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Effects of Dietary Fat on Mammary Development Relative to Age and Hormones in BALB/c Mice (42295)

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Abstract. Earlier studies reported that mammary ducts grew faster if the 10% fat in the diet was composed of oils containing polyunsaturated fatty acids (corn oil: CO) compared to hydrogenated cottonseed oil (HCTO), which is devoid of such fatty acids. These experiments were primarily carried out in immature mice and left unanswered questions regarding the effects of dietary fats on more differentiated stages of mammary development. The use of transplanted ducts permitted the study of mammary growth rates in adult mice. If the diet was started when the animals were adults, there was no difference in the growth rate of those fed HCTO diet compared to those fed CO diet. However, when the diets were fed to immature mice, the mammary gland grew slower in mice fed the HCTO diet, confirming our earlier observations. The HCTO and CO diets caused no difference in the growth rate or morphology of fine ducts and alveoli that developed during pregnancy. Furthermore, no differences were seen in female mice following 6 weeks of progesterone administration begun at 3 week of age. Experiments that used male mice to examine the initial stages of mammary duct growth also showed that the effect of dietary fat was not observed when estrogen (E) or E and progesterone (P) were injected. In addition, there was no effect of dietary fat in ovariectomized 3-week-old females when any dose of E was administered from 0.01 to 1 μg/day. Examination of the ovaries from mice fed either HCTO or CO diets from 3 to 9 weeks or 3 to 13 weeks of age showed that mice fed HCTO diet did not develop corpora lutea, while those fed CO diet had normal appearing ovaries. The HCTO diet inhibits normal maturation of the follicle and corpus luteum formation. We conclude that the effect of the dietary fat on the developing mouse is on the maturation of the ovary and subsequently on mammary growth.

The composition of the fats in the diet has been shown to effect mouse mammary gland growth (1–3). Mammary ducts grew faster and formed more secondary and tertiary ducts in mice fed diets containing corn oil (CO) than in those fed diets with hydrogenated cottonseed oil (HCTO) (2, 3). The CO is high in polyunsaturated fats (60% linoleate) compared with HCTO, in which there is no linoleate. Earlier experiments were performed in sexually immature mice. As a result they did not clearly distinguish between the direct effects of dietary fats on the mammary epithelium and potential secondary effects mediated through endocrine organs such as the ovary.

In order to distinguish between these effects we have performed a series of experiments, some of which used the mammary gland transplant technique (4, 5). This technique permitted the study of duct development, which normally occurs in the first few weeks of life, in adult mice. Additional studies of mammary gland development were conducted on male and female mice which were treated with hormones or underwent pregnancy while being fed the test diets. Both mammary duct transplants and entire mammary glands were used, since our earlier studies had shown comparable response to the test diets of transplants and intact mammary glands (3).

Methods and Materials. *Animals and diets.* All animals used were BALB/c mice that were purchased from Simonsen Laboratories, Gilroy, California, or bred in our laboratory from mice purchased from Simonsen. The mice were held five or six per cage and allowed access to food and water *ad libitum.* The mice were housed in temperature controlled rooms (22–23°C) on a 12-hr light–dark cycle.

Three diets were used in these studies. The stock diet was Purina Laboratory Chow No. 5020 (chow) containing 9% fat of which 44% of the constituent fatty acids was linoleate. The test diets were based on a fat-free dry mixture

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that contained, by weight, 55% glucose, 24% vitamin-free casein, 3.5% mineral mixture as described in AIN recommendation of 1976 (6), 2.2% vitamin mixture (vitamin diet fortification mix, Nutritional Biochemicals, Cleveland, Ohio), 0.3% DL-methionine, 5% cellulose, and 0.01% butylated hydroxytoluene. Enough diet to last 1 week was prepared by the addition of 10 g of either CO or HCTO to each 90 g of fat-free dry mixture. The two test diets were isocaloric and differed only in their fatty acid composition. The fatty acid composition of the CO and the HCTO has been reported previously (2).

Effects of age of host when diets fed. The experiments reported in Table 1 examined the effect on mammary gland growth of the age of the mouse when fed the test diets. The data for groups A and B were taken from an earlier report in which mammary duct growth was examined after 4 and 6 weeks, respectively, during which time the mice were fed either CO or HCTO test diets. In all other groups, 3-week-old mice had their developing glands removed from both inquinal fat pads using a technique described previously (4, 5). The mice were then held until they were 8 or 17 weeks of age, at which time each fat pad was transplanted with a piece of duct (groups C–F) or a portion of hyperplastic outgrowth line $Z5C_1$ (groups G and H). The duct transplants were primary ducts obtained from 6- to 8-week-old BALB/c females. The characteristics of the outgrowth line (HPO) have been described in an earlier publication (7). The mice were then allowed free access to the semipurified diets for 3 or 6 weeks, starting at the time they were either 3, 8, or 17 weeks of age, as indicated in Table I.

At termination, both inguinal fat pads and a portion of the host glands were removed for whole mount preparation (5, 8). The stained

TABLE I. THE EFFECT OF TIME OF DIETARY FAT ADMINISTRATION COMPARED TO TIME OF TRANSPLANTATION ON THE GROWTH OF MAMMARY DUCT AND HYPERPLASTIC OUTGROWTH LINE (HPO) TRANSPLANTS

	Transplant			Diet fed		Growth of mammary epithelium			
Group	Tissue	Age of host (week)	Time in host (week)	Age of host (week)	Kind	Number of takes/ transplant	%	% Pad filled[a]	P^b
A	Duct[c]	3–7	4	3–7	HCTO	16/16	100	23 ± 24	—
					CO	16/16	100	81 ± 12	0.001
B	Duct[c]	3–9	6	3–9	HCTO	14/16	88	60 ± 22	—
					CO	16/16	100	95 ± 6	0.001
C	Duct	8–11	3	3–11	HCTO	11/25	44	30 ± 32	—
					CO	19/20	95	56 ± 33	0.05
D	Duct	8–11	3	8–11	HCTO	7/20	35	11 ± 4	—
					CO	15/20	75	29 ± 26	NS
E	Duct	8–14	6	8–14	HCTO	13/15	87	92 ± 15	—
					CO	9/9	100	100	NS
F	Duct	17–20	3	17–20	HCTO	12/12	100	66 ± 19	—
					CO	9/10	90	49 ± 28	NS
G	HPO	8–11	3	3–11	HCTO	11/11	100	22 ± 11	—
					CO	10/10	100	24 ± 8	NS
H	HPO	8–14	6	8–14	HCTO	15/16	94	69 ± 10	—
					CO	9/9	100	59 ± 11	NS

[a] Mean percentage of mammary fat pad filled by mammary epithelium ± standard deviation.
[b] Probability from Duncan–Kramer multiple range test that the CO group is different from the HCTO group. NS = not significant.
[c] Intact mammary glands were studied in groups A and B.

and cleared preparations were examined using a dissecting microscope and the results are given in Table I.

Effect of diet on mammary development during pregnancy. Mammary lobules transplanted into previously cleared fat pads of adult females will not grow as lobulo-alveolar structures. Initially, lobule transplants grow as ducts and then, if the animal is pregnant, alveoli are formed (9). In order to examine the effect of dietary fat on alveolar growth, mice in the initial days of pregnancy were fed CO or HCTO diets. In these mice the ducts had already filled the mammary fat pads and the test diets would be present only during lobulo-alveolar growth. Ten BALB/c females were isolated on the first day of pregnancy as judged by the presence of a vaginal mucus plug following overnight caging with a male mouse. Half of the isolated mice were placed on CO diet and half on HCTO diet. The mice were maintained on the diets for 18 to 20 days and then sacrificed. The intact inguinal mammary glands were removed and placed in Tellyesniczky's fixative for preparation of whole mounts. The stained whole mounts were evaluated using a dissecting microscope and judged for the extent of lobular development.

Hormone-stimulated male mice. The next experiment examined the initial stage of duct growth of the mammary gland using castrated male mice given hormonal stimulation. A total of 76 BALB/c males were castrated at 3 to 5 weeks of age and divided into eight groups based on three variables: hormones administered, length of time the hormones were administered, and test diet used. The hormones given were either 3 μg estradiol valerate (E) twice a week or the same dose of E plus 3 mg of progesterone (P) twice a week in triolein. The E solution was prepared by diluting Estradiol Valerate Injectable (E.R. Squibb & Sons, Inc., Princeton, N.J.) with triolein. The P was purchased from Sigma Chemical Company, St. Louis, Missouri. The hormones and test diets were given concurrently for 3 or 6 weeks prior to termination of the experiment. The numbers of mice in each group and the results are shown in Table II.

Ovariectomized immature female mice given E. In preliminary studies using E dosages of 1 or 3 μg per day, which were reported to be effective in male C57B1 mice (10), the growth of mammary ducts in immature female mice appeared to be inhibited. Consequently, we decided to examine the effect of smaller doses and shorter times of hormone administration on mammary gland growth in ovariectomized mice. BALB/c mice were ovariectomized when 3 weeks old and then injected with E every third day for 3 or 5 weeks starting at 4 weeks of age. Each subcutaneous injection of 0.1 ml of triolein contained either 3.0, 1.5, 0.75, 0.3, 0.03 μg or no estradiol valerate. The average daily dose as shown in Table III was 1.0, 0.5, 0.25, 0.1, 0.01 or no μg E per day. Half of each group of mice were fed the CO diet and half the HCTO diet. The mice were terminated when 7 or 9 weeks old and the intact inguinal mammary glands removed and fixed for whole mount preparation.

Intact female mice given P. This experiment examines the effect of administration of P on the growth of the mammary glands in intact female mice fed either the CO or HCTO diets. The hormone was dry packed into a piece of Silastic tubing that was sealed at either end with surgical cement and placed in the interscapular subcutaneous tissue (11). Instead of progesterone the synthetic steroid chlormadinone (Syntex Corporation, Palo Alto, Calif.) was used to provide a dose equivalent to 1 mg of progesterone per day. The hormone tubes were inserted in 3-week-old mice which were then started on either CO or HCTO diets. The mice were sacrificed at 9 weeks of age and the intact inguinal mammary glands removed for whole mount preparation. The number of glands studied in each treatment group and the results are shown in Table IV.

Histology of ovaries from mice fed test diets. The ovaries from mice that had been fed CO or HCTO diets from 3 to 9 weeks of age or 3 to 13 weeks of age were studied. The ovaries were removed, paraffin embedded, and sectioned at 6 μm for light microscope examination. One section through the medial plane of each ovary was examined and the number of primary, secondary and mature follicles, as well as corpora lutea, corpora albicans and atretic follicles, were counted. The representative section technique does not give a count of the number of each of the listed structures in the entire ovary, but it does allow a com-

TABLE II. EFFECT OF DIETARY FAT ON MAMMARY GLAND GROWTH IN CASTRATED MALE BALB/c MICE INJECTED WITH ESTROGEN OR ESTROGEN AND PROGESTERONE

Hormone	Time (week)	Diet	Glands Grew/Exam.	%	% Fat pad filled	Probability Diet[c]	Probability Hormone[d]
Estrogen[a]	3	HCTO	2/10	20	10 ± 0	—	—
		CO	5/10	50	22 ± 16	NS	—
	6	HCTO	5/12	42	20 ± 10	—	—
		CO	3/10	30	13 ± 6	NS	—
Estrogen + progesterone[b]	3	HCTO	21/26	81	19 ± 8	—	NS
		CO	18/26	69	18 ± 8	NS	NS
	6	HCTO	20/26	77	72 ± 16	—	0.001
		CO	25/32	78	74 ± 21	NS	0.001

[a] Dose 3 μg E twice weekly.
[b] Dose of 3 μg E and 3 mg P twice weekly.
[c] Probability from Duncan–Kramer multiple range test that the CO group is different from the HCTO group. NS = not significant.
[d] Probability from Duncan–Kramer multiple range test that E vs E + P groups are different in the same time and diet grouping.

parison of structures present in the ovaries from mice fed the CO or HCTO diets. The results are shown in Table V.

Statistical analysis. Comparisons between two means were made with the use of Student's *t* test, whereas comparisons among several means were made by one-way analysis of variance; the Duncan–Kramer multiple-range test was used to determine statistical differences. Only those comparisons in which $P < 0.05$ were considered to be significantly different.

Results. *Age of host.* The results of the experiments in which pieces of duct or hyperplastic outgrowth line $Z5C_1$ were transplanted into hosts of different ages are reported in Table 1. The amount of mammary duct growth in sexually immature hosts fed CO diets was significantly greater than those fed the HCTO

TABLE III. GROWTH OF MAMMARY GLANDS IN BALB/c MICE OVARIECTOMIZED AT 3 WEEKS OF AGE AND INJECTED WITH VARIOUS DOSES OF ESTRADIOL VALERATE AND FED CO OR HCTO DIETS

Approx daily dose of E (μg)	Injection time (week)	HCTO diet Number	HCTO diet % FPF[a]	CO diet Number	CO diet % FPF[a]	Probability Diet[b]	Probability Hormone[c]
0.0	5	12	11 ± 3	6	12 ± 4	NS	—
1.0	5	10	89 ± 10	12	84 ± 12	NS	<0.001
0.5	5	12	98 ± 6	12	99 ± 3	NS	<0.001
0.25	5	12	98 ± 5	12	100	NS	<0.001
0.25	3[d]	12	46 ± 7	12	50 ± 7	NS	—
0.10	3	10	58 ± 13	12	57 ± 7	NS	—
0.01	3	14	60 ± 15	14	59 ± 14	NS	—

[a] Mean percentage of mammary fat pad filled by ducts ± standard deviation.
[b] Probability from Duncan–Kramer multiple range test that the CO group is different from the HCTO group at a given dose and time of hormone administration. NS = not significant.
[c] Probability from Duncan–Kramer multiple range test that the groups injected with E for 5 weeks were different from the no hormone controls.
[d] Probability that the mice given 0.25 μg E per day for 3 weeks were different from those given 0.25 μg E for 5 weeks was <0.01.

TABLE IV. EFFECT OF EXOGENOUS PROGESTERONE ON MAMMARY GLAND GROWTH IN 3-WEEK-OLD BALB/c FEMALE MICE FED EITHER CORN OIL (CO) OR HYDROGENATED COTTONSEED OIL (HCTO) DIETS FOR 6 WEEKS

Diet	Hormone[a]	Number of glands	% Fat pad filled[b]	Probability		Mammary morphology
				Diet[c]	Hormone[d]	
HCTO	None	16	67 ± 30	—	—	Ducts with end buds
	Progesterone	22	99 ± 2	—	0.001	Lobulo-alveolar
CO	None	16	95 ± 7	0.002	—	Ducts
	Progesterone	18	97 ± 7	NS	NS	Lobulo-alveolar

[a] Silastic implants releasing 1 mg P/day.
[b] Percentage of fat pad filled ± standard deviation.
[c] Probability from Duncan–Kramer multiple range test that the CO group is different from the HCTO group when no hormone or progesterone is given. NS = not significant.
[d] Probability from Duncan–Kramer multiple range test that the progesterone-treated group is different from the no hormone group given either diet. NS = not significant.

diet for 4 and 6 weeks (groups A and B). In contrast, no significant difference in the amount of duct growth was detected if the mice were mature (8 to 11 weeks old) when the specialized diets were begun (groups D and E).

There was evidence that the transplants began to grow sooner in the hosts fed CO than in hosts fed HCTO. To be counted as a successful "take" the transplant had to show evidence of growth, usually the formation of end buds. After 3 weeks only 35% of the transplants had grown in the mice fed HCTO, but 75% of the transplants had grown in the mice fed CO (group D). After 6 weeks in the hosts the number of transplants that had grown increased to 87 and 100% in the hosts fed HCTO and CO, respectively. Thus, the time that elapsed before the transplant started to grow could have an effect on the value calculated for the average percentage of fat pad filled in these short term experiments.

Differential effects of dietary fat on duct growth were observed in mature mice only when the diets were fed from the time the animals were weaned. A significant decrease was seen in the number of transplants that grew and the percentage of fat pad filled, when transplants were in the HCTO fed hosts from 3 to 9 or 8 to 11 weeks (groups B and C, respectively), provided the mice were placed on test diets at 3 weeks of age. When groups B and C are compared to groups E and F, the experimental variable was the age of the mice when test diets were started. In groups B and C the mice were fed the diet from 3 weeks,

TABLE V. DEVELOPMENT OF THE OVARY IN BALB/c MICE FED CO OR HCTO DIETS FROM 3 TO 9 OR 3 TO 13 WEEKS OF AGE

Time on diet (week)	Diet	Primary follicles	Secondary follicles	Mature follicles	Corpora lutea	Corpora albicans	Atretic follicles
6[a]	HCTO	7.3 ± 3.4	7.8 ± 2.4	0.8 ± 0.8	0	0	6.2 ± 2.0
	CO	6.2 ± 3.5	9.5 ± 6.8	1.7 ± 1.9	0.7 ± 1.2	0	3.8 ± 2.1
10[b]	HCTO	3.6 ± 1.9[c]	6.1 ± 3.3	3.0 ± 1.4	0[d]	0.9 ± 1.2	4.8 ± 2.2
	CO	6.8 ± 3.2	7.4 ± 2.1	3.2 ± 1.6	1.9 ± 1.8	1.2 ± 1.4	4.3 ± 1.2

[a] Mean of 6 samples ± standard deviation.
[b] Mean of 10 samples ± standard deviation.
[c] Comparison of HCTO to CO, $P = 0.02$.
[d] Comparison of HCTO to CO, $P = 0.01$.

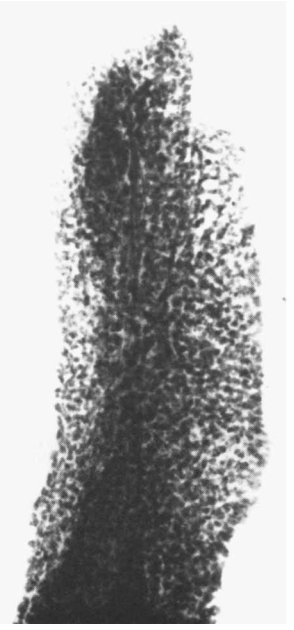
group E, 8 to 14, and group F from 17 to 20 weeks of age. There was no effect on growth of the transplants if the hosts were mature when the diets were begun.

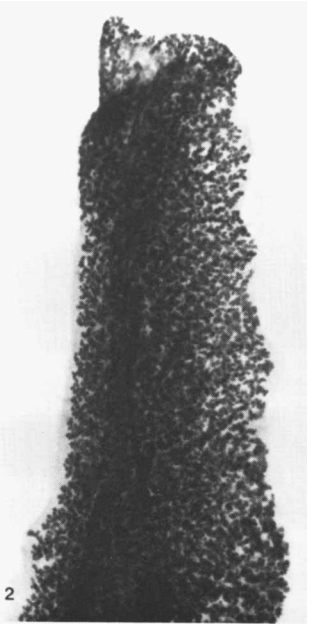
Mice in groups G and H had transplants of HPO line $Z5C_1$ for 3 and 6 weeks, respectively. Results indicate no difference in the number of transplants which grew or the rate of growth with either test diet. These results are similar to those observed in earlier experiments (3).

Effect of diet on mammary development during pregnancy. The observation that diet had no effect on the growth rate of HPO, which are lobulo-alveolar in morphology, prompted us to investigate lobulo-alveolar development in pregnant mice. Whole mounts of the fat pads from the mice fed HCTO or CO diet throughout pregnancy were studied. Each fat pad in both dietary groups was filled by the proliferation of ducts and lobulo-alveolar development commensurate with the mice being in their 18–20th day of pregnancy. There was no detectable difference in mammary gland morphology or growth rate in pregnant mice receiving the HCTO diet compared to those fed the CO diet (Figs. 1, 2).

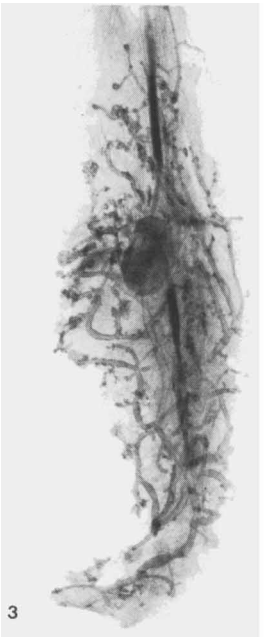
Hormone-stimulated male mice. Since no dietary effects on duct growth or lobulo-alveolar development could be found in mature mice and dramatic effects were seen in immature mice fed a saturated fat diet, we decided to investigate the earliest stages of duct development and used the castrated male mouse stimulated with female steroid hormones as our experimental model. This experiment examined the effect of E alone or E and P in combination with the diets. The amount of mammary duct growth present in the male was measured after 3 and 6 weeks on diets. The results, shown in Table II, indicate very little growth of the ductal tissue in either 3 or 6 weeks following injection of E only. Many of the samples showed no evidence of growth and the number of glands that grew ranged from 20 to 50% (Figs. 3 and 4). There were no significant differences when diets were compared at either 3 or 6 weeks in the mice given E.

When E and P were injected, an average of 75% of the samples grew and filled about 19% of the fat pad in 3 weeks and 78% of the fat pad in 6 weeks (Figs. 5 and 6). There was no

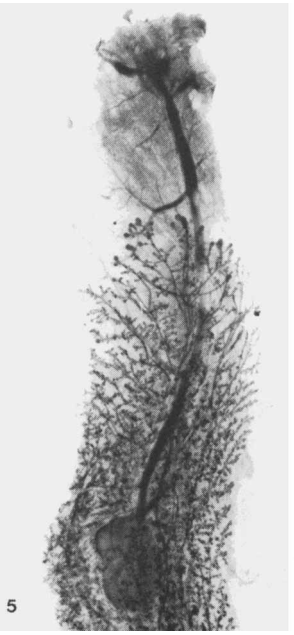


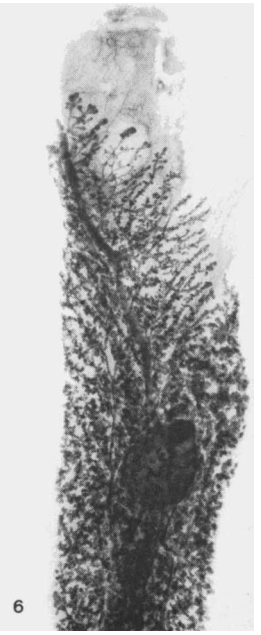


FIGS. 1 AND 2. Inguinal mammary glands from 18 day pregnant mice fed HCTO (1) or CO (2) diets from first day of pregnancy. Note full lobulo-alveolar development in both fat pads. 5.25×



FIGS. 3 AND 4. Early ductal development in the inguinal fat pads of castrated male mice injected twice weekly with 3 μg E and fed either HCTO (3) or CO (4) diets for 6 weeks. 4.9×



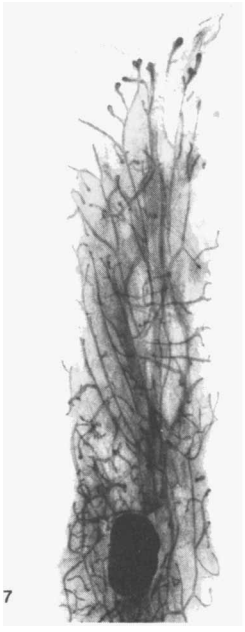


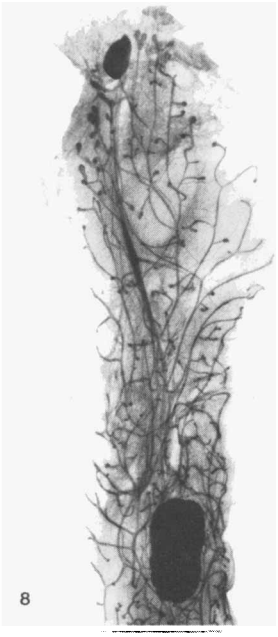
FIGS. 5 AND 6. Beginning lobulo-alveolar development in inguinal glands from castrated male mice injected twice weekly with 3 μg E and 3 mg P and fed either HCTO (5) or CO (6) diets for 6 weeks. 4.9×

significant difference in the results based on the diet fed in either the 3 or 6 weeks groups given E and P. From these experiments we concluded that the effect of dietary fat was mediated through the endocrine system rather than acting directly on the mammary epithelium.

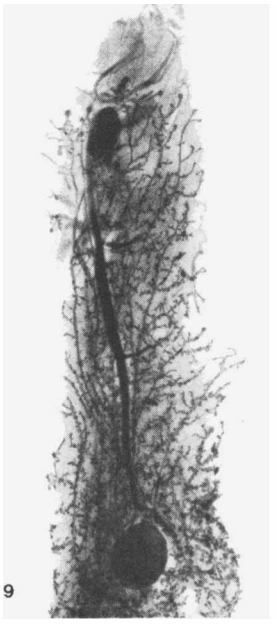
Ovariectomized immature female mice given E. In order to verify the results obtained with E alone in the castrated males we used ovariectomized females to further analyze estrogenic effects with CO or HCTO diets. The amount of growth of the mammary glands in response to injected E with each diet is shown in Table III. The growth at 9 weeks of age with no hormone administered was 12 and 11% on the CO and HCTO diets, respectively. This amount of growth probably had taken place by 3 weeks of age when the mice were ovariectomized. There was no difference in the amount of mammary duct growth after 5 weeks on either diet at any of the doses tested (1.0 to 0.25 μg per day). The amount of ductal growth was significantly less ($P < 0.01$) in the mice treated with E for 3 weeks than in those treated for 5 weeks (Figs. 7 and 8). However, the test diets did not influence the amount of duct growth at any dose of E from 0.25 to as low as 0.01 μg per day. Therefore, E could overcome the effects on the mammary gland of HCTO in the diet.

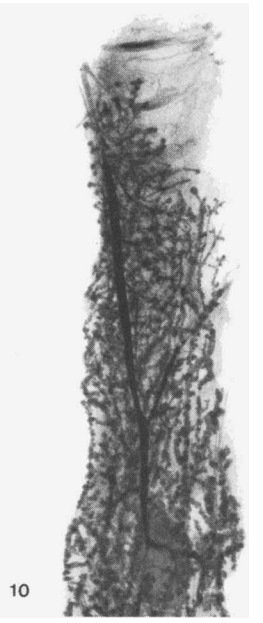
Intact female mice given P. The observation that diet had no effect on mammary growth in pregnant mice or ovariectomized mice given E led to experiments to compare diets in intact virgin females given P. Intact female mice fed the HCTO or CO diet from 3 weeks until 9 weeks of age had ducts which filled an average of 67 and 95% of the inguinal fat pad, respectively (Table IV). These results are significantly different ($P = 0.002$) and confirm that the HCTO diet in young mice inhibits ductal growth compared to the CO diet. The administration of progesterone to the mice fed the HCTO diet reversed that inhibition of growth and the difference was significant at $P = 0.001$. There was no difference in the percent of fat pad filled following P administration when the HCTO diet group was compared to the CO group, i.e., 99 ± 2 and 97 ± 7%, respectively (Figs. 9 and 10). The administration of P was sufficient to initiate lobulo-alveolar





FIGS. 7 AND 8. Mammary glands from ovarectomized female mice injected with 0.1 μg E per day and fed diets for 3 weeks. No difference is seen when those fed HCTO (7) are compared to those fed CO (8). 4.9×

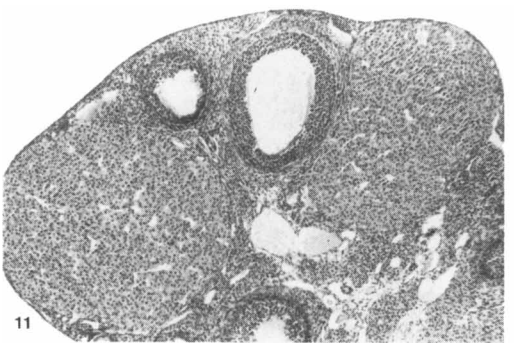


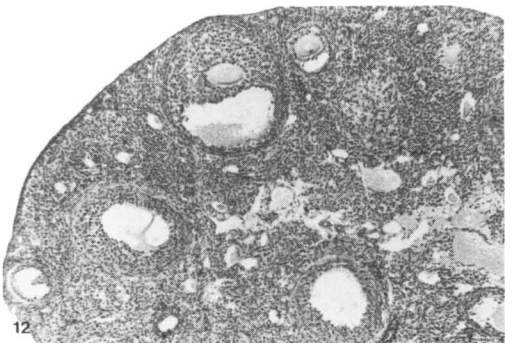


FIGS. 9 AND 10. Beginning lobulo-alveolar development is seen in inguinal glands from intact female mice receiving 1 mg per day of exogenous P and fed either HCTO (9) or CO (10) for 6 weeks. 4.9×

development in female mice on either experimental diet.

Histology of ovaries from mice fed test diets. Examination of representative sections of ovaries taken from mice fed either CO or HCTO diets produced the results shown in Table V. After 6 weeks on the special diets, the number of follicles in each stage of development from primary to secondary to mature were similar in mice on both diets. There were no corpora lutea in any of the HCTO samples, and a few ovaries had corpora lutea present in the CO group. A more striking difference was seen in the number of corpora lutea after 10 weeks on the test diets. There were no corpora lutea in the HCTO group, while the CO group averaged about 2 per ovary, which was significantly higher ($P = 0.01$) (Figs. 11 and 12). The histologic appearance of the ovaries from mice on the CO diet was similar to those





FIGS. 11 AND 12. Histologic sections of ovaries from BALB/c mice fed either HCTO or CO diets for 10 weeks. Note the two corpora lutea in the ovary from the CO fed animal (11) as compared to only follicles in the HCTO (12) fed mouse. 49.8×

from 10 mice fed laboratory chow (data not shown). However, the ovaries from mice fed the HCTO diet appeared to have been blocked at the mature follicle stage and no corpora lutea were formed.

Discussion. The experiments reported in this paper using the BALB/c strain demonstrated that the effect of dietary fat on growth rates of mammary ducts was dependent on the age of the mice when the test diet was started. We and our co-workers in earlier studies showed that ducts grew more slowly in 3-week-old mice which received a diet devoid of polyunsaturated fat than in those mice receiving a diet containing polyunsaturated fat (2, 3). In the present report, we show that dietary fat does not affect the growth of ducts transplanted into mice after they reach maturity unless the hosts are started on test diets at weanling age. We conclude that the effect of dietary fat is not directly on the mammary ducts, but is inhibiting some maturation process, which in turn is affecting the hormonal milieu and mammary development.

There was no effect of different dietary fats on the growth of fine ducts and alveoli in the development of the mammary gland during pregnancy. In addition, the percentage of fat pad filled with gland after 6 weeks of P administration to virgin female mice was the same regardless of the test diet. It is obvious from the data that lobulo-alveolar development, whether pregnancy induced or the result of P administration, is not affected by dietary fat.

There was no difference in the growth rate of HPO in mice ingesting either test diet. The HPO are morphologically similar to normal lobulo-alveoli in mammary glands of pregnant mice. We have, therefore, concluded that polyunsaturated fatty acids are not required for either normal or hyperplastic lobulo-alveolar growth.

The early studies by Cerecedo *et al.* (1) using a diet devoid of fat led to several conclusions. First, mice fed this diet from 4 to 17 weeks of age showed the symptoms of dermatitis, alopecia, and a retardation of growth. Second, if lactating dams were given this diet, these symptoms would develop even faster in their weanlings which were continued on the fat-deficient diet. Third, the inclusion of linoleate in the fat-free diet led to a partial remission of the symptoms. Fourth, a difference in the time necessary for the onset of symptoms was noted in the strains studied. The dba strain was more sensitive to fat deficiency than C_3H or C57 strains.

The diets used in our experiments contained 10% fat, either the polyunsaturated fats of CO or the saturated fats of HCTO. The CO is high in linoleate while HCTO is devoid of it and therefore essential fatty acid (EFA) deficient. In our studies those mice fed the HCTO diet did not show dermatitis, alopecia, or a significant slowing of weight gain in the 3 to 11 weeks of the experiments. In other studies (unpublished observations) we have fed mice the HCTO diet for 40 weeks and these symptoms did not occur. There are several possible explanations for the differences in the results observed. First, the BALB/c strain may be more resistant to effects of EFA deficiency than are C_3H, C57, or dba. Second, the EFA-deficient test diet used by Cerecedo *et al.* was devoid of any fat, while the control diet contained 5% lard and 10% Crisco.

Recent studies by Knazek *et al.* (13) of the effects of EFA deficiency on the development of the mammary gland in C_3H mice, noted that when EFA-deficient diets were started at 5 to 6 weeks of age the duct system developed at a normal rate. Furthermore, when the offspring of females maintained on the diet from midpregnancy are continued on the EFA-deficient diet they failed to develop either mammary ducts or alveoli. Our experiments with BALB/c confirm the observations that it is the time at which the diet is fed that determines the result.

At first glance our observations concerning alveolar proliferation may appear to be different from those of the earlier workers. However, in the study by Cerecedo *et al.* (1), the lactating mothers placed on the diet continued to lactate and raised their young indicating alveolar structures must have been maintained. In the study by Knazek *et al.* (13), the mice placed on EFA-deficient diets in midpregnancy not only went to term and delivered their young but also lactated until weaning. The authors make no mention of examining the glands of the dams but they must have been near normal in development to have supported the young until weaning. In our experiments examination of the whole mounts of pregnant females

fed the test diets throughout pregnancy showed no difference in morphology or degree of lactation when the HCTO group was compared to the CO group. All of the studies have shown that the dietary fat does not affect lobulo-alveolar development.

There are few, if any, reports on the effect of EFA on fertility in mice. However, among the first symptoms observed in EFA-deficient rats were irregular ovulation and impaired reproduction (14). Our observation that the HCTO diet fed to immature mice resulted in poorly developed ovaries in which few, if any, corpora lutea were formed is in keeping with the rat data. We have concluded that the lack of EFA in the diet affects the development of the ovary which subsequently affects mammary duct proliferation.

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