EFFECTS OF NUTRITIONAL DEFICIENCIES IN YOUNG RATS ON GONADAL RESPONSE TO PREGNANT MARES' SERUM

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EFFECTS OF NUTRITIONAL DEFICIENCIES IN YOUNG RATS ON GONADAL RESPONSE TO PREGNANT MARES' SERUM

By

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INTRODUCTION

A considerable but unresolved literature exists on the relation between nutritional deficiencies and reproductive disorders. Inasmuch as the reproductive system is largely under the direct control of the endocrines, it might be expected that the effects of dietary deficiencies would be manifested through the hormones. Thus, the secretion of certain hormones may be reduced, the reaction of target organs to these hormones may be decreased or the metabolism of these hormones may be altered.

With the exceptions of deficiencies of vitamin E and perhaps A, none of the other vitamin deficiencies have been shown to produce any specific effects on reproductive function. There is ample evidence that starvation depresses reproductive activity, and this is believed to be mediated through a depression of anterior pituitary function. It seems probable that many of the reported deleterious effects of vitamin deficiencies on sexual function were actually due to the concomitant inanition produced rather than to any specific vitamin lack per se.

In view of the foregoing, it was of particular interest to read the recent work of Hertz (1945, 1948). This investigator and his collaborators demonstrated that a deficiency of folic acid in chicks, rats and monkeys interfered with the growth-promoting effects of estrogens on the oviducts of these animals. Stimulated by these reports, it was decided to determine whether diets deficient in folic acid or in other factors would interfere with the action of a gonadotrophic hormone on the seminal vesicles and coagulating glands of young male rats.

Young male weanling rats were placed on various deficient diets for a period of twenty-eight days, and during the last four days were injected with a constant dose of pregnant mares' serum. This gonadotrophic substance contains a predominance of follicle stimulating hormone, but also considerable amounts of luteinizing (interstitial cell stimulating) hormone. The rats were sacrificed and the combined weights of the seminal vesicles and coagulating glands were used as a measure of gonadotrophic activity.

For the most part these studies were largely confined to the effects of B-vitamin deficiencies on gonadotrophic action. Although not all the B-vitamins deficiencies were induced, it appears on the basis of the data obtained that specific B-vitamin deficiencies did not interfere with the action of pregnant mares' serum except to the extent that they induced decreases in feed intake. The reduced feed consumption rather than any specific vitamin deficiency appeared to be responsible for the inhibition of gonadotrophic activity observed in these animals.

REVIEW OF LITERATURE

This review does not attempt to cover all or even most of the literature available on the interrelationships between nutritional deficiencies and reproductive function. Rather, it is an attempt to cover principally such literature as is pertinent to this thesis. Each of the nutritional deficiency-reproduction interrelationships is dealt with under a separate heading, although it is obvious that nutritional factors all influence each other to some extent.

Undernutrition

Loeb (1921) noted atrophic changes in the ovaries of guinea pig during chronic underfeeding. Marrian and Parkes (1929) noted ovarian atrophy and anestrus during partial inanition in rats. Moore and Samuels (1931) reported the presence of castrate types of prostates and seminal vesicles during partial inanition in rats. Breneman (1940), Selye and Collip (1936) Kurzrok et al., (1940) and Stephens and Allen (1940) all agreed that atrophy of the sex organs and anestrous conditions in undernourished animals resulted from diminished production of hypophyseal gonadotrophic hormones.

Stephens (1941) reported that chronic undernutrition is accompanied by structural and functional alterations in the hypophysis which result in decreased pituitary activity. He reported that in female guinea pigs, undernutrition of sufficient degree to cause a loss of twenty to thirty percent of body weight in a period of two weeks, resulted in atrophic and retrogressive changes in the ovaries similar in character and degree to those following hypophysectomy. These changes were not affected by

administration of vitamin supplements but were reversible with refeeding.

The ovaries of the undernourished guinea pigs remained responsive to stimulation by at least one of the gonadotrophic principles (LH) of the anterior pituitary. These observations suggested that changes occurring in the ovaries during inanition may be due partly to inability of the anterior pituitary to continue to produce sufficient gonadotrophic hormone to maintain the normal structure and function of the ovaries. (Stephens 1941).

Mulinos and Pomerantz (1939) reported that adult female rats which were acutely starved or chronically underfed ceased to have estrus when the body weight had dropped about fifteen percent. They found that rats could be brought to estrus by injections of estrogen even though the body weight was reduced by inanition to forty percent of normal. Injections of gonadotrophic hormone also brought these starved female rats to estrus. This indicated that the non-appearance of estrus may have been due to diminished production of gonadotrophic hormone rather than to inability of the ovaries to respond.

Mulinos and Pomerantz (1940) also studied the effects of inanition in male rats. Complete inanition unto death resulted in the destruction of an occasional seminiferous tubule, whereas prolonged chronic inanition resulted in severe atrophy of the testes and accessories. This picture resembled exactly that described by Smith (1930) in adult male rats which had been hypophysectomized. The testes and accessories were only about one fifth the normal weight in both conditions. The production of spermatozoa was abolished after hypophysectomy and diminished or absent

during inanition. There was generally no destruction of Sertoli cells or spermatogonia in either condition.

Both Mulinos and Pomerantz (1940) and Breneman (1940) presented evidence indicating that the reduction in size of the gonads and accessories during inanition is not due to loss of sensitivity by these organs to hormone stimulation. The former worker showed that injections of gonadotrophins into starved male rats reinitiated both spermatogenesis and androgen production. Breneman (1940) reported that injection of testosterone into underfed chicks produced a greater comb response than in normal chicks.

Folic acid

Hertz and Sebrell (1944) first noted an impairment in the response of the oviducts of chicks to diethylstilbestrol when fed a diet deficient in folic acid. This failure of estrogens to induce oviductal growth in the absence of folic acid has also been observed in the monkey (Hertz, 1948), frog (Goldsmith et al., 1948) and rat (Hertz, 1949). Andrus and Zarrow (1949) noted that estradiol, given to normal chicks, caused a sixty-eight percent increase in oviduct weight but had no effect on the weight of the oviducts of chicks receiving folic acid antagonist. Phosphatase occurred to a marked degree in the oviducts of the control chicks and only to a slight degree in the birds receiving the antagonist.

Hertz (1945) reported a quantitative relationship between the stilbestrol response and dietary folic acid in the chick. Chicks maintained on stock diet showed a forty-fold hypertrophy of the oviduct following stilbestrol administration, whereas folic acid deficient chicks showed

only a four-fold increase in oviduct weight. In folic acid supplemented chicks, the degree of oviduct-increase varied directly with the level of folic acid ingested. Doses of folic acid considerably in excess of that required for growth and hemopoiesis failed to restore the oviduct response to the level obtained in chicks on a stock diet. Concentrates containing folic acid from yeast and spinach similarly failed to completely replace the stock diet, but liver fractions increased the oviduct weight up to the same levels obtained on the stock diet.

Riboflavin, pyridoxine and pantothenic acid deficient chicks exhibited optimal oviduct responses, indicating that the failure of the estrogen response in folic acid deficient chicks was not simply a corollary of arrested body growth and general debility.

Hertz (1948) confirmed the above results in monkeys. Of eight sexually immature monkeys maintained on a folic acid deficient synthetic diet, six failed to show the characteristic normal response to estrogen administration. Two control monkeys fed the same diet plus liver, showed typical estrogen responses.

Hertz and Tullner (1949) studied the quantitive interference by folic acid antagonist to estrogen-induced tissue growth in rat and chicks. They used eight types of chemically pure folic acid antagonists. Some of the antagonists were relatively more effective in inhibiting the genital tract response to estrogen injections than others.

Haque et al., (1949) studied the effects of various vitamin deficiencies on the response of the oviducts of chicks to estrogen. They found a normal response in all of the vitamin deficiencies studied, except

in the case of folic acid. The response of the oviducts of the folic acid deficient chicks was less than one half that of the oviducts of the chicks receiving the normal diet. This confirms the observations of Hertz and Sebrell (1944) and Franklin et al., (1948).

Goldsmith et al., (1949) reported on the effects of folic acid deficiency in mice. The seminal vesicles and coagulating glands of testosterone-treated mice on a control stock ration showed the characteristic hypertrophy resulting from the androgen treatment. The other group was fed sixty grams of folic acid antagonist for sixty days and showed a reduction in response to testosterone. Therefore these authors concluded that the vitamin antagonist could interfere with the stimulation of the male sex accessories by high doses of male sex hormone.

These authors in a private communication (1949), came to the conclusion that the reduced growth response of the male accessories to testosterone noted in their folic acid deficient mice was probably not due to a lack of folic acid per se but rather to the concomitant inanition. It will be seen later that this view coincides with the results reported in this thesis.

Riboflavin

Warkany and Schraffenberger (1943) reported on reproduction of female rats fed a purified diet without riboflavin. Twenty-one female rats of the Sprague-Dawley strain, reared on an adequate diet and weighing from 170-240 gms. were put at the age of three months, on a purified diet lacking in riboflavin.

The female rats on the purified diet lacking in riboflavin showed regular estrus cycle for several weeks. Nineteen females became pregnant within three weeks. Eleven pregnancies ended in resorption while eight females delivered their litters at term. The pregnancies caused such great losses in eleven animals that they were considered unfit for further breeding. Of the eight that remained in the experiment four did not show estrus cycle within eight weeks after the first pregnancy. Four rats underwent a second pregnancy. One of these resorbed, while three delivered their litters at term.

They repeated these experiments in several other strains of rats and noticed the same congenital malformations on the same purified diet lacking in riboflavin.

About half of the female rats stopped having estrus cycles, lost considerable weight and showed signs of "malnutrition". They were unable to "restore" such animals by giving small doses of riboflavin, but these were unable to reproduce especially when their weight was reduced from the normal average of 200 gms. to 175 gm. or lower. Riboflavin administration caused some gain in weight within twenty-four hours, but after two or three days there was no further increase in body weight.

These authors concluded that the abnormalities described above were caused by a prenatal riboflavin deficiency. The mothers apparently had sufficient riboflavin for maintenance of estrus cycles and for gestation. The fetuses had sufficient riboflavin for growth but not for differentiation.

Thiamin

Mason (1929), Evans (1929) and Parkes (1927) studied the effects of various vitamin deficiencies on the mammalian reproductive system. Many rats were kept on a diet deficient in the antineuritic factor. They found that the testes of such rats were essentially normal, histologically, but the prostate gland and seminal vesicles were castrate in type as if the testicles had been removed some weeks before. It was obvious that the male reproductive accessories were incapable of reacting to the testicular hormone because of the general decline induced by the deficient diet.

Despite the fact that some animals had suffered a weight loss of forty percent, the animals on deficient diet were essentially normal in gross size and appearance. These organs, for the most part, contained histologically normal seminiferous tubules. The majority of the tubules not only showed quantities of mitotic figures but also a normal content of spermatozoa. The majority of rats at death contained spermatozoa in the epididymis that were capable of motility when suspended in normal saline solutions. The interstitial cells appeared somewhat atypical in that the cytoplasm was greatly reduced in amount and nuclei were somewhat atypical.

An examination of other rats made it apparent that similar conditions were produced on an inanition diet with an excess of vitamin B, as well as on unlimited diets lacking in vitamin B. In each group the testes were normal in size, and seminiferous tubules were in active spermatogenesis and showed but little or no destruction of germinal epithelium. The

prostate gland and seminal vesicles however, were castrate in type in most of the animals under experiment. The authors drew the conclusion that a general nutritive deficiency is basically responsible for the peculiar hormone state of these animals rather than any specific effect attributable to the absence of the antineuritic factor.

To summarize, the testes of male rats kept upon a diet deficient in the antineuritic fraction of vitamin B or on insufficient diets containing an excess of vitamin B, were found to consist of normal seminiferous tubules in active spermatogenesis but the reproductive accessories (prostate gland and seminal vesicles) were castrate in type. Daily injections of either testes or anterior pituitary hormone from pregnancy urine, caused the castrate condition to be replaced by the normal state within a period of ten days.

Drill and Burill (1944) noted that the absence of thiamin in a diet otherwise adequate causes a progressive decrease in food intake, resulting in loss of body weight and accompanied by cessation of estrus cycles. The same loss in body weight and cessation of cycles occurred even when thiamin was given, if the total food intake was reduced to correspond with that of the thiamin-deficient rats. Thus the effect of thiamin deficiency on the ovaries is not one of the thiamin deficiency per se, but rather due to the resulting inanition.

Pantothenic acid

The importance of pantothenic acid deficiency for normal reproduction and embryonic development in the chick has been stressed by several investigators (Bauernfiend and Norris, 1939; Gillis, Heuser and Norris, 1943;

and Pearson et al., 1945). Little is known about the effects of pantothenic acid deficiency on reproduction in rats. In general, it may be said that in chronic nutritional deficiencies with prolonged survival, the estrus cycle is irregular or absent (Evans and Bishop 1922). In severe, acute deficiency of pantothenic acid it is known that the majority of animals do not mature before they die (Figge and Allen 1942). Jukes (1940) mentioned the occurrence of resorptions in part of a group of stock rats placed on pantothenic acid deficient diets at mating. Sure (1941) noted that reproduction on purified diets without the addition of calcium pantothenate was very abnormal, since either "sterility" of the mother or a high incidence of stillbirths in the young resulted.

Nelson and Evans (1946) studied the reproductive phenomena in partial prolonged pantothenic acid deficiency in rats. Normal female rats, two to five months of age, were placed on pantothenic acid deficient diet on the thirteenth day of gestation. No significant difference in reproductive performance was noticed when compared to control groups. When the female rats were kept on an experimental diet starting the first day of gestation, marked upsets in reproductive function was noticed. Approximately one third of the rats underwent resorptions instead of littering. This confirmed the findings of Jukes (1940). The remainder of the rats littered but the average weight of the young at birth was significantly decreased.

Nelson and Evans (1946) also ran pair-fed control groups. This experiment indicated that in the deficient group three rats failed to implant, four rats resorbed and four rats littered. In the control group

restricted in calories (pair-fed), only two rats failed to implant and the remainder of the rats cast litters. The reduction in calories varied from forty-three percent to eighty-four percent.

These workers concluded that their experimental data indicated that the disturbance of reproductive phenomena in the deficient female rats was due to pantothenic acid deficiency, and not due to inanition.

It is of interest that many different conditions have been reported to cause resorption in the pregnant female rat: inanition (Barry, 1920) vitamin E deficiency (Evans and Bishop, 1922), vitamin A deficiency (Sure, 1928), a deficiency in essential fatty acids (Burr and Burr, 1930), a low protein intake (Guilbert and Goss, 1932), vitamin B deficiency (Ueno, 1934), filtrate factor deficiency (pantothenic acid) deficiency (Jukes, 1940) and riboflavin deficiency (Warkany and Nelson, 1942). In some of the studies reported the interpretation of results is difficult because of the concurrent effects of inanition and of one or more specific dietary deficiencies.

Pyridoxine

Nelson and Herbert (1946, 1947) reported that when pyridoxine was omitted from a purified diet, starting on the day of breeding in rats there were only slightly adverse effects on reproduction. This was evidenced principally by the occurrence of resorption in about ten percent of the rats. When the rats were placed on this pyridoxine deficient diet ten to twenty days prior to breeding, marked upsets in reproduction were observed. The percentage of resorptions increased directly with

extension of the deficient period. One hundred percent of the rats showed resorption when the pyridoxine deficiency was extended to twenty-two days before breeding.

Vitamin E

A deficiency of vitamin E in males induces degenerative changes in the germinal epithelium and disappearance of spermatogenesis without apparent morphological alteration of the intertitial cells. The failure of vitamin E deficient female rats to become pregnant is apparently due to disturbance of the implantation process rather than the absence of ovulation; there is no direct proof of ovarian dysfunction (Kaunitz et al., 1947; Blandau et al., 1949).

Since normal function of the ovaries and testes is dependent upon the anterior pituitary, several authors have compared the gonadotrophic potency of the anterior pituitary of normal rats with those on diets deficient in vitamin E. In males, Van Wagenen(1925) found castration changes in the basophils of the gland. Her results were extended and confirmed by Koneff (1939). The anterior pituitary of female E deficient rats showed morphological changes characteristics of spaying, according to Joel (1943). Both Koneff (1939) and Joel (1943) described a decrease in the number of oxyphils (acidophils).

Nelson (1931) reported that the increase in the gonadotrophic potency of the pituitary of the deficient E males approached that of the glands of castrates. He detected no difference in the amount of gonadotrophin in the pituitaries of normal and E deficient females. However McQueen-Williams (1934) concluded that glands from E deficient female rats

contain about twice as much gonadotrophin as those from normal rats.

In contrast to the foregoing reports, Biddulph and Meyer (1941) stated that pituitary powder from vitamin E deficient male or female rats produced the same weight change in the ovaries of hypophysectomized rats as pituitary powder from normal rats of the corresponding sex. However, they believed that male pituitaries from E deficient rats contained an increased amount of luteinizing (interstitial cell stimulating) hormone. Rowlands and Singer (1936) found that the pituitaries of E deficient female rats contained less gonadotrophin than normal rats, as determined by the production of ovulation in estrous rabbits.

P'an et al.,(1949) noticed that the weight of testes of immature hypophysectomized rats which received injections of anterior pituitary glands from either male or female vitamin E deficient rats was greater than those of rats receiving corresponding doses of the gland from normal male or female rats. These findings however, did not indicate whether the increased gonadotrophin content of the anterior pituitary of the vitamin E deficient rats was due to an increased production or to a decreased secretion or both.

Ever since Evans and Bishop (1922) established that rats require vitamin E for successful reproduction, the possible relationship of this factor to breeding troubles has been a subject of much interest to livestock breeders. There is a vast literature concerning vitamin E in its relation to reproduction in cattle. This has been recently reviewed by Asdell (1949). The information available indicates that it has not been established that cattle and other ruminants need vitamin E for successful

reproduction. It is significant that most of the studies reporting improvement in reproducing ability following vitamin E administration were conducted over relatively short periods with cattle on normal rations (Bay et al., 1934; Gunn, 1941; Jones and Ewalt, 1936). Frequently the treatments followed or occurred simultaneously with other forms of therapy.

Gullickson et al., (1949) performed experiments in Holstein and grade Jersey cattle to determine whether vitamin E is needed for successful reproduction. They fed the animals throughout their lives on rations containing liberal amounts of all known essential nutritional factors except vitamin E. They fed thirty calves of mixed breeding, twenty-two females and eight males. The data showed that reproducing ability of the cattle was not affected adversely by feeding vitamin E poor ration continuously through three generations. However, about a dozen animals died suddenly from the characteristic heart ailment described by Gullickson and Calverley (1946).

Vitamin A

The course of pregnancy may be severely altered by feeding female rats a diet restricted in vitamin A. The severity of the alteration appears to be roughly proportional to the degree of vitamin deprivation. Death and resorption of the fetuses, prolongation of pregnancy and delivery of stillborn young were reported by Mason (1935) and Cannon (1940), who employed deficient diets with little or no supplement of vitamin A or carotene.

Warkany and Schraffenberger (1944, 1946), using similar diets supplemented with small amounts of carotene, also noted a high incidence of

fetal resorption in rats. By interrupting pregnancy at various times from the thirteenth day to term, they were able to obtain a number of living and recently dead fetuses suitable for histologic studies. The eyes of these offsprings were found to bear several developmental anomalies, notably: folding and eversion of the retina, persistence of the choroidal fissure, absence of the ciliary body, and postlenticular fibroplasia. Thus deficiency of vitamin A in the diet of female rats prior to and during pregnancy not only reduces fecundity by causing fetal death and resorption, but also causes developmental anomalies in many of the fetuses that escape intrauterine death.

Vitamin D

Haque et al.,(1949) used chicks in experiments to find out whether vitamin deficiencies would interfere with the response to male and female sex hormones. The vitamin deficiencies were induced by giving low levels of a particular vitamin for a period of about six weeks. Male and female sex hormones were injected every alternate day during the last twelve days of the experiments. Testosterone propionate and alpha-estradiol were used as the sex hormones. Forty-eight hours after the last injection the birds were killed, and the combs and testes of males and oviducts of females were removed and weighed.

The results indicated that comb-growth by testosterone propionate was not prevented by any of the vitamin deficiencies except in the case of vitamin D. These authors suggested that the slightly smaller response in comb growth from testosterone propionate injections in the absence of vitamin D indicates that this vitamin is necessary to secure full androgenic stimulation.

Protein

Yamamoto and Chow (1950) studied the effects of low protein and poor protein diets on the ability of the gonads of rats to react to injections of gonadotrophic hormone. They found that the degree of hypertrophy of the sex organs was related to the nutritive value of the protein and to the amount of protein intake. Low protein or poor quality protein intakes resulted in poor gonadal responses to gonadotrophic hormone. It will be seen that these results, reported only in abstract form, are similar to the findings in the rats on low protein diets reported in this thesis.

PROCEDURE

The experiments reported were performed on weanling male rats of the Michigan State College and Carworth strains. In each experiment uniform groups of rats were placed on special diets for a period of twenty-eight days. During the last four days of the experimental period, all except the control rats were each injected with one-half Cartland-Nelson unit (ten International Units) of pregnant mares' serum (P.M.S.). The animals were sacrificed on the twenty-eighth day, and body weights as well as wbights of the seminal vesicles and coagulating glands were recorded. The testes weights were not recorded, since previous work (Meites and Chandrashakar, 1949) had indicated that P.M.S. had little effect on testes size.

A semi-synthetic diet was used in all except a few preliminary experiments in which it was desired to determine the effects of a commercial diet (Purina Laboratory Chow). The composition of the semi-synthetic diet was as follows:

Cerelose	62 gms.
Alcohol washed casein.....	25 "
Corn oil.....	5 "
Salt Mix. NO 2.....	4 "
Cod liver oil.....	5 "
Choline.....	100.0 mgm.
Ca Pantothenate.....	2.8 "
Niacin.....	1.0 "
Riboflavin.....	0.5 "
Thiamin.....	0.2 "
Pyridoxine.....	0.2 "
2,Me,1,4 Napthoquinone.....	0.04 "

The effects of low caloric intake on the response of the seminal vesicles and coagulating glands to P.M.S. were determined by giving

experimental groups of rats either 3/4 or 1/2 of the amount of feed consumed daily by ad libitum fed controls. The effects of low protein intake were produced by reducing the casein content of the semi-synthetic diet from twenty-five to either fifteen or ten grams per 100 grams of diet. Vitamin deficiencies were induced by omitting a particular vitamin from the diet, or by adding sulfasuccidine or antagonists of folic acid and biotin.

All animals were housed in an air conditioned room at a constant temperature of 75 degrees Fahrenheit. Fresh water was available at all times. The data relative to the effects of P.M.S. on the combined weights of the seminal vesicles and coagulating glands (henceforth referred to as 'seminal vesicles') were treated statistically. The standard error of the mean was determined by the following formula:

$$S.E. = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

Significant differences between means were determined by the following formula:

$$S.D. = \frac{m_1 - m_2}{\sqrt{E1^2 + E2^2}}$$

RESULTS

Effects of reduced caloric or protein intake on seminal vesicle response to P.M.S. in Michigan State College rats.

The results of this experiment are given in Table I. The first four groups of rats were fed Purina laboratory chow. Group 2, which received P.M.S., showed approximately a 100 percent increase (110.0±13.4 mg.) in the weight of the seminal vesicles as compared to group 1, which received no P.M.S. (48.1±3.4 mg.). Groups 3 and 4, which received respectively, only 3/4 and 1/2 of the feed consumed by the ad libitum fed rats of group 1, showed decreases in body weight and seminal vesicle response. However, on a 100 gm. body weight basis, the seminal vesicle response was the same as in the ad libitum fed rats.

The effects of the semi-synthetic diet on the reaction of the seminal vesicles to P.M.S. were similar to those obtained by feeding Purina laboratory chow. When caloric intake was reduced (groups 7 and 8), the body weights and seminal vesicle weights were correspondingly reduced, but the response of the latter to P.M.S. remained the same on a 100 gm. body weight basis. The reduction in protein (casein) intake also produced decreases in body growth and seminal vesicle response to P.M.S., but again the response of the latter to the gonadotrophin was the same on a 100 gm. body basis.

Effects of vitamin deficiencies on seminal vesicle response to P.M.S. in Michigan State College rats.

These data are given in Table II. Each of the vitamin deficiencies produced a decrease in growth, with the exception of group 9 which

TABLE I

EFFECTS OF REDUCED CALORIC OR PROTEIN INTAKE ON SEMINAL VESICLE RESPONSE TO P.M.S. IN MICHIGAN STATE COLLEGE RATS

Group	No. of rats	Diet fed	Was P.M.S. given?	Average initial body weight gm.	Average final body weight gm.	Average seminal vesicle weight mg.	Average seminal vesicle weight / 100 gm. body weight mg.
1	7	Purina chow	No	43.7	168.0	81.6	48.1 ± 3.4 *
2	7	" "	Yes	43.9	151.0	160.9	110.0 ± 13.4
3	5	3/4 " "	"	43.2	127.0	125.6	98.8 ± 17.2
4	6	1/2 " "	"	43.9	84.9	91.7	107.3 ± 6.2
5	7	Semi-synthetic	No	43.0	156.0	65.5	41.6 ± 2.0
6	6	"	Yes	43.0	154.0	156.4	99.2 ± 12.0
7	5	3/4 "	"	43.2	113.0	102.3	90.1 ± 10.9
8	7	1/2 "	"	43.4	89.7	91.5	102.6 ± 12.4
9	8	15% Casein	"	43.7	134.9	138.1	103.1 ± 12.9
10	7	10% "	"	43.7	86.3	87.6	95.4 ± 14.4

* Standard error of mean.

TABLE II

EFFECTS OF VITAMIN DEFICIENCIES ON SEMINAL VESICLE RESPONSE TO P.M.S. IN MICHIGAN STATE COLLEGE RATS

Group	No. of rats	Diet fed	Was P.M.S. given?	Average initial body weight gm.	Average final body weight gm.	Average seminal vesicle weight mg.	Average seminal vesicle weight 100 gm. body weight mg.	
1	7	Semi-synthetic	No	43.0	156.0	85.5	41.6±2.0 *	
2	9	"	Yes	43.6	150.0	160.2	107.0±10.5	
3	8	No Vitamin A	"	43.5	83.9	135.7	157.2±10.9	X
4	8	No Vitamin D	"	43.0	73.0	81.5	107.8±18.2	
5	6	No Thiamin	"	43.3	78.7	57.8	71.8±13.1	X
6	4	No Riboflavin	"	43.3	54.0	26.6	42.8±10.9	X
7	9	No Pyridoxine	"	42.6	91.9	85.2	93.0± 7.1	X
8	8	No Pantothenic Acid	"	43.9	90.7	72.7	80.3± 4.5	X
9	9	No Niacin	"	43.7	161.7	150.2	90.7± 8.0	
10	9	Biotin Antag. 1%	"	43.7	117.6	103.1	86.6± 6.9	X
11	10	Folic Acid Antag. 1%	"	43.0	92.4	60.3	64.4± 7.2	X
12	8	Sulfasuccidine	"	43.9	100.0	65.6	65.6± 6.2	X

* Standard error of mean.

X Significant differences:
groups 2&3-2.09 group 2&8-2.34
groups 2&5-2.09 group 2&11-3.35
groups 2&6-4.25 group 2&10-1.62
groups 2&7-1.10 group 2&12-3.39

received no niacin. Inasmuch as this group, like the others in the experiment, received twenty-five percent casein, it seems doubtful that a niacin deficiency was produced. Tryptophan which is present in large quantities in casein, has been shown to be converted to niacin in the body.

The omission of vitamin A from the semi-synthetic diet evoked only a small decrease in the response of the seminal vesicles to P.M.S. (group 3). On a 100 gm. body weight basis, it appeared to be responsible for a significant increase in the response to P.M.S.

The omission of thiamin and panthothenic acid (groups 5 and 8) or riboflavin (group 6) from the diet, or the induction of folic acid deficiency by feeding one percent of folic acid antagonist (group 10) or sulfasuccidine (group 12), all elicited significant decreases in the reaction of the seminal vesicles to P.M.S. The greatest inhibition in a seminal vesicle response to P.M.S. was produced in the riboflavin deficient rats. On a 100 gm. body weight basis, these rats appeared to show no response at all to P.M.S. The folic acid deficient rats showed the next greatest decrease in seminal vesicle response, while the effects of thiamin and panthothenic acid deficiencies were just on the borderline of significance.

Effects of folic acid antagonist and paired-feeding on a seminal vesicle response to P.M.S. in Carworth rats.

The previous experiment demonstrated that folic acid and riboflavin deficiencies were particularly effective in inhibiting the response of seminal vesicles to P.M.S. This and the following two experiments were designed to determine whether deficiencies of these vitamins per se or

the concomitant inanition evoked by their absence were responsible for the observed inhibition of seminal vesicle response to P.M.S. Inasmuch as Michigan State College rats were no longer available for these experiments, due to a failure in the animal room breeding program, Carworth rats were purchased and used.

Folic acid deficiency was induced by feeding the antagonist. The same amount of feed consumed daily by the vitamin deficient rats was fed to controls, but without folic acid antagonist or sulfasuccidine. The results are shown in Table III.

The weight of the seminal vesicles of the controls (group 1) was 123.1±14.0 mg. on a 100 gm. body weight basis, while the seminal vesicles of the P.M.S. injected rats weighed 253.1±14.2 mg. on a 100 gm. body weight basis. The rats which received 1/2 percent of the folic acid antagonist showed a marked decrease in seminal vesicle response to P.M.S. (group 3). However, the pair-fed rats (group 4) showed a similar reduction in seminal vesicle response, indicating that the decrease in feed intake alone could account for these results.

Effects of folic acid antagonist and paired-feeding on seminal vesicle response to P.M.S. in Carworth rats.

This experiment (Table IV) represents an extention and confirmation of the data obtained in the previous experiment. The folic acid antagonist (group 3) appeared to completely inhibit the response of the seminal vesicles to P.M.S. The pair-fed rats (group 4) showed a small response to P.M.S. which was just on the border line of significance.

TABLE III

EFFECTS OF FOLIC ACID ANTAGONIST, AND PAIRED-FEEDING ON SEMINAL VESICLE RESPONSE TO P.M.S. IN CARWORTH RATS

Group	No. of rats	Diet fed	Was P.M.S. given?	Average initial body weight gm.	Average final body weight gm.	Average seminal vesicle weight mg.	Average seminal vesicle weight / 100 gm. body weight mg.
1	6	Semi-synthetic	No	52.3	154.7	183.9	123.1±14.0 *
2	8	"	Yes	51.9	158.1	401.4	253.1±14.2
3	10	Folic Acid Antag. 0.5%	"	52.8	93.9	148.8	157.4±10.7
4	10	Pair-fed as above group	"	51.5	98.3	179.4	179.0±17.3 X

* Standard error of mean.

X Significant differences: groups 3 and 4 -- 1.06

TABLE IV

EFFECTS OF FOLIC ACID ANTAGONIST AND PAIRED-FEEDING ON SEMINAL VESICLE RESPONSE TO P.M.S. IN CARWORTH RATS

Group	No. of rats	Diet fed	Was P.M.S. given?	Average initial body weight gm.	Average final body weight gm.	Average seminal vesicle weight mg.	Average seminal vesicle weight / 100 gm. body weight mg.
1	8	Semi-synthetic	No	52.8	152.7	170.0	123.3±12.3*
2	7	"	Yes	52.8	153.4	309.8	202.3±12.8
3	8	Folic Acid Antag. 0.5%	"	52.1	101.6	121.0	117.7±4.3
4	10	Pair-fed as above group	"	51.1	99.4	138.4	139.0±9.7
5	8	Folic Acid Antag. 0.5%	"	52.5	95.6	124.0	127.5±9.5
6	9	Pair-fed as above group, plus 10-fold increase in B vitamins	"	52.5	99.4	148.5	149.8±10.8

* Standard error of mean.

Significant differences:
groups 3 and 4 -- 2.00
groups 5 and 6 -- 1.55

In order to insure that pair-fed rats received an adequate B vitamin intake despite reduced caloric intake, these vitamins were increased in the semi-synthetic diet ten-fold above the amounts ordinarily given (see Procedure) and fed to group 6. Although the response in this group was slightly greater than in folic acid deficient rats (group 5), there was no statistically significant difference between the two groups.

Effects of riboflavin deficiency and paired-feeding on seminal vesicle response to P.M.S. in Carworth rats.

This experiment was performed in order to determine to what extent inanition was responsible for the reduced response to P.M.S. previously noted in riboflavin deficient rats. The results are given in Table V. It can be seen that when riboflavin was omitted from the diet (group 3) there was a significant reduction in the body weight and in the response of the seminal vesicles to P.M.S. However, the pair-fed rats (group 4) showed a comparable response to P.M.S. on a 100 gm. body weight basis.

The effects of riboflavin deficiency and paired-feeding were further compared (groups 5 and 6), except that in the pair-fed rats the B vitamins were increased ten-fold to insure an adequate vitamin intake. The response of the seminal vesicles to P.M.S. in the two groups of rats was the same on a 100 gm. body weight basis.

TABLE V

EFFECTS OF RIBOFLAVIN DEFICIENCY AND PAIRED-FEEDING ON SEMINAL VESICLE RESPONSE TO P.M.S. IN CARWORTH RATS

Group	No. of rats	Diet fed	Was P.M.S. given?	Average initial body weight gm.	Average final body weight gm.	Average seminal vesicle weight mg.	Average seminal vesicle weight / 100 gm. body weight mg. *
1	8	Semi-synthetic	No	52.8	152.7	170.0	121.3±12.3
2	7	"	Yes	52.8	153.4	309.8	202.3±28.1
3	10	No Riboflavin	"	51.1	83.2	126.9	148.7±15.3
4	10	Pair-fed as above group	"	52.2	99.0	146.2	147.2±14.1
5	9	No Riboflavin	"	52.8	80.2	138.7	167.3±14.4
6	9	Pair-fed as above group, plus 10-fold increase in B vitamins	"	52.8	91.2	152.8	166.3± 7.5

* Standard error of mean.

DISCUSSION

In these experiments it was found that a reduction in feed allowance, or omission of individual vitamins which resulted in reduced feed intake, both caused a depression in the rate of body growth. With the exception of deficiencies of folic acid and riboflavin (and probably thiamin and pantothenic acid) the decreases in the response of the seminal vesicles to P.M.S. were in direct proportion to the reduced body growth of the rats.

In the rats made deficient in folic acid and riboflavin, the decreases in the response of the seminal vesicles to P.M.S. could not be accounted for solely on the basis of reduced body growth. On a 100 gm. body weight basis, the response of these rats was significantly lower than in the normally-fed rats. However, by using the paired-feeding technique, it was shown that the reduction in caloric intake was primarily responsible for the adverse effects of the folic acid and riboflavin deficiencies on gonadotrophic action.

Insofar as the effects of folic acid deficiency on P.M.S. action is concerned, these results would seem to be in agreement with the report of Goldsmith et al., (1949, private communication) that inanition rather than folic acid deficiency caused the reduced response of the male accessories to testosterone in their mouse experiment. Apparently the male accessories, unlike the female oviduct, do not need folic acid in order to respond to their specific growth hormone (androgen).

It is unfortunate that the same strain of rats could not be used throughout these experiments. The Michigan State College and Carworth strains were not strictly comparable in their reactions to the various

vitamin deficiencies and P.M.S. administration. Thus, in the case of the Michigan State College strain, a one-half reduction in caloric intake decreased the gain in body growth (to 91.5 grams instead of 154.0-156.0 grams) and produced a corresponding reduction in the reaction of the seminal vesicles to P.M.S. The body growth of the Carworth strain on folic acid or riboflavin deficiency and in the pair-fed groups was similarly reduced (to 95-100 grams instead of 153.0-159.0 grams), but the seminal vesicle response to P.M.S. was depressed below that which could be accounted for on the basis of body weight alone. This only further emphasizes the importance of using uniform strains of rats in such experiments.

It seems well established that reduced caloric intake results in a decrease in anterior pituitary function. However, in these experiments it seems unlikely that the pituitary could have been an important factor, since it has been demonstrated that the target organs of undernourished animals do not decrease in responsiveness to injected hormones. It must be recognized that the action of P.M.S. on the seminal vesicles is not a direct one, but is mediated through stimulating the production of androgen by the interstitial tissue of the testes.

It cannot be said that the question of whether vitamin or other nutritional deficiencies can affect gonadotrophic action in male rats has been definitely answered by these experiments. Except in the case of thiamin, riboflavin and vitamin D, extreme deficiencies were not produced as judged by the decreases in body weight gains. Also the age of the animals and the length of time they were on deficient diets must be

considered. Alterations in any of these factors could have led to different results from those reported here.

It does not seem unreasonable to suppose that tissues may need certain vitamins in addition to sufficient caloric intake in order to respond to endogenous or exogenous hormones. Although it appears that inanition rather than any B-vitamin deficiency was responsible for the decreased response of the seminal vesicles to P.M.S., the fact remains that the deficiencies of B vitamins were responsible for the inanition. In short, these animals were unable to obtain the full energy present in the carbohydrates, fats and proteins of the diet without the aid of the B vitamins. Thus, it would seem reasonable to conclude that, although B vitamins may not be needed for specific reproductive functions (with the possible exception of folic acid for estrogen action on the oviduct), they probably are needed by the body as a whole in order to obtain the calories from the energy producing foodstuffs.

SUMMARY

1. The effects of nutritional deficiencies on the response of seminal vesicles and coagulating glands to pregnant mares' serum were studied in weanling male rats of the Michigan State College and Carworth strains. These animals were placed on various caloric, protein or vitamin deficient diets for a period of twenty-eight days, and during the last four days they were injected with a constant dose of pregnant mares' serum (P.M.S.). The combined weights of the seminal vesicles and coagulating glands were used as a measure of gonadotrophic action.

2. In the first experiment the effects of caloric and protein deficiencies were determined. The ad libidum fed controls showed approximately a 100 percent increase in the response of the seminal vesicles and coagulating glands to P.M.S. The rats which were fed either 3/4 or 1/2 of the amount of feed consumed by the ad libidum fed controls showed a reduced response to P.M.S. However, when computed on 100 gm. body weight basis, the response of the male accessories to P.M.S. in these rats was the same as in the ad libidum fed controls.

3. A reduction in protein (casein) intake from twenty-five to either fifteen or ten percent resulted in a reduced response to P.M.S. However, on a 100 gm. body weight basis, these animals responded as well to P.M.S. as the controls which received twenty-five percent protein.

4. In another experiment the effects of various vitamin deficiencies were determined. It was found that deficiencies of the following

vitamins induced a decrease in the rate of body growth and a corresponding decrease in the response of male accessories to P.M.S.: vitamins A, D, biotin and pyridoxine. Deficiencies of riboflavin and folic acid (and possibly thiamin and pantothenic acid) produced reductions in the response of the male accessories which could not be accounted for by the reduction in body weight alone.

5. In two experiments it was decided to determine whether the reduced response to P.M.S. obtained by a folic acid deficiency could be accounted for on the basis of the concomitant inanition. A folic acid antagonist (x-methyl-folic acid) was used to produce the folic acid deficiency. The rats which were restricted to the same caloric intake as the folic acid deficient rats, responded almost the same to the P.M.S. In two of the three paired-feeding experiments there was no significant differences in the response of the seminal vesicles to P.M.S., while in the third paired-feeding experiment the folic acid deficient rats showed a lower response. Increasing the B-vitamin intake ten-fold in one of the pair-fed groups did not increase the response to P.M.S.
6. The effects of riboflavin deficiency and paired-feeding were compared in four groups of rats. The response of the seminal vesicles to P.M.S. under both nutritional treatments was the same. Increasing the B-vitamin intake ten-fold in one of the pair-fed groups did not increase the response to P.M.S.
7. It is concluded that none of the nutritional deficiencies tested in the above experiments induced a significant reduction in the response

of the seminal vesicles and coagulating glands to pregnant mares' serum when computed on a 100 gm. body weight basis, with the exception of folic acid and riboflavin deficiencies. When rats were pair-fed in accordance with the daily feed intake of the folic acid and riboflavin deficient animals, the response of the seminal vesicles and coagulating glands to P.M.S. of the two groups was essentially similar. Thus the effects of deficiencies of these two vitamins on the seminal vesicle response to P.M.S. could be attributed to the concomitant inanition.

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THESIS ABSTRACT

EFFECTS OF NUTRITIONAL DEFICIENCIES IN YOUNG RATS ON GONADAL RESPONSE TO PREGNANT MARES' SERUM

By

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The effects of nutritional deficiencies on the response of seminal vesicles and coagulating glands to pregnant mares' serum were studied in weanling male rats of the Michigan State College and Carworth strains. These animals were placed on various caloric, protein or vitamin deficient diets for a perief of twenty-eight days, and during the last four days they were injected with a constant dose of pregnant mares' serum (P.M.S.). The combined weights of the seminal vesicles and coagulating glands were used as a measure of gonadotrophic action.

In the first experiment the effects of caloric and protein deficiencies were determined. The ad libidum fed controls showed approximately a 100 percent increase in the response of the seminal vesicles and coagulating glands to P.M.S. The rats which were fed either 3/4 or 1/2 of the amount of feed consumed by the ad libidum fed controls showed a reduced response to P.M.S. However, when computed on 100 gm. body weight basis, the response of the male accessories to P.M.S. in these rats was the same as in the ad libidum fed controls.

A reduction in protein (casein) intake from twenty-five to either fifteen or ten percent resulted in a reduced response to P.M.S. However

on a 100 gm. body weight basis, these animals responded as well to P.M.S. as the controls which received twenty-five percent protein.

In another experiment the effects of various vitamin deficiencies were determined. It was found that deficiencies of the following vitamins induced a decrease in the rate of body growth and a corresponding decrease in the response of male accessories to P.M.S.: vitamins A, D, biotin and pyridoxine. Deficiencies of riboflavin and folic acid (and possibly thiamin and pantothenic acid) produced reductions in the response of the male accessories which could not be accounted for by the reduction in body weight alone.

In two experiments it was decided to determine whether the reduced response to P.M.S. obtained by a folic acid deficiency would be accounted for on the basis of the concomitant inanition. A folic acid antagonist (x-methyl-folic acid) was used to produce the folic acid deficiency. The rats which were restricted to the same caloric intake as the folic acid deficient rats, responded almost the same to the P.M.S. In two of the three paired-feeding experiments there was no significant differences in the response of the seminal vesicles to P.M.S., while in the third paired-feeding experiment the folic acid deficient rats showed a lower response. Increasing the B-vitamin intake ten-fold in one of the pair-fed groups did not increase the response to P.M.S.

The effects of riboflavin deficiency and paired-feeding were compared in four groups of rats. The response of the seminal vesicles to P.M.S. under both nutritional treatments was the same. Increasing the B-vitamin intake ten-fold in one of the pair-fed groups did not increase the response to P.M.S.

It is concluded that none of the nutritional deficiencies tested in the above experiments induced a significant reduction in the response of the seminal vesicles and coagulating glands to pregnant mares' serum when computed on a 100 gm. body weight basis, with the exception of folic acid and riboflavin deficiencies. When rats were pair-fed in accordance with the daily feed intake of the folic acid and riboflavin deficient animals, the response of the seminal vesicles and coagulating glands to P.M.S. of the two groups was essentially similar. Thus the effects of deficiencies of these two vitamins on the seminal vesicle response to P.M.S. could be attributed to the concomitant inanition.

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