Summary. Teat ligation of 3 abdominal-inguinal mammary glands of lactating rats markedly decreased DNA and RNA content and RNA/DNA ratio when compared with similar estimates of either contralateral, non-ligated glands or glands from normal rats lactating the same length of time. Suckling stimulus, without milk removal, did not prevent cellular loss and did not maintain level of protein synthesis. DNA and RNA content and RNA/DNA ratio of contralateral, non-ligated glands were greater than those of glands of normal rats lactating comparable periods of time.

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Relationship Between Fluoride Deposition and Metastatic Calcification in Soft Tissues of Rat and Guinea Pig. (28472)

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The relationship, if any, between fluoride metabolism and the deposition of fluoride in soft tissues of the rat is not known. In fact, only recently has it been recognized that fluoride may be deposited in soft tissue, it being thought previously that ingested fluoride was either retained in the skeleton (and teeth to a much lesser degree) or excreted. Several factors are now recognized which may influence fluoride deposited in the soft tissues, among which are degree of skeletal fluoride saturation(1,2), dietary fat level(3,4), age of the animal(2), dietary ascorbic acid content(5), and presence or absence of dietary molybdenum(6). However, little information is available concerning the mechanism by which this deposition of fluoride occurs. It seemed reasonable that soft tissue-retained fluoride may be deposited as calcium fluoride or a calcium fluoride-complex of some nature. These studies concern the relationship between the deposition of calcium, phosphorus, and fluoride in the soft tissues of albino rats and guinea pigs.

Methods. In Series I, 40 Sprague-Dawley strain albino rats from our breeding colony and ranging in age from 47 to 55 days were divided equally into 4 groups according to sex and body weight. The animals were housed in pairs in raised wire screen cages in an air-conditioned room and received redistilled fluoride-low water (F = 0.01 μg/ml) *ad libitum*. The animals in Groups A and B received a low fluoride stock corn diet* (F = 0.08 μg/g) while those animals in Groups C and D received the same diet plus 2% $CaCO_3$, 1% NaH_2PO_4, and 0.1% granular calciferol (850,000 units/gram) added at the expense of yellow corn meal. In addition, one-half of the animals in Groups B and D received 1.0 mg of fluoride (as aqueous NaF in 1.0 ml of solution) daily by stomach tube. After 30 days, all animals were sacrificed by ether inhalation and the hearts, kidneys, and livers removed and prepared for fluoride(6,7), calcium(8), and phosphorus(9) analyses. Each tissue was divided into approximate halves, one of which was placed in a 30 ml porcelain evaporating dish for calcium and phosphorus analysis and the other in a platinum crucible for fluoride analysis. Both portions were dried to a constant weight by means of a

* Composition of the diet (in %) was as follows: yellow corn meal, 64.0; powdered whole milk, 30.0; alfalfa meal, 4.8; iodized salt, 1.0; and irradiated yeast, 0.2.

vacuum desiccator. The tissue fraction in the porcelain dish was charred under infra-red lamps for one hour and ashed in a muffle furnace at 600°C for 6 hours. The ash was dissolved in 3 ml of 3N hydrochloric acid, quantitatively transferred to a 10 ml volumetric flask, and diluted with double-distilled fluoride-free water. Aliquots of this solution were analyzed for calcium and phosphorus. The tissue portion in the platinum crucible was treated with 75 mg of low-fluoride MgO, covered with redistilled fluoride-free water, dried in an oven, and ashed in a muffle furnace. In addition to the soft tissues, both femurs were removed, pooled, and ashed for fluoride. Fluoride determinations were performed using a modified Willard-Winters distillation and Smith Gardner titration as previously described(10).

In Series II, 32 weanling male guinea pigs were divided into 4 equal groups according to body weight. The animals were housed in solid bottom wire cages and received redistilled fluoride-free water *ad libitum*. The diet employed in Groups A and B was a rolled oat diet.† Animals in Groups C and D received the same diet to which 2.0% $CaCO_3$, NaH_2PO_4, and 0.1% crystalline calciferol was added at the expense of the rolled oats. Both diets were given *ad libitum*. In addition, Groups B and D received 1.0 mg of fluoride (as sodium fluoride) by daily intraperitoneal injection. After 25 days, all of the animals were sacrificed by ether inhalation and the hearts, livers, lungs, kidneys, and salivary glands removed for calcium, phosphorus, and fluoride analyses. All analytical determinations were performed as described previously. The femurs were removed, prepared, and analyzed for fluoride as in Series I.

The termination of each series was indicated by weight loss in the animals receiving the calcification-inducing diets with the animals being sacrificed 5 to 7 days after this loss of weight became apparent.

Results. The results obtained for the soft tissue analyses in the Series I animals are shown in Table I. All values are expressed

† Composition of the diet (in %) was as follows: rolled oats, 59.0; skim milk, 30.0; corn oil, 8.0; cod liver oil, 2.0; and iodized salt, 1.0.

TABLE I. Amount of Fluoride, Calcium, and Phosphorus in the Rat (Series I).

Group	Livers			Hearts			Kidneys		
	Fluoride*	Calcium*	Phosphorus*	Fluoride	Calcium	Phosphorus	Fluoride	Calcium	Phosphorus
A†	2.17 ±.21‡	831 ± 122	7963 ± 515	2.72 ±.18	971 ± 159	9165 ± 258	3.55 ± .29	916 ± 177	2190 ± 100
B	2.49 ±.30	834 ± 211	9666 ± 554	3.01 ±.34	1295 ± 317	9070 ±1043	7.80 ± .98	977 ± 177	2760 ± 350
C	3.71 ±.32	1650 ± 248	8528 ± 348	3.49 ±.29	2181 ± 332	8904 ± 984	5.54 ± .62	4950 ±1987	2450 ± 140
D	6.34 ±.72	1541 ± 293	9765 ± 311	6.73 ±.57	2141 ± 185	9981 ± 660	235.5 ±32.4	11,314 ±2631	3510 ± 410

* All values are given in μg/g of dry tissue.
† A = stock diet; B = stock diet + 1.0 mg F daily; C = calcification-inducing diet; D = calcification diet + 1.0 mg F daily.
‡ Standard deviation.

TABLE II. Femur Fluoride Data in Rats (Series I).

Group	Final No. animals	Avg final body wt (g)	Femur data		
			Avg ash wt (g)	Avg μg F/g	Avg total μg F⁻
A*	10	263	.5599 ± .1260†	67.6 ± 11.0	36.9 ± 6.6
B	10	267	.5537 ± .0835	2782 ± 341	1570 ± 245
C	10	151	.4810 ± .0797	77.7 ± 11.2	37.0 ± 7.3
D	9	137	.4812 ± .0802	3644 ± 214	1725 ± 214

* A = stock diet; B = stock diet + 1.0 mg F; C = calcification diet; and D = calcification diet + 1.0 mg F.
† Standard deviation.

as mean micrograms of fluoride, calcium, or phosphorus per gram of dry tissue weight. Livers from the control animals contained 2.17 μg/g fluoride while livers of the animals which received the same diet plus 1.0 mg fluoride daily contained 2.49 μg/g fluoride. Although calcium concentrations in the livers of the 2 groups of animals were nearly identical, an increase in phosphorus content of the Group B animals was found. The livers from the animals in Group C contained 3.71 μg/g F in spite of the fact that no supplemental fluoride was administered. When fluoride was administered (Group D), the fluoride content was further elevated to 6.34 μg/g. The calcium data indicate that the calcification-inducing diet (Groups C and D) resulted in nearly a 2-fold increase in the liver calcium concentration, while in comparison only minor changes in the liver phosphorus content occurred.

The data obtained from the analysis of the hearts show the same general trends as found in the livers. As with the liver tissue, the hearts from those animals which received the calcification-inducing diet and fluoride were found to contain nearly twice as much calcium and fluoride as those of the comparable control animals.

The kidney data indicate again the same general trends but to greater degree. Comparison of the data in Groups A and B indicates that those animals which received the supplemental fluoride and the stock corn diet showed a 2-fold increase in fluoride content associated with an increase in both the calcium and phosphorus concentration. In Group C, which received the calcification diet, a 5-fold increase in calcium was found. In Group D, in which the calcification diet plus fluoride was fed, a 30-fold increase in fluoride resulted in an 11.5 times greater concentration of calcium.

The femur fluoride data from the Series I animals are shown in Table II. Groups A and C which received no supplemental fluoride were found to contain almost equal amounts of fluoride. The femurs of the Group D animals were found to contain 1725 μg F while those from Group B contained 1570 μg of fluoride.

Addition of 1.0 mg of fluoride to the animals receiving the stock corn diet resulted in no adverse effect on growth as judged by body weight gain (Table II). However, the animals which received the calcification-inducing diet failed to gain normally. Those which received the calcification-inducing diet plus fluoride were affected to the greatest degree, weighing approximately one-half the weight of comparable animals receiving the same amount of fluoride but the stock corn diet (Group B).

The data obtained from the soft tissues of the Series II animals are shown in Table III. Livers from the guinea pigs receiving the control diet contained 1.17 μg/g F, 1136 μg/g Ca and 9751 μg/g phosphorus. Livers from the animals which received the same diet but supplemental fluoride showed slightly reduced values for each of these elements. In the animals receiving the calcification-inducing diet (Group C) concentration of both calcium and fluoride in the liver were elevated to 1.62 μg/g F and 1647 μg/g Ca in spite of the fact that the animals received no supplemental fluoride. When supplemental fluoride was administered (Group D), the concentration of calcium did not change while that of fluoride was elevated even further, with values of 8.78 μg/g F and 1561 μg/g Ca being found. Use of the calcification

TABLE III. Tissue Data in Guinea Pig (Series II).

Group	Livers			Hearts			Kidneys		
	Fluoride*	Calcium*	Phosphorus*	Fluoride	Calcium	Phosphorus	Fluoride	Calcium	Phosphorus
A†	1.166 ±.141‡	1136 ± 88	9751 ± 445	1.699 ±.673	869 ± 101	4780 ± 250	6.49 ± .78	1240 ± 150	7195 ± 508
B	.963 ±.132	951 ± 72	9094 ± 541	1.655 ±.306	1279 ± 171	4540 ± 304	6.08 ± 1.23	1147 ± 101	7160 ± 571
C	1.622 ±.297	1647 ± 368	9897 ± 499	2.742 ±.908	6152 ±1595	4877 ± 630	25.50 ± 5.70	10,240 ±2449	9462 ±1553
D	8.775 ±.665	1561 ±223	9123 ± 413	3.829 ±.633	12,384 ± 248	6599 ±1011	121.0 ±34.8	10,935 ±1138	9830 ± 878

* All values are given in μg/g of dry tissue.
† A = stock diet; B = stock diet + 1.0 mg F; C = calcification diet; and, D = calcification diet + 1.0 mg F.
‡ Standard deviation.

diet with and without fluoride appeared to have no significant effect on concentrations of phosphorus in the liver, although a numerical decrease was noted when fluoride was administered.

The hearts of the animals on the control diet contained 1.70 μg/g F, 869 μg/g Ca, and 4780 μg/g P. The supplementation of fluoride (Group B) did not appreciably alter the tissue fluoride level although there was an increase in tissue calcium concentration and a slight decrease in tissue phosphorus level. The hearts from the Group C animals showed an increase in the tissue fluoride level to 2.74 μg/g concomitant with a marked increase in calcium concentration to 6152 μg/g. When fluoride was administered to animals receiving this diet (Group D), the hearts contained an even greater amount of fluoride, 3.83 μg/g F, and a 2-fold increase in concentration of calcium to 12,384 μg/g. In this instance an increase in the tissue phosphorus level was also noted.

As in the case of rat kidney, the effect of the calcification diet on fluoride retention in the guinea pig kidney was most dramatic. Introduction of the calcification diet in the absence of supplemental fluoride (Group C) produced a 4-fold increase in tissue fluoride to 25.50 μg/g and a 9-fold increase in tissue calcium to 10,240 μg/g with a 31.5% increase in phosphorus. When supplemental fluoride was given (Group D) a much greater level of fluoride, 121.0 μg/g, was noted with no additional changes in tissue concentrations of calcium and phosphorus when compared to Group C animals.

In the salivary glands controls were found to contain 2.58 μg/g F, 3213 μg/g Ca, and 5830 μg/g P. Administration of fluoride did not appreciably alter the tissue fluoride, calcium or phosphorus levels. In the presence of the calcification diet, a 75.6% increase in the tissue fluoride level was noted, accompanied by marked increases in tissue calcium and phosphorus levels to 8850 and 7550 μg/g, respectively. In the presence of supplemental fluoride (Group D), the tissue fluoride level was increased further to 6.08 μg/g with no additional increases in calcium and phosphorus levels.

The analyses of the lungs showed levels of 1.33 μg/g F, 1727 μg/g Ca, and 9890 μg/g P in the control group. Administration of fluoride markedly increased the concentration of fluoride to 6.36 μg/g but tended to decrease the level of calcium and phosphorus. In Group C, levels of 8.16 μg/g F, 5660 μg/g Ca, and 8170 μg/g P were found while in Group D only the tissue fluoride level was markedly changed in comparison to the Group C animals showing a concentration of 30.34 μg/g.

The femur data from Series II animals were identical to that found in the rat. Similarly, the animals receiving the calcification inducing diet failed to gain weight in comparison to comparable controls.

Discussion. Two studies have been conducted in which the soft tissues were analyzed for fluoride, calcium, and phosphorus, one of which was conducted in the rat and the other the guinea pig. The data found are similar in both species in the soft tissues studied, in that no significant correlations exist between amount of calcium and fluoride found in the tissue. The exception to this is the kidney. The kidneys from rats on a control stock corn diet contained a significantly greater amount of fluoride when supplemental fluoride was given but with no pronounced accompanying changes in calcium and phosphorus levels. In the guinea pig, the kidney and the lung had an elevated soft tissue fluoride content under similar experimental conditions. One might suspect that there could be a minor increase in tissue fluoride level since the animal was not perfused. The relationship, if any, between the presence of fluoride and the calcium or phosphorus content of the soft tissues in either species when the stock corn diet is used is certainly not directly evident from these data.

The more dramatic findings occurred in both species of animals receiving the calcification-inducing diet. In every instance in both the rat and guinea pig a marked increase in concentration of calcium in all tissues studied was noted. This was accompanied by a consistent increase in concentration of fluoride in spite of the fact that no supplemental fluoride was administered. With administration of fluoride to the diet, a still greater increase in tissue fluoride concentrations was found in all of the tissues examined, with the most dramatic increase in the kidney of both rat and guinea pig. In general, these additional increases in fluoride content were not accompanied by a further increase in tissue calcium concentration. The phosphorus content was somewhat elevated in the guinea pig heart and kidney, but no consistent trends were found.

The data fail to provide direct evidence as to how fluoride is retained in soft tissues, although they do show clearly that fluoride can be retained in a variety of different soft tissues. In every instance, in both rat and guinea pig, feeding a calcification inducing diet increased both the soft tissue fluoride and calcium content even in the absence of added dietary fluoride when compared to animals receiving the same amount of fluoride but receiving a stock corn diet. This would suggest that there is some relationship between the presence of soft tissue fluoride and the calcium content of the tissue, but the mechanism is certainly not a simple one. Administration of fluoride to animals receiving the calcification-inducing diet significantly increases the fluoride content of every tissue studied in both rat and guinea pig, but this is not directly related to an increase in calcium content of all tissues. It does suggest, however, that the nature of the diet has a pronounced effect on soft tissue fluoride levels, and, as such, needs further investigation.

Summary. Two studies have been conducted to investigate whether there is a relationship between the deposition of fluoride in various soft tissues of rats and guinea pigs and the levels of calcium and phosphorus in the tissues. The results suggest that when rats receive a stock corn diet there is a tendency toward an increase in the calcium, phosphorus, and fluoride content of livers, hearts, and kidneys "in the presence of supplemental fluoride." In the guinea pig these relationships are not true in all of the tissues studied. When calcification inducing diets were used, in both types of experimental animals a marked increase in tissue calcium was accompanied by a marked increase in fluor-

ide concentration in all soft tissues studied while changes in the phosphorus content were less pronounced.

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Effects of Pancreatectomy on Development of Atherosclerosis in the Rabbit.* (28473)

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Because of the known association of diabetes and a higher incidence of severe atherosclerosis in man, experiments were undertaken to observe the effect of pancreatectomy on atherogenesis in the rabbit. It was postulated that pancreatectomized animals would be unable to metabolize lipids normally and that the feeding of small amounts of cholesterol to these animals would enhance the formation of atheromata. However, contrary to expectation, pancreatectomy resulted in a limitation in hypercholesteremia and atherosclerosis.

Materials and methods. Adult, male, Belgian hares were used. The normal diet consisted of Rockland Rabbit Ration Pellets (17.41% protein, 1.61% fat, 65.8% carbohydrate, choline 110 mg/100 g, fiber, ash, vitamins, and minerals). Two groups of rabbits, B. and E, received a cholesterol-enriched diet prepared by coating pellets with a solution of cholesterol in ether, calculated to give a final pellet cholesterol concentration of ¼%. The average daily intake of pellet per animal was 250 g, resulting in a daily intake of 625 mg of cholesterol. Each day the pancreatectomized animals received an additional 500 mg of orally administered choline to prevent deficiency changes (1); this resulted in a total average daily choline intake of 725 mg. Pancreatectomy with splenectomy was performed under ether-oxygen anesthesia in 2 stages, according to the method described by Greeley(2). Because of the diffuse distribution of the pancreas in a rabbit, total pancreatectomy is an extremely difficult procedure. The post-operative mortality rate was very high and explains the small number of animals that survived to the end of the experiment. Only animals that survived for a postoperative period of at least 6 months are included in this report. Total serum cholesterol(3) and blood sugar(4) were analyzed weekly in control and pancreatectomized rabbits for the first month after pancreatectomy and monthly thereafter until they were sacrificed. Twenty-four hour collections of urine were examined for glucose in pancreatectomized animals and the amount of insulin for replacement was calculated from the degree of glycosuria. The animals were weighed at regular intervals. Terminally, the animals were sacrificed, and heart, aorta, great vessels, brain, lungs, liver and kidneys were examined grossly and microscopically. A thorough search was made for residual pancreatic tissue but none was found. Bile is known to be essential for absorption of fat from the

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