed an average carcass weight of 42 per cent as compared to the Protamone-stilbestrol groups which showed average carcass weights of approximately 32 per cent; the stilbestrol groups showed average carcass weights of approximately 23 per cent.

Since a rise in water consumption was noted for both groups in this experiment (Figure 5) we might attribute the higher weights of the Protamone carcasses to the higher amount of food intake.

So far in the discussion one point has been studiously avoided and that is, -- in this food-water-weight balance, what part does diuresis as well as water retention play? It is obvious that an interpretation based on exclusion of this factor must be quite inadequate. However, we have now arrived at the point where we can pursue this factor with logic and deductive reasoning. Since no record was kept of urinary excretion it was useless to postulate what percentage of body weight was increased or lost by urine retention or excretion. With the data now before us it should be possible to reconstruct some of the physiological functions which resulted in the observable behavior.

First, the problem as first visualized, was based on the assumption that there is a direct relationship of

body weight with food and water intake. This handicap was eliminated at the outset in the philosophical discussion which recognized that endocrine behavior, like any system, reacts according to the components of its variables. Therefore high food intake and high water intake may be so offset by high urine excretion as to result in weight loss; and this assumes a static calorigenic situation.

The difficulty then of assessing one variable in a system which is completely re-orientated as a result of that variable, is not to be solved by the mere observance of a factor which is the resultant of a series of adjustments. Therefore, in itself, the data collected is of little, or no value. On the other hand, if we recognize the data in the light expressed, it becomes meaningful, since it is not removed as a static factor from a dynamic system.

The original premise can be restated here in this question -- where is the "antagonism" of a drug which when administered to its "antagonist" produces death in an individual that formerly withstood each drug? This "antagonism" is based on a superficial observation of the "living-sick" and the "healthy-dead".

This study shows that Protamone will increase food consumption, water intake and body weight (Figure 1). It is important to note that this is true up to a point of diminishing returns. Beyond this point, output begins to

exceed intake. This study also shows that stilbestrol will decrease food consumption, water intake and body weight (Figure 2). Again it is important to note that there is a point where this relationship changes. Assuming these phenomena to be results of a linear progression, one is justified in expecting these hormones to be counterparts, each to the other. The fact is, that both hormones together had a much more devastating result than either acting singly (Figure 3). This can be explained on a dialectic basis.

The increase in water intake resulting from the Protamone administration followed from the increase in metabolic rate. A comparable increase in urinary excretion was also to be expected as a result of increased metabolic water formation.

In the case of stilbestrol administration it was logical to expect a decrease in food and water intake as a result of an inhibited metabolism. By the same token, it follows that urinary excretion would be decreased.

If we made a unilateral assumption that Protamone stimulates the metabolism and stilbestrol inhibits the same function, we could logically expect a cancelling of the effects; but while one drug produces its effect via the thyroid mechanism, the other produces its contrary effect via a different mechanism. The result is that the Protamone increases the metabolism in an animal whose food intake is reduced via another mechanism. This burns the

candle at both ends. The increased metabolism which is now a predominately "katabolic" affair demands increased water intake. In addition, metabolic water is piling up in the system of an animal which due to the stilbestrol effect, is not "anabolizing". The resultant effect of these forces is to consume more quickly the animal's energy "reserve" and replace it with the "left-over" water. The water, like the food reserve, degenerates in two directions at once. While the Protamone causes an increase (Figure 1), the stilbestrol causes a retention (Figure 2). From the standpoint of body weight, this may appear like an antagonism (Figure 5), since the mice treated with both drugs are heavier than those treated with stilbestrol alone (Figure 5). This seems to be confirmed by the fact that the dry carcass weights (Table I) show a greater loss of actual body substance on the stilbestrol-treated mice as against those "antagonistically-treated". However, when we consider the bone structure and luxurious hair and heavy integument of this group, we must bear in mind that the substance for this maintenance was derived from the body substance of the animal itself. The groups having the most effective stilbestrol doses outlasted by far the mixed groups. This was accomplished by the normal physiological adjustments accompanying malnutrition. Energy was conserved by retarding of growth, decrease in activity, decrease in amount of nourishment to hair, skin and other of the lesser essential structures.

Of course the preceding, while presented as a positive argument, is not intended as fact. The writer is fully aware of the lack of conclusive evidence to confirm his point. Therefore this rationalization is offered for confirmation, denial, debate or just as a stimulant for discussion. If this paper provokes any of these, it will have adequately served its purpose.

DATA

All figures indicate the total weights for each group. Since all groups consisted of ten mice, a shift of the decimal point one integer to the left gives the average weight per mouse. As mice died the weights were divided by the total number of survivors in each group and multiplied by 10, giving a figure which could continually be compared to the controls.

TABLE I

THE RECIPROCAL EFFECTS OF DIETHYLSTILBESTROL AND THYROPROTEINS ON BODY WATER DISTRIBUTION OF FEMALE MICE

Weights below are shown in grams	Group 1	2	3	4	5	6	7
Percentage of Protamone in diet	.0	.1	.1	.1	.0	.0	.0
Percentage of Stilbestrol in diet	.0	.0125	.00625	.003125	.0125	.00625	.003125
Av. wt. before experiment	22.4	22.4	22.4	22.4	22.4	22.4	22.4
Av. wt. after experiment	23.1	17.3	19.0	18.0	20.6	17.1	18.7
Av. wt. of carcass	15.2	10.4	11.0	11.0	10.4	10.4	11.4
Av. wt. of carcass, dehydrated	11.5	7.6	8.0	8.0	7.6	6.2	7.1
Av. wt. of carcass water	3.7	2.8	3.0	3.0	2.8	4.2	4.3
Percentage of water to carcass weight	24	27	27	27	27	38	37
Av. wt. of viscera	7.9	6.9	8.0	7.0	10.2	6.7	7.3
Percentage of viscera to body weight	34	40	42	39	50	40	40
Av. wt. in percentage of dry carcass matter to body weight	42	33	31	34	24	22	23

CHAPTER VIII

SUMMARY AND CONCLUSIONS

As the literature indicates, diethylstilbestrol produced a decrease in the amount of food consumed by the experimental animals. A corresponding effect was produced on the water consumption, except in the range of the minimal effective doses.

Furthermore, the animals exhibited loss of body weight at all dosage levels, while hair and integumentary development showed definite deterioration in direct response to dosage.

The activity of the experimental animals was retarded; while the general appearance was indicative of marked malnutrition.

On the other hand, the administration of the thyroprotein, Protamone, produced an increase in the activity of the experimental animals and an increase in both food and water consumption. However, the quantity of these increases were directly proportional to dosage levels only within a prescribed range. Beyond this optimally effective range, responses were affected by the Laws of Diminishing Returns.

The Protamone-treated animals also exhibited fine sleek coats in contrast to the estrogen-treated mice. Also,

the body weights of these mice were increased and the general appearance and condition of these animals ranged from normal to enhanced.

From the apparent diametrically opposite effects produced by diethylstilbestrol and Protamone, each administered separately, it was presumed that given <u>together</u> there should be some measure of antagonism produced which would reflect in the responses of the experimental animals.

At first, it was gratifying to find that such was the case. Where diethylstilbestrol had formerly produced emaciation, those mice "fortified" with Protamone withstood the deleterious effects of the diethylstilbestrol. However, and this is significant, <u>in every case the animals so treated died within, or shortly after, the experimental period</u>; while the animals given the estrogen only, had few fatalities and showed a high percentage of recovery.

The seeming paradox of healthy-appearing animals being consistently survived by emaciated animals, is explained on the following basis:

1. The Protamone achieves its effect via the thyroid-endocrine system relationships.

2. The diethylstilbestrol achieves its effect via the ovary-endocrine system relationships.

3. Therefore, it does not follow that there need be a <u>direct</u> antagonism, since each drug operates via its own avenues. However, this does not preclude the possibility of an <u>indirect</u> antagonism.

4. If we assume an indirect antagonism, then how can we explain the fact that animals given both drugs show a radical decrease in survival ability?

5. The fact is, that the animals given both drugs show a significant loss of body weight when dehydrated; as compared to the controls. In other words, much of the apparent "weight" was simply due to high water retention.

6. In addition to the preceding circumstances, the Protamone produced an increase in metabolic activity which tended to further consume an already starved animal.

7. The combination of the foregoing factors explains the high mortality in the groups treated with both drugs. Furthermore, it indicates that there is no essential antagonism between the two drugs, though in some respects either drug may have had a dominant effect. For example, the Protamone seemed to have a dominant effect in regard to hair growth, while the diethylstilbestrol seemed to have a dominant effect in regard to water retention.

This study indicates that the behavior exhibited as a result of thyroid-estrogen balances is a vector of a great many inter- and intra-related responses which must be viewed not en toto; but rather from the standpoint of the artist who examines the leaves and grass before sketching the panorama of the forest.

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