I. EFFECTS OF HORMONE ADMINISTRATION UPON MILK PRODUCTION IN UNDERFED RATS

II. EFFECTS OF UNDERFEEDING ON MAMMARY GLAND DEVELOPMENT

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ABSTRACT

I. EFFECTS OF HORMONE ADMINISTRATION UPON MILK PRODUCTION IN UNDERFED RATS

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by Albert Ratner

1. Considerable data are available which indicate that the functions of the endocrine system are largely dependent upon the nutritional state of the animal. The objects of the present experiments were to determine the effects of inanition upon mammary growth and lactation in the rat, and to see if the administration of hormones could overcome the decrease in lactational performance as a result of underfeeding.

2. Experiment 1. The effects of administering growth hormone, thyroxine, anterior pituitary powder, oxytocin, prolactin, hydrocortisone acetate; and oxytocin, prolactin, and hydrocortisone acetate in combination, on lactational performance of underfed rats were studied. A total of 105 mature, female Carworth rats of the CFH strain were bred. On the 4th day postpartum the litters were reduced to eight pups per dam, and the food intake of the dams was reduced to 1/2 normal consumption. The dams were injected daily with a particular hormone or hormones from the 4th to 17th days postpartum. All injections

were given subcutaniously once daily, except oxytocin which was given four times daily. The dams and litters were weighed daily, and the lactational response was measured by the daily litter growth rate. Since the pups received only milk during this period, this was used as a measure of milk production. On the 18th day postpartum the dams were sacrificed and the pituitary, thymus, thyroid, adrenals, ovaries, and uteri were removed, trimmed of extraneous tissue and weighed.

Each group of mother rats was treated as follows:

1. Controls, saline; ad libitum fed.
2. Controls, saline; 1/2 feed.
3. Bovine anterior pituitary, 15 mg; 1/2 feed.
4. Bovine anterior pituitary, 25 mg; 1/2 feed.
5. Growth hormone, 1 mg; 1/2 feed.
6. Thyroxine, 10 µg/100 gm B.Wt.; 1/2 feed.
7. Hydrocortisone acetate, 0.5 mg; 1/2 feed.
8. Hydrocortisone acetate, 1 mg; 1/2 feed.
9. Prolactin, 1 mg; 1/2 feed (20 I.U./mg).
10. Oxytocin, 1 I.U.; 1/2 feed.
11. Hydrocortisone acetate, 1 mg; prolactin, 1 mg; and oxytocin 1 I.U.; 1/2 feed.

3. The 1/2 fed control group gained about half as much as the full-fed controls; 92 gm as compared to 186 gm. None of the hormones injected into the 1/2 fed lactating rats produced any significant change in litter weight

gains during the entire period of lactation (4th-18th days postpartum) or during the early part of lactation (6-10th days postpartum). However, during the later part of lactation (11th-18th days postpartum), prolactin, the higher dose of hydrocortisone acetate and oxytocin produced a slight but significant increase in litter weight gains. When prolactin, oxytocin, and hydrocortisone acetate were injected in combination, the response was about the same as when each of these hormones was given separately.

4. Dams receiving growth hormone and the higher dose of anterior pituitary powder lost less weight than the 1/2 fed controls; 35 gm and 34 gm as compared to 60 gm. However, these hormones produced no beneficial effect insofar as increasing milk production was concerned. Hydrocortisone acetate, which showed some beneficial effect on litter weight gains during the later part of lactation, caused an 80 gm loss of body weight by the dams. There seemed to be no correlation between mother's weight and the amount of milk produced.

5. On an absolute weight basis all the endocrine organs of the 1/2 fed groups were decreased in weight. On an organ-body weight ratio they remained about the same, except for the adrenals which increased in weight and the thymus which decreased in weight. Administration of hydrocortisone acetate decreased the weight of the adrenals and thymus on both an absolute and relative body weight basis.

6. Experiment 2. The effects of underfeeding upon mammary development were studied. Mature, female Carworth rats of the CFH strain were divided into three groups of eight rats each. Group I was fed ad libitum and groups II and III received 1/2 feed and no feed for 14 days. At the termination of the experiment, the rats were sacrificed and the right inguinal mammary pad was removed and prepared for gross mounting. The glands were rated for development according to the following scale:

I. Few ducts; few or no end buds

II. Moderate duct development; moderate number of end buds

III. Numerous ducts with many branches; many end buds

IV. Numerous ducts with many branches; with moderate lobulo-alveolar (L-A) growth

The pituitary, thymus, ovaries, uteri, and adrenals were removed, cleaned and weighed.

7. Mammary glands from the full-fed controls had an average rating of 1.2. Glands from the 1/2 fed group averaged 2.2. Glands from the group given no food averaged 3.0, with three rats exhibiting moderate lobulo-alveolar development.

8. On an absolute weight basis, the pituitary, thymus, uteri, and ovaries were all decreased considerably as a result of the various degrees of inanition. The

adrenals, however, increased over their original weight during the period of complete food withdrawal. On an organ-body weight ratio the weights of the ovaries and uteri remained about the same, and the pituitary increased.

9. Experiment 3. The effect of underfeeding on mammary involution was studied. Mature, female Carworth rats of the CFN strain were bred. On the 4th day postpartum the litters were removed and the mother rats were divided into two groups of eight rats each. The experimental animals were fed 1/3 the food consumed by the ad libitum fed controls. On the 15th day postpartum the dams were sacrificed and the right inguinal mammary gland was removed for histological examination.

10. As compared with the full-fed controls, underfeeding retarded mammary involution as judged histologically. The glands from the underfed group showed better maintenance of alveolar structure and in some areas secretory material was present.

11. It is concluded that:

a. The decrease in milk production of underfed suckled lactating rats cannot be overcome by the administration of hormones. This decrease is due primarily to the lowered food intake, and not to reduced endocrine function. It is thought that the suckling stimulus is able to maintain the secretion

of those hormones necessary for milk production at a near optimal level.

b. Underfeeding can induce mammary development in the mature cycling rat, and retard involution of the postpartum gland. It is thought that severe underfeeding can promote the secretion of ACTH and prolactin from the anterior pituitary in amounts adequate to induce the observed effects.

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INTRODUCTION

It is well known that nutrition plays an important role in the functional integration of body organs. Furthermore, the endocrine system is not divorced from nutritional influences. The functions of the anterior pituitary gland and its target organs are partially dependent upon proper nutrition. Thus, inanition or lack of adequate specific dietary constituents, such as vitamins or proteins leads to a reduction in activity of most endocrine glands. Mulinos and Pomerantz (1941) termed the condition associated with inanition "pseudohypophysectomy" because of its resemblance to the condition which normally follows surgical removal of the pituitary gland.

Sub-normal nutrition depresses the release of a number of anterior pituitary hormones. The primary cause of endocrine hypofunction during malnutrition is considered to be a diminished level of circulating hypophyseal hormones, rather than a refractory state of the target organs. The target organs readily respond to administration of anterior pituitary hormones (Ershoff, 1952). The reduction in endocrine function leads to a number of endocrine disturbances such as growth depression, reduced thyroid activity and reproductive failures. Many of these disturbances have been overcome by the administration of hormones (Ershoff,

1952). Nelson (1959) has shown that pregnancy can be maintained even when the diet is completely lacking in protein by the administration of estrogen and progesterone.

Restrictions of food intake, protein or other nutrients in lactating animals results in a decrease in milk production (Reece, 1959). It is known that the maintenance of milk production also depends upon several hormones, particularly prolactin, ACTH, and oxytocin (Meites, 1959). One of the primary objectives of the present study, therefore, was to determine to what extent, if any, the decrease in milk production in rats which results from underfeeding is due to reduced endocrine function, and whether the fall in milk yields can be overcome by administration of hormones.

Another aspect of the relation of nutrition to mammary function which was of interest to investigate was based on reports of development of gynecomastia in chronically undernourished men (Keys, et al., 1950). Numerous reports indicate that the ACTH-adrenal cortical mechanism during chronic inanition or acute starvation is not depressed (Boutwell et al., 1948; Quimby, 1948; D'Angelo et al., 1948). It appears that during periods of severe food restriction the pituitary is able to respond with synthesis and release of ACTH. Srebnik et al., (1961) have also shown that the pituitary, even in the complete absence of protein, can respond to the stimulus of ovariectomy with increased synthesis and release of gonadotropins.

The release of ACTH from the anterior pituitary can be induced by a number of factors other than stress, including the suckling stimulus; numerous drugs such as serotonin, chlorcpromazine and reserpine; adrenalectomy; and thyroxine administration (Meites et al., 1962). These factors have also been shown to produce physiological manifestations in the rat which have been interpreted as being indicative of increased secretion of prolactin from the anterior pituitary. Thus, all these factors induce pseudopregnancy or lactation in the rat (Meites et al., 1962). Since both ACTH and prolactin are released together under many conditions, it is possible that inanition could induce secretion of both these hormones from the anterior pituitary.

Food restriction has been reported to retard mammary growth in rats and mice (Sykes et al., 1948; White, 1944). Trentin and Turner (1941) and Astwood (1937) have shown that the mammary duct system is much less sensitive to estrogen during inanition. In these experiments the degree of inanition was not severe and the experiments were of short duration. Since only long periods of chronic inanition or shorter periods of complete starvation increase the secretion of ACTH, it was of interest to determine the effects of similar degrees of inanition upon mammary growth and involution. It was considered possible that the resultant rise in ACTH and prolactin secretion might increase mammary growth and retard mammary involution.

LITERATURE REVIEW

Nutrition and Reproductive Function

Since reproductive function is primarily controlled by the endocrine system, and particularly by the gonadotropic and gonadal hormones, it is necessary to consider in detail the relation of gonadal function to nutrition. The relationship between the function of the gonads and nutrition has received a great deal of attention, and some progress has been made towards elucidating the mechanisms involved.

Inanition and nutritional deficiencies. Evans and Bishop (1922) demonstrated in the female rat that underfeeding results in atrophy of the ovaries, follicular atresia and failure to ovulate. They were the first to emphasize that a diet which is adequate for body growth may be grossly deficient for normal reproduction. Ovarian hypofunction with resultant uterine atrophy and anestrous vaginal smears have been observed by numerous workers during inanition (Ershoff, 1952). Similar changes have been observed in the male. Atrophy and hypofunction of the testes have been repeatedly observed during inanition (Jackson, 1925; Quimby, 1948). The male accessory sex organs (prostate and seminal vesicles) are particularly sensitive to restrictions of caloric intake, resulting in atrophy of

these structures before the testes are appreciably reduced in size (Moore and Samuels, 1931). The promptness with which the male accessory organs react makes them a very useful object of study in nutritional deficiencies which primarily affect the testis.

In general, observations on human populations during periods of food restriction are in accord with the above findings. Gonadal atrophy and loss of sexual function were found in many undernourished prisoners of war (Keys et al., 1950). Goyen (1945) studied the endocrine status of adults suffering from chronic undernutrition. He reported that sexual function was markedly decreased, and that loss of libido and impotence were frequent. Testicular biopsies revealed decreased size of the tubules, hypospermatogenesis and testicular fibrosis. In adult females, amenorrhea was present in 50% of young women; the vagina and the endometrium were atrophic, and there was a low excretion of urinary FSH.

As early as 1932, Guilcent and Goss showed that diets containing 7 per cent protein, or less, resulted in atrophy of the gonads, reproductive failures and disturbances of the estrous cycles in rats. In male rats, diets deficient in protein caused atrophy of the seminal vesicles and prostate (Samuels, 1940; Leathem, 1949).

Nelson and Evans (1943) showed that a 5% casein level (equivalent to 5.5 protein) was apparently the critical amount

of protein for normal reproductive function in the rat. Below the 6% casein level, fetal death and resorption consistently occurred in the majority of the animals. Reproductive failure on the protein poor diet was characterized by signs of sex hormone inadequacies, as shown by the early loss of the estrous cycle in unbred rats maintained on a similar diet. Normal reproductive performances in pair-fed controls eliminated caloric restriction and other dietary deficiencies as causes for reproductive failure.

Impaired gonadal functions have also been observed on diets deficient in specific amino acids. Gonadal damage has been reported in rats fed diets deficient in phenylalanine, leucine, histidine, and tryptophan (Ershoff, 1952). But the effects observed appear to be due, at least in part, to factors other than inanition since testicular atrophy of phenylalanine, leucine, and histidine deficient rats was more severe than that of pair-fed controls (Maun et al., 1946). It is possible that the gonadal injury observed on diets deficient in essential amino acids may be due to direct tissue injury or decreased sensitivity of target organs to pituitary stimulation. It has been demonstrated that protein synthesis occurs only if all the essential amino acids are present (Geiger, 1950). Hence, a deficiency of any one of the essential amino acids may cause an animal to go into negative nitrogen balance. One might expect then, that a deficiency in any one of the essential

amino acids, if sufficiently prolonged, would result in many of the typical effects of quantitative protein deficiency. Whether the gonadal injury as a result of feeding diets deficient in a single essential amino acid is characteristic of the deficient amino acid or is due to general impaired protein metabolism, remains to be investigated.

Deficiencies of B vitamins have been shown to elicit similar effects on gonadal function as reduced food intake. Delayed sexual maturity, anestrus and atrophy of the ovaries and uteri or testis and secondary sex organs have been observed in animals deficient in thiamine, riboflavin, pyridoxine, pantothenic acid, biotin and vitamin B_{12} (Ershoff, 1952). Since in most cases food intake was reduced in these animals, it is not clear to what extent the results were due to inanition as distinct from the vitamin deficiency. Moore and Samuels (1931) demonstrated that although the secondary sex glands of the male rat atrophied when rats were fed a thiamine-deficient diet, atrophy also occurred in rats fed a complete diet but restricted in calorie intake to the same extent as consumed by the thiamine deficient rat. Drill and Burrill (1944) observed similar effects in female rats.

Atrophy of the testis and seminal vesicles and degenerative changes in the semeniferous tubules have been reported as a result of vitamin E and A deficiency (Mason, 1933; Lutwak-Mann, 1952). However, female rats deficient

in these vitamins appear to show no impairment of ovarian function (Mason, 1939), although resorption of the fetus is induced.

Gonadal and reproductive failures have been observed in rats on fat deficient diets. Evans et al. (1939) showed that fat deficient diets caused atrophic changes in the testis and sex accessory organs, and a failure of spermatogenesis. In female rats Terrondo et al. (1935) reported that vaginal smears showed no signs of estrous and the rats became sterile.

Hormonal administration. Numerous studies have shown that the changes in gonadal function and reproductive processes as result of deficient diets are due to a low level of circulating gonadotropins, since atrophic changes in the reproductive system and reproductive failures can be corrected by administration of gonadal or gonadatropic hormones.

Marian and Parkes (1929), Stephens and Allen (1941), and Benaldi (1949) showed that the ovaries of underfed rats and guinea pigs were responsive to injections of gonadotropic hormones. Similar results were obtained in underfed male rats, where the testes and secondary sex organs were maintained by administration of gonadotropic hormones (Moore and Samuels, 1931; Yadu, 1930).

The administration of gonadotropins stimulated the gonads and accessory glands in both male and female rats

fed protein deficient diets (Samuels, 1950; Leathem, 1959). The high sensitivity of the gonads to gonadotropic administration of protein deficient rats was demonstrated by Srebnik et al. (1958). They showed that the protein deficient rats required the same amount of FSH for follicular repairment as hypophysectomized rats, and showed greater sensitivity to ICSH.

Elimination of protein from the diet from the onset of pregnancy throughout gestation results in loss of the young (Nelson, 1959). Nelson and Evans (1954) fed protein-free diets to pregnant rats and gave those hormones which were shown to maintain pregnancy in hypophysectomized-ovariectomized rats (Lyons, 1943). Pregnancy was maintained in 30 per cent of the rats given estrone, in 70 per cent of the rats given progesterone, and in 100 per cent of the animals given a combination of estrone and progesterone.

Drill and Burrell (1944) showed that the administration of gonadotropic extracts to thiamine deficient rats restored gonadal function in both the male and female. Nelson et al. (1957) reported similar effects with vitamin B_6-deficient rats. The feeding of pyridoxine, B_1, or B_6 deficient diets to pregnant rats resulted in a high incidence of resorptions. Nelson (1957, 1959) was able to maintain pregnancy in these rats by administration of estrone and progesterone.

Yadu (1950) showed the omission of thiamine,

pantothenic acid, and riboflavin or folic acid produced a decrease in the response of the seminal vesicles to DES on a body weight basis. Pair-fed experiments showed that inanition was responsible for the observed results with the exception of folic acid deficiency. Hertz (1946) and Kline and Dorfman (1951) found that the response of the oviduct to estrogens was impaired when the diet was deficient in folic acid. Velardo and Hisaw (1953) also showed that a deficiency of folic acid inhibits the action of progesterone on the uterus, and prevents decidual development. A critical examination of the relationship between folic acid deficiency and estrogen hormones (Silver, 1954) indicates that the action of the estrogenic stimuli to the target organ is not primarily interfered with, but rather that the proliferation is deranged and therefore the response of the organs from deficient animals differs from the response in the controls. This is not surprising since folic acid is bound up with the synthesis of nucleic acids (Shive, 1951). It is possible that folic acid functions in fundamental metabolic reactions linked with the synthesis of nucleic acids needed for the tissue proliferation in response to such stimulants as ovarian hormones.

With respect to the effects of vitamins A and E on the reproductive system, the secretion of gonadotropin is apparently reduced in the vitamin A deficient animal since the atrophic changes in the male testis and tract

can be restored by administering physiological levels of gonadotropin. However, it seems that dysfunction of the pituitary is not the cause of testis injury or resorption during gestation in E-deficient rats, since gonadotropic hormones were ineffective in repairing the testis injury of E-deficient rats. Endocrine therapy has also proved ineffective in preventing the reproductive pathology which occurs in vitamin E-deficient female rats (Lutwak-Mann, 1958).

Pituitary function. Mulinos and Pomerantz (1941) presented a series of observations concerning the effects of inanition upon the endocrine status of the rat. From their observations they concluded that inanition leads to a physiological state somewhat comparable to that which normally follows surgical removal of the pituitary. They termed this condition "pseudohypophysectomy." Data have been accumulating on the effects of nutritional deficiencies upon the endocrine system for some time prior to these observations, but for the first time the emphasis was placed on the pituitary gland.

Since the gonads and accessory organs retain apparently normal sensitivity to gonadotropic stimulation under conditions of inanition and dietary deficiencies, it appeared that hypophyseal gonadotropins were secreted in insufficient amounts to maintain normal endocrine

function by the gonads. In order to gain information on the functional state of the pituitary, the pituitaries from nutritionally deficient animals were assayed by a number of workers. Mason and Wolfe (1930) implanted pituitaries from chronically starved rats into immature female rats and found that the resulting increase in ovarian and uterine weight was significantly less than from pituitaries obtained from littermates fed the same diet ad libitum. Similar results were reported by Weiner (1939). Later workers found either no change or an increase in pituitary gonadotropin content. Maddock and Heller (1947), after starving rats for 12 days, reported that the pituitaries were more potent than those from normal rats even though the weight of the anterior pituitary had fallen about 40%. Renaldi (1949), employing hypophysectomized young female rats as assay animals, showed that the ovaries of animals injected with pituitaries of underfed rats were four times as large as those obtained from animals injected with pituitaries of normal rats. Similar findings have been reported by Meites and Reed (1949). Injecting the equivalent of one pituitary into immature female rats they found that the gonadotropic content of the pituitary from those rats on restricted diets was equivalent to the controls fed ad libitum.

Studies with diets deficient in a particular nutrient have shown similar results. Nelson (1959) assayed the

pituitaries from rats whose diets were depleted in protein for one month, and found increased FSH with no decrease in LCSH activity per gland. Bioassay of pituitaries from female rats subjected to vitamin B_6 deficiency showed greater increases in FSH activity per gland (Wooten et al., 1955).

It appears that inanition and certain vitamin or protein deficiencies are not associated with a decreased hypophyseal content of gonadotropins. On the contrary, the pituitary content is more likely to be increased. This, together with the response of nutrient-deficient rats to low levels of injected gonadotropins, strongly suggest that the gonadal inadequacies of deficient rats result from a decreased secretion of pituitary gonadotropins. The increased gonadotropic potency in underfed rats may result from:

1. A normal rate of hormone synthesis and a decreased rate of release, with subsequent storage.
2. A decreased rate of hormone synthesis with limited or no release, resulting in accumulation of the hormone.

Though data on hormone content of a gland may serve as indicators of function under some conditions, such data are very hard to interpret. A better indicator of the functional state of a gland is the blood level of the hormone. Sribnik et al. (1961) have reported slight increases in

pituitary content of PBI in rats on protein deficient diets, without a detectable rise in blood levels.

Nutrition and Thyroid Function

A decrease in thyroid activity has been demonstrated during periods of food restriction. Atrophy, involution and degeneration of the thyroid gland have been described in the rat during inanition (Mulinos and Pomerantz, 1940; Stephans, 1940). In chronically undernourished men the basal metabolic rate is low (Keys, 1950). Keites and Wolterink (1950) showed that the amount of I^{131} taken up by the thyroid was reduced in proportion to the severity of food restriction. D'Angelo (1951) demonstrated a reduction in thyrotropic level in the blood of acutely starved rats and mice. Evidence for lowered levels of PBI (Blincoe and Brody, 1955) and a depressed rate of release of thyroidal I^{131} (Reichlin, 1957) have also been reported.

During inanition Stephans (1940) observed that undernourished guinea pigs were 10 times more sensitive to thyrotropic hormone than normal guinea pigs. The thyroid responds to thyrotropic hormone administration, suggesting that the condition has a pituitary origin. It appears that the reduced secretion of thyrotropin is responsible, at least in part, for the regressive changes in the thyroid during inanition. The sensitivity of the pituitary-thyroid axis to mild underfeeding has been studied by Pipes et al.

(1960). They observed that three-fourths of normal food intake significantly decreased thyrotropic hormone without an effect on body weight.

Nutrition and Growth Promoting Function

If an immature animal is placed on a restricted diet, growth as represented by skeletal elongation and gain in body weight is reduced. Li et al. (1949) found that the administration of STH to such animals results in more rapid growth, associated with reduced nitrogen excretion. Frandsen et al. (1954) found that in complete absence of dietary protein, the tibial proximal epiphyses was sealed by bone, which is known to occur after hypophysectomy.

Srebnik et al. (1959) assayed pituitaries of protein deficient rats for growth hormone activity to determine whether growth arrest was due to decreased synthesis of growth hormone. An absence of dietary proteins for five weeks reduced the growth hormone content of the pituitaries to one-half normal amount per mg of tissue. There was a parallel decrease in number and size of pituitary acidophils. But failure of growth hormone synthesis in the absence of dietary protein may not be the only cause of growth retardation; lack of amino acids as building blocks and the inability of the skeleton to respond to growth hormone may also be a factor in growth arrest in protein deficient rats.

Nutrition and Adrenal Cortical Function.

Though most anterior pituitary hormones appear to be secreted in reduced amounts during dietary restrictions, this is not entirely true for ACTH. Boutwell et al. (1948) reduced the caloric intake of mice to one-half their normal consumption for a period of 3 weeks. They found that overian and uterine weight on an organ-body weight ratio was reduced by 40% or more, while adrenal weight was increased more than 50% and showed no change in absolute weight. An increase in weight of adrenals on a body weight basis during long periods of chronic inanition and shorter periods of acute starvation have also been reported in the rat (Quimby, 1948), and guinea pig (D'Angelo et al., 1948). These workers also observed (1) involution of the thymus, (2) decrease in lymphocyte count and (3) increased gluconeogenesis. Such findings are indicative of increased pituitary-adrenocortical function during chronic inanition or acute starvation.

Further proof of an increase in adrenal cortical function during underfeeding has been observed by D'Angelo et al. (1948) in the guinea pig. When subjected to acute starvation or chronic inanition the guinea pig responded with an absolute increase in adrenal size and solid content, which was proportional to the degree of body weight loss and duration of the inanition. Hypophysectomized animals under the same conditions did not show adrenal

hypertrophy, the adrenals being atrophic. Upon histological examination, the adrenal hypertrophy during inanition involved the zona fasciculata, whereas the zona glomerulosa was atrophic. These findings are similar to those observed in the hypophysectomized animal following the administration of ACTH.

In contrast to the above findings, Rulinos and Pomerantz (1941) described atrophic changes in adrenals of rats half-fed for 14 days. Since pituitary implants restored the atrophic adrenals to normal size, they interpreted the atrophy as indicative of reduced ACTH secretion. Meites (1953) also observed that adrenal size of rats decreased on an absolute weight basis when rats were fed up to 1/4 food intake for 14 days. But both groups of workers found that complete food withdrawal for periods of 7-13 days, induced increases in both the relative and absolute weight of the adrenal, indicating that the degree of inanition is an important factor in determining its effects on the adrenals. Selye (1946) believed the apparent shift in the production of anterior pituitary hormones during severe inanition was due to the response of the pituitary-adrenal system (general adaptation syndrome), resulting in an increased secretion of essential ACTH at the expense of less critical anterior pituitary hormones.

Data are conflicting concerning the functional capacity of the pituitary to respond to stress during conditions

of dietary deficiencies. Sayers et al. (1950) reported that neither the formation nor liberation of ACTH is significantly altered from normal in the protein-deficient, acutely-fasted or chronically underfed rat. No significant differences were observed between the rate of ACTH secretion as judged by decrease in ACTH content of the pituitary, increase in adrenal weight and reduction in adrenal ascorbic acid after scalding. It was concluded by these authors that submutrition affects the functional capacity of the pituitary-adrenal system only slightly, in contrast to its marked effect on the pituitary-gonad system. Other workers have reported conflicting results. Koya et al. (1948) observed that the reduction in adrenal ascorbic acid concentration was significantly greater after exposure to cold in rats fed a high protein diet than after receiving a low protein diet. Handler and Bernheim (1950) demonstrated that diets poor in protein were incapable of maintaining the elevated blood pressure induced by nephrectomy. Hypertension was restored by the administration of ACTH. These latter two findings indicate that the elaboration of ACTH is markedly reduced on a low protein diet. Handler and Bernheim (1950) later reported that diets deficient in choline were equally effective in retarding the elevation of blood pressure brought about by nephrectomy. It may be that the effects of a low protein diet were due to a deficiency of a specific amino acid, or of labile methyl

groups and not protein per se.

Evidence for activation of the pituitary-adrenal system has been reported for animals fed diets deficient in ascorbic acid, pantothenic acid, thiamine, choline, and pyridoxine (Ershoff, 1952). If the deficiency is sufficiently prolonged, however, a state of adrenal cortical insufficiency occurs, possibly due to adrenal cortical exhaustion. This response is a characteristic manifestation of non-specific stresses and typical of the general adaption syndrome (Selye, 1946).

Nutrition and Mammary Growth and Lactation

Mammary growth. Sykes et al. (1948) found that the mammary glands of rats fed 70% of its normal intake showed less development than controls. White (1944) reported similar effects, using virgin female mice. However, this lack of growth was believed to be due to cessation of estrous cycles, since mice fed the same sub-caloric diet, but kept in continuous estrous by a subcutaneously inplanted pellet of diethylstilbestrol, showed considerable mammary development.

Normally rats on unrestricted diets respond to estrogen treatment with mammary growth. When rats were placed on a restricted diet and were injected with 5 mg estrone daily for 14 days, which normally elicited good mammary growth, Astwood et al. (1937) found that there was a lack

of growth. Trentin and Turner (1941) reported similar effects on the responsiveness of the male mouse mammary gland to estrogen during inanition. As the food intake was decreased to one-half normal consumption, the amount of estradiol required to produce a minimum degree of duct growth was proportionally increased. They explained these results as due to the indirect action on the mammary gland by the pituitary; the pituitary being physiologically depressed during inanition and therefore it responded to estrogen with a smaller output of the pituitary mammogenic factor. Meites and Reed (1949) measured the prolactin content of pituitaries of rats after various degrees of inanition, and found that prolactin was reduced below that of the full-fed controls. On the basis of hormone concentration per mg of pituitary, there was only a slight decrease.

Atrophy of the breasts is common in undernourished women, but a considerable number of soldiers developed an enlargement of the breasts during the war, where they were both underfed and malnourished. Histological examination of the glands showed hyperplasia of the ducts, and in some cases a secretion similar to colostrum was present (Keys et al., 1950). In most cases it was reported that gynecomastia occurred after prolonged malnutrition, but in many cases it did not develop until after the resumption of a normal diet. It is well known that gynecomastia may develop in liver diseases. Jacobs (1943) felt the inability of

the diseased liver to inactivate estrogens was the cause of gynecomastia. The appearance of the gynecomastia after the resumption of an adequate diet was thought to be due to an impairment of endocrine function, with an increase in estrogen production. Conclusive evidence of actual liver impairment and increased estrogen levels was not evident.

Lactation. There has been virtually no work concerning the nutrition endocrine interrelations during lactation. This can be partly explained by the difficulty in measuring milk production in rats.

It is well established that a decrease in food intake or caloric intake during lactation results in a reduction in milk yield. Nelson (1959) showed that lactational performance, as judged by weight of the young at weaning, decreased proportionately with progressive restriction of an optimal lactation diet. Similar results have been reported in the cow (Reece, 1959).

The predominant role of protein in regulating milk production has long been known. Meigs (1922) review of the literature, although limited to dairy cows, showed that the quantity of milk yield depended upon the quantity of protein supplied. Mueller and Cox (1946) showed that this was also true for the lactating rat. Nelson and Evans (1961) found that the weaning weight of young rats increased progressively with increased casein levels. The casein requirement for optimal lactation appeared to be between 24

and 30 per cent (20-25 per cent protein).

Diets deficient in sodium, potassium, or thiamine (Nelson and Evans, 1961) have severe effects on lactation. Any of these deficiencies beginning at parturation resulted in low weaning weights of the young. Average daily food intake was also markedly decreased. Whether these deficiencies have a direct effect on lactation or an indirect effect resulting from restriction in caloric and protein intake, requires further investigation. For the majority of dietary factors, accurate data on the changes in the milk yield as a result of deficiencies are lacking.

EXPERIMENTAL PROCEDURE AND RESULTS

Experiment I. Effects of Hormonal Administration Upon Milk Production in Underfed Rats

Procedure. One hundred five mature, female Carworth rats of the CFN strain were bred. Once pregnancy was established by palpatation of the abdomen, the rats were placed in individual cages. These cages were designed to make the dams' food inaccessible to the young throughout the experimental period, and the young's food intake consisted only of milk from the mother. The animals were kept in an air conditioned room at a temperature of 74± 1° F. The rats were fed a basic laboratory ration, and water was always available.

On the 4th day postpartum, the litters were reduced to eight pups per dam, and the food intake of the dams was reduced to 1/2 normal consumption. This was determined by measuring the daily food intake of ad libitum fed lactating rats. It was observed that as the period of lactation progressed, the amount of food consumed per day increased.

From the 4th to the 15th day postpartum, the dams and litters were weighed daily, at approximately the same time each day, and the dams were injected with a particular hormone or hormones. On the 15th day postpartum, the dams were sacrificed. The ovaries, uteri, adrenals, thymus,

thyroid, and pituitary were removed, cleaned, and weighed to the nearest 0.1 mg on a Roller-Smith balance.

The groups were treated as follows:

1. Controls, saline; fed ad libitum.
2. Controls, saline; 1/2 feed.
3. Bovine anterior pituitary (A.P.) powder, 15 mg; 1/2 feed.
4. Bovine anterior pituitary powder, 25 mg; 1/2 feed.
5. Growth Hormone, 1 mg; 1/2 feed.
6. Thyroxine, 10 ug/100 gm B.Wt.; 1/2 feed.
7. Hydrocortisone acetate, 0.5 mg; 1/2 feed.
8. Hydrocortisone acetate, 1 mg; 1/2 feed.
9. Prolactin, 1 mg; 1/2 feed (20 I.U./mg).
10. Oxytocin, 1 I.U.; 1/2 feed.
11. Hydrocortisone acetate, 1 mg; prolactin, 1 mg; and oxytocin, 1 I.U.; 1/2 feed.

All injections were given subcutaneously once daily, except for oxytocin which was given at uniform intervals four times daily. All solutions were made up to the required concentration by diluting with physiological saline.

It is difficult to measure accurately the amount of milk produced in small laboratory animals such as the rat, and it was necessary to employ indirect indices. In this experiment, litter growth rates were employed. This is only a relative index of lactational performance, since

the daily weight loss due to excreta and respiration were not measured.

Cowie (1947) used a logistic equation and plotted a mean growth curve for litters of lactating dams from birth to 16 days of age and obtained a sigmoid curve. From the 6th to 11th days of lactation, the points on the curve were on a straight line. He found that during this 5-day period the average increment in litter weights was approximately constant as well as at a maximum for each rat. The weight gain of the litters during this period, Cowie termed the "litter growth index" and he used it as a quantitative measure for lactational response in rats.

In this experiment the average litter weight gain was determined for the following periods:

1. 6th to 10th days postpartum (Cowie's litter growth index).
2. 11th to 13th days postpartum.
3. 6th to 13th days postpartum.

Results. The results of this experiment are tabulated in Tables 1-4. Tables 1 and 2 represent litter weight gains. The ad libitum fed controls (group 1) showed a greater gain during the 11-13 day period than in the 6-10 day period, whereas the 1/2 fed controls (group 2) gained less during the 11-13 day period. This indicated that the effect of underfeeding was more pronounced during the later part of lactation. Over the entire experimental period the litters

of the 1/2 fed control group (group 2) gained about 1/2 as much as the ad libitum fed controls (group 1).

Average litter weight gains during the 6-10 day, 11-18 day, or entire experimental period for the 1/2 fed groups which received A.P. powder (groups 3 and 4), growth hormone (group 5), and thyroxine (group 6) were not greater than the 1/2 fed controls (group 2). A.P. powder (group 4) and thyroxine (group 6) caused significant reductions in litter weight gains. In studies on ad libitum fed rats Meites (1959) reported that injections of 1 mg growth hormone daily to rats had no galactopoietic effect. Combinations of STH with prolactin and cortisone in hypophysectomized rats were no more effective than the latter two hormones alone (Meites, 1959). Similar results were obtained by Cowie (1957) using thyroxine.

The groups which received prolactin (group 9), the higher dose of hydrocortisone (group 8) and oxytocin (group 10), showed no significant increases over the 1/2 fed controls during the 6-10 day period or the entire experimental period. However, a slight but significant increase over the 1/2 fed controls occurred in these groups during the 11-18 day period. The litters of these groups gained about 23% more during this period than the 1/2 fed controls. The rats given prolactin, hydrocortisone acetate, and oxytocin in combination (group 11) showed the same trend as when these hormones were given individually. Thus, these hormones did not appear to have any synergistic effects

in producing average litter growth. Johnson (1937) reported that prolactin, cortisone acetate, and oxytocin, each could increase litter weight gains of ad libitum fed rats. However, Talwalkar (1959), using a similar experimental plan, found that only hydrocortisone acetate increased milk secretion significantly.

Tables 3 and 4 show the effects of the hormones administered on the body weights of the 1/2 fed lactating rats. The 1/2 fed controls (group 2) lost 23 per cent of their original weight during the experimental period, as compared to a gain of 9 per cent by the ad libitum fed controls (group 1). The administration of growth hormone (group 3), and the higher dose of A.P. powder (group 4) caused the dams to lose less weight than the 1/2 fed controls. Yet, these hormones showed no beneficial effects on milk production. The higher dose of hydrocortisone (group 8), which showed some beneficial effect on milk yield, induced a greater loss of body weight by the dams. There appeared to be no correlation between the dams' weight and litter growth rate.

Table 5 summarizes the effects upon the endocrine organs. The weights of the uteri, adrenals, thymus, thyroids, and pituitary of the 1/2 fed group decreased on an absolute weight basis. On an organ-body weight ratio they remained about the same, except for the adrenals which

increased, and the thymus which decreased in weight. This indicated increased ACTH adrenal cortical secretion. Administration of hydrocortisone acetate (groups 7 and 8) decreased the weight of the adrenals and thymus on both an absolute and relative body-weight ratio, indicating pituitary inhibition of ACTH secretion.

Table 1. Effects of A.P. Powder, Growth Hormone, and Thyroxine on Litter Weight Gains of Half-Fed Lactating Rats

Group Number and Treatment	Average Litter Growth Rate					
	6-10th days		11-15th days		4-18th days	
	Total gms.	Daily gms.	Total gms.	Daily gms.	Total gms.	Daily gms.
1.--Controls full-fed (10)*	57.5 ± 1.8**	11.5 ± 0.4	91.6 ± 2.1	11.4 ± 0.3	136.5 ± 5.9	12.4 ± 0.3
2.--Controls half-fed (10)	35.3 ± 1.3	7.1 ± 0.3	29.8 ± 1.7	3.7 ± 0.2	92.2 ± 3.0	6.1 ± 0.2
3.--A.P. Powder 15 mg.daily (5)	34.2 ± 2.9	6.8 ± 0.6	22.4 ± 1.3	2.8 ± 0.1	86.0 ± 4.2	5.7 ± 0.3
4.--A.P. Powder 25 mg.daily (5)	19.7 ± 3.2	4.0 ± 0.6	12.7 ± 3.0	1.6 ± 0.6	59.9 ± 7.1	4.0 ± 0.4
5.--Growth Hormone 1 mg.daily (10)	29.8 ± 1.7	6.0 ± 0.4	20.1 ± 2.2	2.5 ± 0.3	75.4 ± 5.6	5.0 ± 0.4
6.--Thyroxine 10 μgms/100 gms B.Wt. (10)	23.1 ± 1.1	4.6 ± 0.2	10.6 ± 2.1	1.3 ± 0.3	58.4 ± 2.1	3.9 ± 0.1

*No. of litters per group, 8 pups per litter.
**Standard error of the mean.

Table 2. Effects of Hydrocortisone Acetate, Prolactin, and Oxytocin Individually and in Combination on Litter Weight Gains of Half-Fed Lactating Rats

Group Number and Treatment	Average Litter Growth Rate					
	0-10th days		11-13th days		4-13th days	
	Total gms.	Daily gms.	Total gms.	Daily gms.	Total gms.	Daily gms.
1.--Controls Full-fed (10)*	57.5 ± 1.8**	11.5 ± 0.4	91.6 ± 2.1	11.4 ± 0.3	106.5 ± 5.9	12.4 ± 0.3
2.--Controls Half-fed (10)	35.3 ± 1.3	7.1 ± 0.3	29.8 ± 1.7	3.7 ± 0.2	92.2 ± 3.0	6.1 ± 0.2
7.--Hydrocortisone 0.5 mg. daily (5)	28.8 ± 1.8	5.8 ± 0.4	28.0 ± 0.8	3.5 ± 0.1	80.8 ± 2.7	5.4 ± 0.2
8.--Hydrocortisone 1 mg. daily (5)	35.0 ± 1.6	7.0 ± 0.3	36.0 ± 2.5	4.5 ± 0.3	96.8 ± 4.8	6.4 ± 0.3
9.--Prolactin 1 mg. daily (10)	33.7 ± 1.1	6.8 ± 0.2	36.4 ± 2.1	4.5 ± 0.3	96.4 ± 2.3	6.5 ± 0.1
10.--Oxytocin 1 I.U. 4 x daily (10)	32.3 ± 2.3	6.5 ± 0.5	38.7 ± 2.3	4.8 ± 0.3	93.0 ± 8.3	6.2 ± 0.3
11.--Hydrocortisone 1 mg. daily; Prolactin 1 mg.; daily; Oxytocin I.U. 4 x daily	32.6 ± 1.3	6.6 ± 0.3	37.3 ± 2.4	4.6 ± 0.3	96.7 ± 6.2	6.3 ± 0.2

*No. of litters per group, 8 pups per litter.
**Standard error of the Mean.

Table 3. Effects of A.P. Powder, Growth Hormone, and Thyroxine on the Body Weights of Half-Fed Lactating Rats

Group Number and Treatment	Body Weight--4 days Postpartum gms.	Body Weight--18 days Postpartum gms.	Change in Body Weight gms.	Per Cent Change Body Weight
1.--Controls Full-Fed (10)*	238.4 ± 6.5**	259.2 ± 5.8	+ 20.8 ± 3.4	+ 8.9 ± 1.4
2.--Controls Half-Fed (10)	256.0 ± 8.3	195.3 ± 5.6	- 60.7 ± 5.5	- 23.1 ± 1.6
3.--A.P. Powder 15 mg. daily (5)	254.2 ± 7.7	194.4 ± 4.0	- 58.8 ± 3.8	- 22.4 ± 1.1
4.--A.P. Powder 25 mg. daily (5)	253.2 ± 8.7	218.5 ± 5.6	- 34.7 ± 11.1	- 13.9 ± 3.9
5.--Growth Hormone 1 mg. daily (10)	256.7 ± 9.2	221.7 ± 5.0	- 35.0 ± 6.9	- 12.0 ± 2.4
6.--Thyroxine 10 μgms./100 gms. b.w.t. (10)	256.9 ± 3.8	184.0 ± 2.6	72.9 ± 3.6	- 27.3 ± 1.1

*No. of litters per group, 8 pups per litter.
**Standard error of the mean.

Table 4. Effects of Hydrocortisone Acetate, Prolactin, and Oxytocin Singularly and in Combination on the Body Weights of Half-Fed Lactating Rats

Group Number and Treatment	Body Weight--4 days Postpartum gms.	Body Weight--18 days Postpartum gms.	Change in Body Weight gms.	Per Cent Change Body Weight
1.--Controls Full-Fed (10)	238.4 ± 6.5	259.2 ± 5.8	+ 20.8 ± 3.4	+ 8.9 ± 1.4
2.--Controls Half-Fed (10)	256.0 ± 8.3	195.3 ± 5.6	- 60.7 ± 5.5	- 23.1 ± 1.6
7.--Hydrocortisone 0.5 mg. daily (5)	240.5 ± 9.6	183.2 ± 5.0	- 57.2 ± 7.7	- 22.8 ± 2.7
8.--Hydrocortisone 1 mg. daily (5)	251.6 ± 11.3	171.4 ± 5.3	- 80.2 ± 7.3	- 31.2 ± 1.5
9.--Prolactin 1 mg. daily (10)	237.3 ± 9.5	193.4 ± 2.7	- 43.9 ± 8.3	- 17.1 ± 3.0
10.--Oxytocin 1 I.U. 4 x daily (10)	257.6 ± 7.1	204.3 ± 4.0	- 54.4 ± 5.4	- 20.4 ± 1.9

Table 5. Effects of Hormones on Endocrine Organ Weights of Half-Fed Lactating Rats

Group Number and Treatment	Uterine Wt.		Ovarian Wt.		Adrenal Wt.	
	mg	mg/100 gms. B. Wt.	mg	mg/100 gms. B. Wt.	mg	mg/100 gms. B. Wt.
1.--Controls Full-Fed (10)*	200.2 ±8.9**	77.2 ±3.0	62.2 ±4.7	24.1 ±1.8	63.7 ±2.9	24.1 ±0.3
2.--Controls Half-Fed (10)	162.0 ±9.0	83.5 ±3.0	45.2 ±3.0	23.4 ±1.1	51.2 ±2.8	27.0 ±1.4
3.--A.P. Powder 15 mg daily (5)	239.5 ±7.5	123.8 ±3.4	42.6 ±2.1	22.0 ±0.6	50.4 ±2.4	26.5 ±2.1
4.--A.P. Powder 25 mg daily (5)	197.1 ±21.6	94.5 ±11.2	53.5 ±4.1	28.0 ±3.3	46.3 ±2.7	21.5 ±1.4
5.--Growth Hormone 1 mg daily (10)	211.7 ±10.9	98.1 ±6.6	45.9 ±1.7	21.2 ±0.9	55.3 ±4.0	25.5 ±1.8
6.--Thyroxine 10 γ/gms/100 gms. B. Wt. (10)	150.2 ±6.1	83.3 ±3.8	40.5 ±1.8	22.7 ±1.2	48.0 ±2.5	26.9 ±1.4
7.--Hydrocortisone 0.5 mg daily (5)	160.1 ±5.7	89.0 ±4.6	43.6 ±2.8	24.1 ±1.3	37.3 ±3.6	20.6 ±2.1
8.--Hydrocortisone 1 mg daily (5)	192.2 ±9.9	116.0 ±2.9	42.2 ±1.9	26.0 ±1.2	30.3 ±0.8	18.8 ±1.2
9.--Prolactin 1 mg daily (10)	158.0 ±8.2	82.3 ±3.5	43.6 ±3.2	22.7 ±1.7	50.6 ±2.9	26.3 ±1.4
10.--Oxytocin 1 I.U. 4 x daily (10)	172.8 ±12.6	88.7 ±7.4	51.4 ±3.2	26.3 ±1.8	49.7 ±7.2	25.3 ±1.2

*No. of litters per group, 8 pups per litter.
**Standard error of the mean.

Table 5 (continued)

Group Number and Treatment	Thymus wt. mg	Thymus wt. mg/100 gms.B.wt.	Thyroid wt. mg	Thyroid wt. mg/100 gms.B.wt.	Pituitary wt. mg	Pituitary wt. mg/100 gms.B.wt.
1.--Controls Full-Fed (10)	63.9 ±3.5	24.8 ±2.0	13.9 ±0.4	5.4 ±0.2	10.5 ±0.2	4.0 ±0.1
2.--Controls Half-Fed (10)	29.8 ±2.9	13.5 ±1.4	11.7 ±0.3	6.1 ±0.2	10.0 ±1.1	5.2 ±0.1
3.--A.P. Powder 15 mg daily (5)	26.9 ±3.9	13.7 ±1.9	13.6 ±1.4	7.2 ±0.9	9.3 ±0.7	4.9 ±0.8
4.--A.P. Powder 25 mg daily (5)	72.7 ±7.5	34.7 ±4.2	14.0 ±0.8	6.8 ±0.4	8.8 ±0.3	4.2 ±0.2
5.--Growth Hormone 1 mg daily (10)	51.3 ±5.3	14.4 ±1.2	11.2 ±0.4	3.1 ±0.4	9.0 ±0.4	4.1 ±0.2
6.--Thyroxine 10 µgms/100 gms.B.wt. (10)	22.4 ±3.3	12.6 ±1.9	9.3 ±0.5	5.3 ±0.3	8.3 ±0.3	4.7 ±0.2
7.--Hydrocortisone 0.5 mg daily (5)	11.3 ±0.2	6.0 ±0.4	11.0 ±1.1	6.1 ±0.6	9.4 ±0.4	5.2 ±0.3
8.--Hydrocortisone 1 mg daily (5)	13.2 ±0.9	8.0 ±0.5	10.4 ±0.5	6.2 ±0.2	8.7 ±0.5	5.2 ±0.2
9.--Prolactin 1 mg daily (10)	31.5 ±0.8	16.5 ±0.6	10.1 ±0.5	5.7 ±0.3	8.5 ±0.3	4.4 ±0.2
10.--Oxytocin 1 I.U. 4 x daily (10)	30.3 ±2.3	15.4 ±0.7	10.5 ±0.7	5.4 ±0.4	9.4 ±0.6	4.8 ±0.3

Experiment 2. Effect of Underfeeding Upon Mammary Growth

Procedure. Mature, female Carworth rats of the CFN strain were divided into the following groups of eight rats:

1. Controls, ad libitum fed.
2. Fed 1/2 of the controls food consumption per day for 14 days.
3. Complete food withdrawal for 14 days.

Each rat was weighed at the beginning and at the end of each experiment. Upon termination of the experiment, the rats were sacrificed and the ovaries, uteri, adrenals, thymus, and pituitary were removed, cleaned of extraneous tissue, and weighed on a Roller-Smith balance. The right inguinal mammary gland was dissected free, fixed in Bouins fluid, and prepared for gross mounting. The gross mounts were rated under a dissecting microscope for degree of development according to the following scheme:

I -- few ducts; few or no end buds

II -- moderate duct development; moderate number of end buds

III -- numerous ducts with many branches; many end buds

IV -- numerous ducts with many branches; limited lobulo-alveolar growth

Results. The effects on the mammary glands are

summarized in Table 6. The mammary glands from the ad libitum fed controls (group 1) consisted of fat pads with few or no end buds (Fig. 1); they were assigned a mean rating of 1.2. The mammary glands from the rats which received 1/2 feed for 14 days (group 2) were given a mean rating of 2.2. The 1/2 feeding in most cases induced moderate to good duct development with few to many end buds (Fig. 2). Complete food withdrawal for 14 days (group 3) induced good duct development, with many end buds; in some cases the glands exhibited limited lobulo-alveolar growth (Fig. 3). They were given a mean rating of 3.0.

Studies in ovariectomized-hypophysectomized or in adreno-ovariectomized-hypophysectomized rats have indicated that one hormone from the ovary and either prolactin or growth hormone are necessary for inducing ductal growth and end bud development, and prolactin as well as ovarian hormones are essential for inducing mammary lobulo-alveolar growth (Lyons, 1958). The adrenals are believed to be of secondary importance, although it is recognized that they secrete several steroids which can influence mammary growth. In intact or ovariectomized rats, injections of ACTH, cortisone or hydrocortisone can induce mammary growth and initiate mammary secretion (Selye, 1954; Johnson and Meites, 1955). Recently, Clifton and Furth (1960) observed complete lobulo-alveolar development in adreno-gonadectomized rats after implanting "mammatropic" pituitary tumors intramuscularly.

These pituitary tumors appear to secrete both STH and prolactin (Clifton and Furth, 1960). Talwalker and Meites (1961) attempted to see if large doses of these two hormones could induce lobulo-alveolar growth in adreno-ovariectomized and adreno-ovariectomized-hypophysectomized rats. Under these conditions lobulo-alveolar growth was induced by combined treatment with STH and prolactin, in the absence of ovaries and adrenals, while treatment with prolactin or STH alone induced mainly duct and end bud development. From these experiments, it was concluded that prolactin and STH in the absence of the ovaries and adrenals can induce mammary L-A development.

The results in Table 7 show that the weights of the thymus, uteri, and ovaries were all decreased considerably on an absolute basis as a result of inanition. The adrenals, however, maintained their original weight during the period of complete food withdrawal (group 2). On an organ body weight ratio the weights of the uteri and ovaries remained about the same as the ad libitum fed controls (group 1); the pituitary and adrenals increased, while the thymus decreased.

Table 8. Effects of Underfeeding on Mammary Growth in Rats

Group Number and Treatment	Av. wt. loss gms	% wt. loss	Mammary rating*				Av. rating
			I	II	III	IV	
1.--Controls Full-Fed (8)	--	--	6	2			1.2
2.--Half-Fed 14 days (8)	31	13	1	4	3		2.1
3.--No Food 14 days (8)	75	30	1	1	3	3	3.0

*See text for mammary rating system.

Table 7. Effect of Underfeeding on Endocrine Organ Weights of Rats

Group Number and Treatment	Adrenal Wt. mg/100 gms. B.wt.		Thymus Wt. mg/100 gms. B.wt.		Uterine Wt. mg/100 gms. B.wt.		Ovarian Wt. mg/100 gms. B.wt.		Pituitary Wt. mg/100 gms. B.wt.	
1.--Controls Full-Fed (8)	42.1 ±4.2	18.0 ±1.5	131.5 ±6.6	56.9 ±3.8	536.0 ±12.9	230.6 ±4.7	53.8 ±6.6	23.2 ±3.2	14.0 ±0.4	6.0 ±0.1
2.--Half-Fed 14 days (6)	39.3 ±4.9	20.5 ±2.7	91.3 ±9.3	26.8 ±5.2	464.5 ±18.3	241.8 ±9.1	41.3 ±2.4	21.5 ±1.6	13.3 ±0.7	6.9 ±0.1
3.--No Food 14 days (8)	51.5 ±2.9	34.6 ±1.4	44.0 ±2.5	25.2 ±1.7	322.6 ±25.8	176.7 ±15.7	32.4 ±2.2	18.4 ±1.3	12.1 ±0.4	6.9 ±0.1

Figure 1. Gross mount of a mammary gland from a rat fed ad libitum for 14 days. 7X

Figure 2. Gross mount of a mammary gland from a rat half-fed for 14 days. 7X

Figure 3. Gross mount of a mammary gland from a rat starved for 14 days. 7X

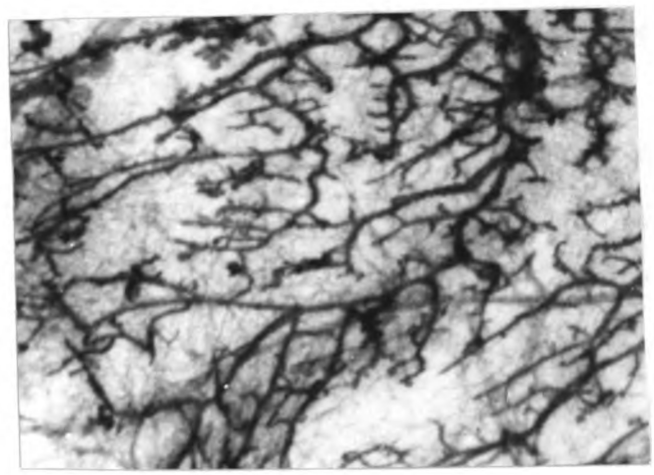

Figure 1

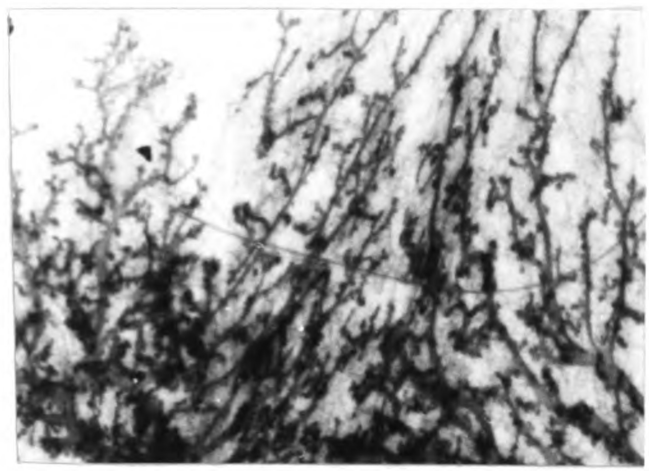

Figure 2

Figure 3

Experiment 3. Effects of Underfeeding Upon Involution of Post-partum Mammary Glands

Procedure. Mature, female Carworth rats of the CFH strain were bred. Once pregnancy was established, the rats were placed in individual cages. On the 4th day post-partum the young were removed and the mother rats were divided into two groups of eight rats. The mother rats were fed 1/3 the food consumed by ad libitum fed controls. On the 10th day postpartum, the dams were sacrificed and the right inguinal mammary gland was removed, fixed in Bouins, and stained with hematoxylin and eosin.

Results. Histological examination of the mammary glands revealed significant differences between the ad libitum and 1/3 fed groups. The mammary glands from the ad libitum fed controls (Fig. 4) 10 days after the young were removed showed marked involutionary changes. The alveoli were collapsed or in an advanced state of disintegration, and there was marked fibrosis of the lobules. One-third feeding for the same period of time maintained alveolar structure in six out of eight rats, when compared to the controls. The alveolar epithelium was more intact than in the controls, and in many areas secretory material was present in the alveoli; while none was seen in the controls. Prolactin (Hooker and Williams, 1941), oxytocin (Benson and Folly, 1956), and cortisone (Johnson and Bates, 1953)

have been shown to reverse mammary involution in the rat or rats. Prolactin and ACTH or cortisol demonstrated a similar ability to retard mammary involution in hypophysectomized rats (Meites and Hopkins, 1961).

Figure 4. Mammary gland from a rat fed ad libitum for 10 days after young have been removed. 140X. Stained with hematoxylin and eosin.

Figure 5. Mammary gland from a rat one-third fed for 10 days after young have been removed. 140X. Stained with hematoxylin and eosin.

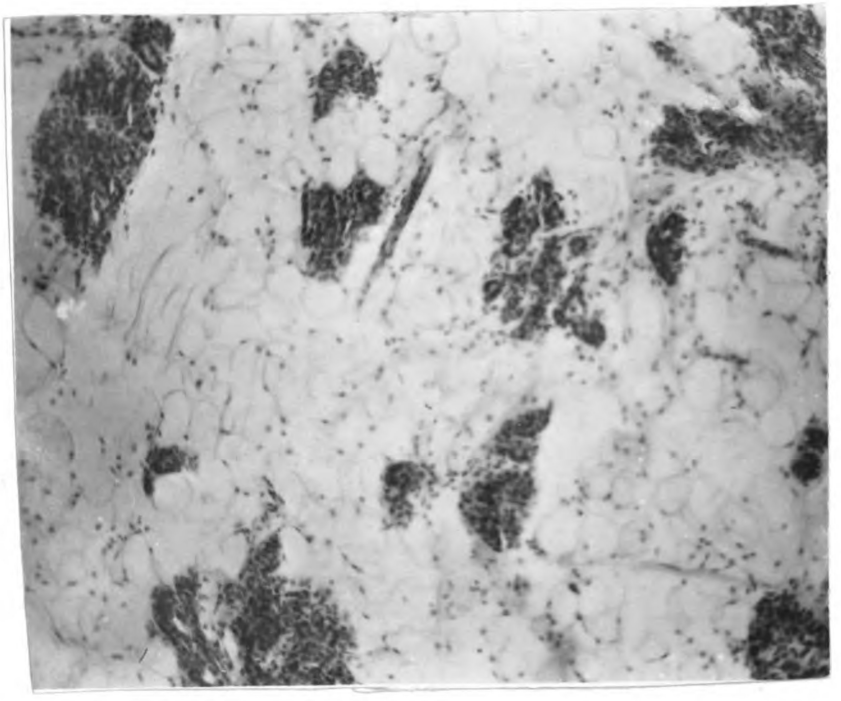

Figure 4

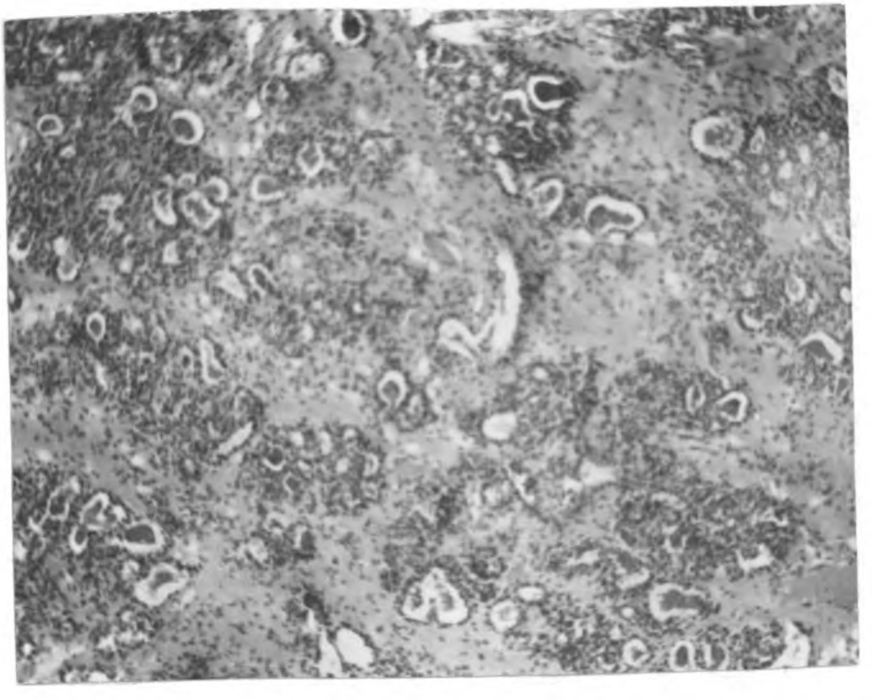

Figure 5

DISCUSSION

It is not surprising that underfeeding causes a marked reduction in milk yield as shown in Experiment 1, since the formation of milk depends upon the synthesis of a complex and energy rich substance. As a result of underfeeding, not only is the nutritional and caloric intake decreased, but there are probable alterations in metabolic processes. Some observed effects of reduced food intake are: a decrease in metabolic rate, a decrease in metabolic pools in tissues and organs, and alterations of levels of tissue and body fluids (Maynard, 1951). The maintenance of milk production has also been shown to be dependent upon the endocrines. Studies with hypophysectomized rats have shown that oxytocin, prolactin, and ACTH or glucocorticoids are the most essential hormones necessary to maintain lactation (Lyons, 1958; Chuvarungsin et al., 1957). To what extent, then, if any, are the endocrines responsible for the reduction of milk yield as result of underfeeding?

Secretion of most anterior pituitary hormones has been shown to be decreased by underfeeding and various nutritional deficiencies (Ershoff, 1952). Many of the hormonal malfunctions occurring as result of nutritional inadequacies have been corrected, to some extent, by the administration of hormones. Most notable of these has been

the ability to maintain pregnancy in deficient rats by the administration of estrogen and progesterone (Nelson, 1939). Here, as in milk production, we have a complex process of development (in this case an embryo), which is controlled by nutritional and endocrine factors. Yet, even in the case where protein was completely omitted from the diet, pregnancy was maintained by the administration of hormones. Would this apply to lactation, which is also a hormone dependent process?

In Experiment 1, the administration of hormones to underfed lactating rats failed to have any effect on litter weight gains during the entire period of lactation. However, the three hormones, prolactin, oxytocin, and hydrocortisone, which have been shown to be most essential for milk production, each demonstrated a slight ability to increase milk yields during the later part of lactation (11-18th days postpartum). These results suggest that the decrease in milk production from underfeeding was due primarily to lowered food intake and not to reduced endocrine function.

Srebnik et al. (1961) demonstrated that ovariectomy in protein-deficient rats greatly increased pituitary FSH and ICSH content, raised the circulating levels of FSH and brought about castration changes in the pituitary. These morphological and physiological changes show that the pituitary gland, even in the complete absence of protein, retained

its capacity to respond to the stimulus of ovariectomy with increased syntnesis and release of gonadotropins. The stress stimulus resulting from severe inanition similarly appears to act upon the pituitary to cause formation and liberation of ACTH (Selye, 1946). It seems then that the functional capacity of the pituitary is not completely inhibited by certain forms of sub-nutrition, and that it can respond to some stimuli with the synthesis and release of hormones.

The suckling stimulus during lactation has been shown to cause the release of oxytocin from the posterior pituitary (Petersen and Ludwick, 1942), and prolactin (Meites and Turner, 1948), and ACTH (Gregoire, 1946) from the anterior pituitary. It is believed that in the present study, the pituitary gland responded to the stimulus of suckling with the production of prolactin, ACTH, and oxytocin at optimal or near optimal levels for milk production. The slight positive effects of administering these three hormones during the later part of lactation could possibly have been due to the decrease in the strength and duration of the suckling stimulus as result of the malnourished condition of the pups and dams.

In Experiment 2, it was shown that mammary development can be induced in rats as result of underfeeding. Since the growth of the gland is dependent upon hormonal stimulation, some hormonal factor or factors were apparently secreted in increased amounts as result of inanition. In

contrast to most anterior pituitary hormones, ACTH has been shown to be secreted in increased amounts during periods of severe food restriction. Since ACTH secretion is increased in response to various forms of stress, this has been explained by considering severe food restriction as a stress condition (Selye, 1946). Nonspecific stresses have also been shown to cause the secretion of prolactin (Nicoll et al., 1960). In addition many other specific factors have been shown to induce ACTH and prolactin release (Meites et al., 1962), as indicated by initiation of mammary secretion in rats. In view of the ability of so many factors, including stress, to promote the release of ACTH and prolactin, it seems probable that prolactin was released together with ACTH as result of underfeeding.

Though the adrenals are believed to be of secondary importance in mammary growth, it is recognized that they secrete several steroids which can influence mammary growth. Johnson and Meites (1955) and Selye (1954) showed that the administration of ACTH and glucocorticoids induced growth of the mammary gland in intact and ovariectomized rats primed with estrogen. The increase in mammary growth which was observed during inanition could be attributed to the increased secretion of ACTH. The additional secretion of prolactin in response to dietary stress is indicated by the experiment of Nicoll et al. (1960). They found that starvation could induce limited mammary secretion in

estrogen-primed rats. The better development induced by complete food withdrawal is probably due to a difference in the amount of hormones released. Complete food withdrawal apparently acted as a stronger stress stimulus with a resultant greater hormone release than partial food withdrawal.

In Experiment 3, it was shown that underfeeding was able to retard mammary involution of the postpartum gland. Prolactin, ACTH, and oxytocin have each been shown ability to inhibit mammary involution in the rat (Meites, 1959). The increased secretion of ACTH and prolactin is believed responsible for the observed effects. It appears that the increased secretion of ACTH and prolactin in sufficient amounts to induce mammary development and retard mammary involution may depend upon the degree and length of food restriction. During periods of moderate restriction probably all the anterior pituitary hormones are secreted in reduced amounts. But this effect is not absolute, since under periods of severe restriction, the stress stimulus is apparently great enough to promote the secretion of ACTH and prolactin.

The close interrelationship between the central nervous system and anterior pituitary function (Harris, 1955) make it probable that inanition acts through the central nervous system. The pituitary gland, through its vascular or nervous connections with the nervous system, is responsive

to the stimuli from without, and it in turn affects its "target organs."

SUMMARY

1. In Experiment 1, the effects of administering anterior pituitary powder, growth hormone, thyroxine, oxytocin, prolactin, hydrocortisone acetate, and oxytocin, prolactin, and hydrocortisone acetate in combination, on the lactational performance of underfed rats were studied. The growth rate of litters of 8 young each during the 4th-15th days postpartum was used as an index of milk production.
2. None of the hormones injected into the underfed lactating rats produced any significant change in litter weight gains during the entire period of lactation.
3. There seemed to be no correlation between the mother's weight and the amount of milk produced. On an absolute weight basis all the endocrine organs of the underfed groups were decreased in weight. On an organ-body weight ratio they remained about the same, except for the adrenals which increased in weight and the thymus which decreased in weight. This indicated increased ACTH-adrenal cortical secretion.
4. These results suggest that underfeeding of suckled, lactating rats does not result in reduced endocrine function, and that the decrease in milk production cannot be overcome by hormone injection. It is thought

that the suckling stimulus is able to maintain the secretion of those hormones necessary for milk production at a near optimal level.

5. In Experiment 2, the effects of underfeeding upon mammary growth were studied. Mature, female rats received half-feed or no food for 14 days. Half-feeding induced mainly duct and end bud development; complete food withdrawal induced similar development with some rats showing moderate lobulo-alveolar growth.

6. On an absolute weight basis, all the endocrine organs decreased in weight during the periods of underfeeding, except for the adrenals, which increased their weight during the period of complete food withdrawal.

7. In Experiment 3, the effects of underfeeding on mammary involution were studied. On the 4th day postpartum litters from mother rats were removed and the mothers were fed 1/3 the food consumed by ad libitum fed controls for 10 days.

8. As compared with full-fed controls, underfeeding retarded mammary involution as judged histologically. The glands from the underfed group showed better maintenance of alveolar structure and in some areas secretory material was present.

9. It is thought that severe underfeeding can promote the secretion of ACTH and prolactin from the anterior pituitary in amounts adequate to induce mammary development and retard involution of the postpartum gland.

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