

REPRODUCTIVE CAPACITY OF FEMALE RATS AS AFFECTED BY KINDS OF CARBOHYDRATES IN THE RATION¹

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INTRODUCTION

When experimental rats used in a study of galactose metabolism² failed to reproduce normally, it was decided to determine the character of the abnormality and also, if possible, the relation of the carbohydrate in the ration to the reproductive capacity of the rat.

LITERATURE

The effects on reproduction of moderate amounts of sucrose and lactose in the rations of rats have been reported by several workers. Mitchell (6)³ found that lactose, when constituting not more than 30 percent of the ration or 50 percent of the total carbohydrate in the ration, did not interfere with growth or well-being. Skinner, Van Donk, and Steenbock (8) found that ovarian function, as measured by length and regularity of oestrus cycles, was improved by the addition of 10 percent of sucrose to a ration of mineralized milk. Keil, Keil, and Nelson (4), however, reported interference with reproduction as a result of the addition of 5 percent of sucrose to a differently mineralized milk ration.

Evans (3) stated that a lack of vitamin E has no effect on the ova or ovarian tissue. In a comparison of vitamin E deficiency with that of vitamins A and B, he stated that vitamin A deficiency is characterized by a continuous cornified smear.

Smith and Engle (10) found that anterior pituitary transplants stimulated the ovaries of immature and mature rats, producing an excess of follicles. Ovulation occurred in the young animals. In older animals there were some follicular cysts, and there was also some luteinization. The cysts were sometimes twice the size of normal follicles. Engle and Smith (2), in a study of corpus luteum formation in pituitary-stimulated animals, found that follicles did not rupture, but formed cysts which transformed directly into corpora lutea. Sometimes eggs were found "trapped" as a result of luteinization of the follicular wall. Smith (9) observed that hypophysectomy of the rat resulted in a regression of the ovaries. If corpora lutea were present, they persisted for a long time. Transplants of anterior pituitary stimulated these regressed ovaries to renewed activity but follicular and lutein cysts resulted. Casida and Hellbaum (1), using 21- to 25-day-old rats, produced large follicles and

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² These animals were under the care of G. N. Woodruff of Kansas State College.

³ Reference is made by number (*italic*) to Literature Cited, p. 532.

corpora lutea by injections of extracts from adrenal glands of horses. They obtained ovulation in about 50 percent of the treated animals. They made no attempt to determine whether this effect was direct or indirect. Moore and Price (7) concluded from their work that the only gonad-stimulating hormones are hypophyseal or hypophyseallike substances.

MATERIALS AND METHODS

The rations fed were designed to be adequate in every known respect. One group of rations contained a minimum amount of galactose while the other group contained lactose which provided a liberal supply of readily available galactose.

The regular experimental rations (designated according to the characteristic carbohydrate) are shown in table 1.

TABLE 1.—Composition of experimental rations

Food product	Sucrose	Lactose	Starch
	Percent	Percent	Percent
Yeast.....	5	5	4
Osborne and Mendel salt mixture.....	4	4	5
Cod-liver oil.....	1	1	1
Casein.....	20	20	20
Butter oil.....	20	20	20
Starch.....	30	30	50
Sucrose.....	20		
Lactose.....		20	

Commercial lactose was used for all but one group of lactose-fed rats. The substitution of Mallinkrodt's "analytical reagent" lactose for this group produced no change in the results. No further attempt was made to differentiate lactose from factors which might be associated with it as the important constituent of the ration.

These rations were fed to many groups of rats during a period of more than 2 years. The early groups included several females for which the reproductive record is not complete enough to justify detailed description but which furnished the incentive for the detailed records set forth in this paper.

Some of the rats were mated in order to test fertility. Ages at which the vaginas opened were determined. Microscopic observations were made on the ovaries of some animals, and the uteri of these animals were examined macroscopically. Serial sections of the ovaries were made for microscopic study. In some cases one ovary and part of one horn of the uterus were removed by laparotomy. Since little difference was noted between the remaining ovaries of these animals and ovaries from other animals receiving the same feed, they have been included in the appropriate age and feed groups.

OBSERVATIONS

Seven animals fed lactose, five fed sucrose, and two fed starch were kept with fertile males for 40 days or until pregnancy was detected. During the time of this test all the animals were changed from the experimental rations to the regular stock ration. The results are presented in table 2.

TABLE 2.—The effect of feeding various carbohydrates on the reproductive ability of rats

Animal no. ¹	Carbohydrate fed	Age of animal		Size of litter	Condition of young
		When placed with male	When litter was born		
		Days	Days	Number	
70a.....	Lactose.....	79	107	10	Normal.
78a.....	do.....	79	105	5	All died within 3 days.
84b.....	do.....	79	102	7	Normal.
90b.....	do.....	79	105	5	Do.
121c.....	do.....	56	80	4	1 died, 3 normal.
125c.....	do.....	56	93	8	Normal.
129d.....	do.....	48	85	8	Do.
1.....	Sucrose.....	80	-----	0	
2.....	do.....	70	-----	0	
3 ²	do.....	70	111	3	2 died, 1 weakly.
87b.....	do.....	79	-----	0	
119c.....	do.....	56	80	-----	Abortion.
123c.....	Starch.....	56	94	10	Normal.
127c.....	do.....	48	87	7	Do.

¹ The animals having like letters were litter mates.

² After producing 1 litter, this female was kept with a fertile male for 90 days without producing another litter.

As table 2 shows, each lactose- and starch-fed animal reproduced. All these litters, except possibly one born to a lactose-fed female, were normal. The one sucrose-fed female which produced a live litter was afterward sterile.

The age at which the vagina opened and the type of vaginal smears were determined for 17 sucrose-, 4 starch-, 6 stock-, and 18 lactose-fed animals. The results are presented in table 3. The sucrose-fed

TABLE 3.—Effect of feeding various carbohydrates on age at which vaginas of rats opened, and types of smears secured

Ration fed	Animals	Age at which vagina opened			Standard deviation	Vaginal smear record
		Youngest	Oldest	Average		
	Number	Days	Days	Days		
Sucrose.....	17	32	51	38	4.8	1-3 cycles, then dioestrus.
Starch.....	4	45	53	49	3.8	Not observed.
Stock.....	6	51	60	55	3.4	Normal cycles.
Lactose.....	18	45	64	59	6.7	Do.

animals showed sexual activity, as measured by the age at which the vaginas opened, earlier than any of the other groups. There is considerably less than 1 chance per 1,000 that this difference is due to chance. The smears of the sucrose-fed animals, however, showed only a few oestrus cycles. The lactose-fed animals were slightly late in reaching sexual maturity but showed normal cyclic changes in the smears. The starch- and stock-fed groups consisted of only a few animals, but these showed no abnormalities. They closely resembled the lactose group.

The observations made on the ovaries of the animals fed sucrose and those fed lactose are shown according to age groups in table 4.

TABLE 4.—Effect of feeding sucrose and lactose in relation to microscopic observations of the ovaries of rats in various age classes

Animals (number)	Age	Carbohydrate fed	Corpora lutea	Luteinization of follicles	Atresia ¹
	<i>Days</i>				
3.....	37	Sucrose.....	+	+	—
2.....	37do.....	—	+	—
1.....	37	Lactose.....	—	—	+
5.....	44-47	Sucrose.....	+	+	—
2.....	44-47	Lactose.....	—	—	+
10.....	64-72	Sucrose.....	+	+	+
10.....	64-72	Lactose.....	² +	+	+
6.....	64-72do.....	—	—	+

¹ Atresia indicates that follicles are so degenerated that they should soon disappear.

² 3 animals in this group had fresh rupture points in some of the follicles. Eggs were found in the Fallopian tubes of 2.

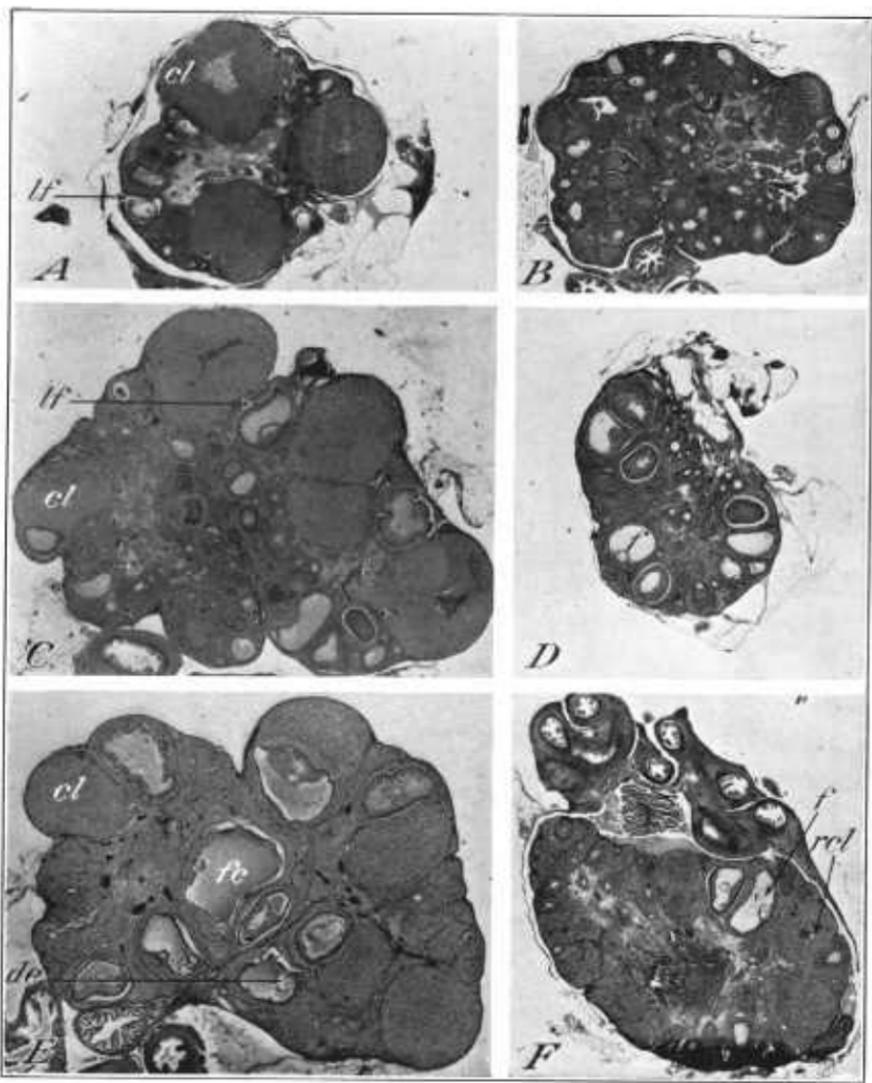
Photomicrographs of typical sections from ovaries of sucrose- and lactose-fed animals of each age group are shown in plate 1, *A* to *F*. These sections all have the same magnification, but they do not represent the relative sizes of the ovaries from different animals.

The presence of luteinized follicles in the sucrose-fed animals of all age groups would account for the lack of atretic disappearance of follicles. Luteinization of immature follicles would also explain the failure of the follicles to rupture.

As table 4 shows, the sucrose-fed animals were precocious. Corpora lutea were found in the ovaries of some of these animals at 37 days of age (pl. 1, *A*). In sharp contrast to this, the lactose-fed animal of this age was practically in an infantile sexual condition. Small follicles only were present in the ovary (pl. 1, *B*).

At 44 to 47 days of age similar differences existed as in the 37-day-old animals but they were even more striking. The sucrose-fed animals showed many more corpora lutea, luteinization of the larger follicles, and only a few small follicles (pl. 1, *C*). There was some indication that the follicles were being transformed into corpora lutea without a rupture occurring; at least no rupture points were observed. Some of the large luteinized follicles contained eggs that were degenerating. The lactose-fed animals, on the other hand, contained ovaries with follicles of all sizes (pl. 1, *D*). There was no luteinization of follicles among lactose-fed animals.

In the age group 64 to 72 days, many large corpora lutea were found in the ovaries of the sucrose-fed animals. There were no cyclic ages of corpora lutea, and no small follicles were present. Some cysts due to luteinization of the large follicles were found, but none of the corpora lutea showed signs of degenerating as would be expected if normal cycles were occurring (pl. 1, *E*). There was considerable variation among the lactose-fed animals. Some had not yet ovulated, showing some indication of retardation. In most cases, however, fresh corpora lutea and normal follicles of different sizes were found (pl. 1, *F*). In some of the lactose-fed animals the rupture points were visible, and eggs were found in the tubes of two animals. More than one age of corpora lutea was observed in the ovaries of some animals. There was no luteinization of follicles.



A. Left ovary from a 37-day-old sucrose-fed rat, showing large corpora lutea (cl) and small luteinized follicles (lf). B. Left ovary from a 37-day-old lactose-fed rat, showing many small follicles (f). There is no luteinization of any follicles. Some atresia is present. C. Right ovary from the same animal as A, at 45 days of age. A sucrose-fed rat, showing large corpora lutea (cl) and luteinized follicles (lf). No normal-appearing follicles of any size. D. Right ovary from the same animal as B, at 45 days of age. A lactose-fed rat, showing follicles of different sizes (f). No corpora lutea and no luteinization of follicles. E. Ovary from 70-day-old sucrose-fed rat, showing many corpora lutea (cl) and luteinized follicles, some containing degenerating eggs (de). Some follicles are so heavily luteinized that cysts have resulted (jc). F. Ovary from 70-day-old lactose-fed rat, showing follicles of different sizes (f) and recent corpora lutea with blood clots (rcf). There is no luteinization of follicles.

The uteri of the sucrose-fed animals of all age groups were generally larger than those of the corresponding lactose-fed animals. Some were distended with fluid and some were highly vascular.

Ovaries of three lactose-fed animals were observed about 30 days after the animals had given birth to litters. The normal cyclic ages of corpora lutea were present. Ovaries of four other animals aged about 200 days were also observed. These had been on a sucrose ration until about 70 days of age. Afterward they had been on the regular stock ration. The ovaries were made up almost entirely of masses of corpora lutea and follicular cysts. No evidence of cycles was seen, and there was indication of the cysts transforming into corpora lutea.

DISCUSSION

With our present knowledge of nutrition it is difficult to understand how the replacement of either the 20 percent lactose, or 20 of the maximum 50 percent starch, with 20 percent sucrose in the ration could have produced the observed sexual precocity and luteinization of follicles. A few instances have been reported in which sucrose in the ration was associated with abnormal reproduction. The observed abnormalities, however, were not linked with either abnormal ovarian structure nor with any pituitary disturbances. The absence of galactose might possibly be associated with the results produced by the sucrose ration. Sorensen and Haugaard (11) have shown that casein contains a small amount of galactose. It is possible that the additional cornstarch contained sufficient galactose to meet the need and make possible the observed relations of the three rations studied. It might, on the other hand, be assumed that the sucrose affected some gland which secreted a gonad-stimulating substance. Since most work indicates that the pituitary is the only gland which could have produced this effect, there is the possibility that the sucrose either directly or indirectly affected the activity of the pituitary.

The results obtained were similar to those produced by pituitary implants (10). One difference, however, is that no excessive number of follicles or corpora lutea were present. The luteinization of follicles and formation of cysts and the persistence of the corpora lutea were more like the results produced by implanting pituitaries into hypophysectomized rats (9). The transformation of follicular cysts into corpora lutea is similar to results obtained by Engle and Smith (2). The similarity of the ovarian abnormalities in the writers' animals to defects noted by other workers when the pituitary function was altered is strong evidence that some pituitary disturbance existed.

The possibility that a vitamin deficiency might have brought about the abnormalities noted is very small. The symptoms observed were not the characteristic symptoms of any vitamin deficiency. The smear record and ovarian observations are far from indicating a deficiency of either vitamin A or E.

When the mixture of purified amino acids recently announced (5) becomes available as a substitute for proteins, it may be practical to determine more definitely what characteristics of the carbohydrates studied are associated with the results secured in this investigation.

SUMMARY

In this study of the relation of the carbohydrate in the ration to the reproductive capacity of rats, three carbohydrates were fed, sucrose, lactose, and starch.

The sucrose-containing ration was found to be inadequate for normal reproduction in rats, even when replaced during adult life by a normal ration.

Rats receiving the sucrose ration were precocious in sexual development.

Extreme luteinization of follicles and persistence of corpora lutea were characteristic of the ovaries of the sucrose-fed animals. This adequately explains the failure of these animals to reproduce.

The ovarian abnormalities of the sucrose-fed animals indicate pituitary disturbances.

Animals fed the lactose- and starch-containing rations reproduced normally and had normal ovarian structures.

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