circumstances it may be the route of choice.

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Radiophosphorus Uptake by Normal, Hyperplastic, and Tumorous Mammary Tissues of Mice.* (22383)

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The hyperplastic nodules of the mammary gland of high-cancer strains of mice have been the object of limited investigation over the past 15 years(1). In general, most of the available evidence points to these nodules as potential sites of tumor formation (hence preneoplastic), although it is important to emphasize that there seems to be a "family" of structures of varying morphology, and possibly of varying growth potential, which are described as hyperplastic nodules. As part of a comprehensive study of these nodules in the C3H/He mouse, we have been attempting to characterize them definitively, both morphologically and physiologically. The present study is concerned with an investigation of a general metabolic characteristic of hyperplastic nodules, based upon their uptake of radiophosphate (P^{32}). Phosphate, playing a key role in cellular metabolism, enters a wide variety of compounds within the cell, and to some extent the rate of incorporation of phosphorus may be used as an index of metabolic activity. In general, the rate of accumulation of phosphate is greater in actively metabolizing and growing cells and tissues(2).

The experiments reported here were designed to answer two questions: (a) Can normal, hyperplastic, and tumorous mammary tissues selected from a single mouse on the basis of their morphologic characteristics also be distinguished on the basis of their P^{32} uptake? (b) Can the relative P^{32} uptake of normal, hyperplastic, and tumorous mammary tissues be altered by varying the endocrine state of the mouse? The results obtained allow us to describe the hyperplastic nodule as a structure metabolically intermediate, as indicated by P^{32} uptake, between normal mammary tissue and frank mammary tumor.

Materials and methods. 81 multiparous female mice of the C3H/He strain, 8-11 months of age, were used in these studies. All mice bore spontaneous mammary tumors of various sizes. They were separated into groups and treated as follows (Table I): (1) 12 normal (untreated, non-pregnant); (2) 15 pregnant (14th-18th day); (3) 14 bilaterally ovariectomized (2 weeks before sacrifice); (4) 16 estrogen-treated (implanted with 2-3 mg pellets of estradiol 2 weeks before sacrifice); (5) 12 cortisol-treated (injected every 2 days with 0.5 mg hydrocortisone acetate in saline suspension beginning 2 weeks before sacrifice); (6) 12 androgen-treated (im-

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planted with 4-6 mg pellets of testosterone 2 weeks before sacrifice). An additional 16 lactating, untreated, non-tumorous mice were employed to determine the P^{32} uptake of actively secreting mammary tissue. Most of the lactating mice were free of the milk factor to decrease the possibility of selecting hyperplastic nodules as normal lactating tissue. Animals were injected intraperitoneally 7 hours prior to sacrifice with 2 μc of P^{32} (as sodium phosphate dissolved in isotonic KH_2PO_4) per g body weight. Generally 5 samples of each tissue—normal, hyperplastic, and tumorous—were taken from each mouse. Occasionally, because of the small size of the nodules, 2 or 3 nodules comprised a single sample of hyperplastic tissue. The nodules were selected, removed, and dissected as free as possible from surrounding tissue with the aid of a low-power microscope. Only those nodules were selected in which dense clusters of alveoli could be identified. Tumor tissue samples were selected from non-necrotic areas of small palpable tumors. Tissue samples averaged 400 μg (dry weight, unextracted). Five to 10 samples of mammary gland were removed from the lactating mice, and 5 samples of adipose tissue (generally mesometrial) were taken from each of 11 animals among the several groups. Individual samples were placed on previously weighed, small aluminum foil squares, and were dried at 32-37°C for approximately 15 hours. After drying, the samples on the foil squares were weighed to $\pm$ 2 μg on a calibrated quartz-fiber helix microbalance. Fat was then extracted by placing each sample in 1.5 ml of absolute ether-ethanol (1:3) for 11-15 hours. After extraction, the samples were again weighed, and the % loss in weight (% fat) was calculated. The samples were then placed in individual aluminum planchets and counted (1600-6400 counts) in a flow counter, usually on the second day after sacrifice. Whenever any deviation from this routine occurred, counts were corrected for decay. Final values are expressed in counts per min. per 100 μg (dry, fat-extracted weight) of tissue. The paired data within each of the experimental groups were analyzed to determine the significance of the differences between normal, hyperplastic, and tumorous mammary tissue. The standard error of each mean value was also computed for both P^{32} uptake and % loss in weight. While an attempt was made to inject as closely as possible 2 μc P^{32} per g body weight, the measurement of radioactivity of our original P^{32} preparations was not sufficiently accurate to avoid some error. To control partly for this, members of each experimental group were staggered so that animals from more than one experimental group received P^{32} from each of the preparations used. In addition, the variation in the size of the tumors possessed by individual animals introduced some question as to the reliability of the "body weight" as a figure from which to compute total dosage of P^{32} for each animal. For these reasons and because of the presence of variable amounts of adipose tissue, the data do not lend themselves well to comparisons between experimental groups, but only within groups.

Results. Table I gives the P^{32} uptake in counts per min. per 100 μg of water-free, fat-free mammary tissue. It is clear that hyper-

TABLE I. Radiophosphorus Uptake and Fat Content of Mammary Tissues in Various Endocrine States.

		Counts (P^{32})/min./100 μg†			% loss in wt (% fat)		
		Mammary tissue*					
Animal group	No. of mice	Normal	Hyperplastic	Tumorous	Normal	Hyperplastic	Tumorous
Normal	12	409 ± 23	548 ± 46	909 ± 70	81.8 ± 2.0	72.3 ± 2.7	13.8 ± 2.1
Pregnant	15	799 ± 51	861 ± 51	1047 ± 74	77.1 ± 2.9	58.9 ± 3.3	17.7 ± 2.5
Ovariectomized	14	425 ± 47	644 ± 51	1070 ±100	66.8 ± 8.2	47.0 ± 5.6	10.3 ± 1.0
Estrogen-treated	16	368 ± 27	619 ± 41	801 ± 78	62.0 ± 7.1	42.1 ± 5.1	11.1 ± 1.7
Cortisol-treated	12	347 ± 33	571 ± 48	806 ± 51	87.3 ± 3.1	67.3 ± 4.6	13.1 ± 1.1
Androgen-treated	12	394 ± 42	557 ± 53	970 ±102	66.5 ±10.6	50.7 ± 7.9	10.4 ± 1.3
Lactating	16	965 ± 89			33.2 ± 3.4		

* Mean ± $S.E._m$ † Values based on dry, fat-free wt.

plastic nodules show a greater uptake than normal mammary tissue and a lower uptake than tumorous mammary tissue (adenocarcinoma). The paired values within each experimental group are all significantly different at the 1% level of confidence, except within the group of pregnant animals where normal (*i.e.*, prelactating) is significantly different from the hyperplastic tissue (*i.e.*, lactating areas) at the 5% level. Examination of the % loss in weight (Table I) reveals a relationship between these paired values inverse to what is seen with P^{32} uptake, *viz.*, the tumorous tissue loses the least weight, the normal tissue the most. By this criterion, as well as by the metabolic criterion of P^{32} uptake, the nodule is seen to occupy an intermediate position in comparison with normal gland and mammary tumor.

Attempts to alter the relative P^{32} uptake of the three mammary tissue types by endocrine manipulations were essentially unsuccessful. However, during pregnancy the P^{32} uptake of both normal and precancerous tissue is greatly increased, and in general the P^{32} uptakes of various mammary tissues show the relation: nonlactating normal $<$ nonlactating hyperplastic nodule $<$ prelactating normal $<$ prelactating hyperplastic nodule $\leqq$ lactating $=$ tumorous.

Discussion. In general, the untreated hyperplastic nodule approaches normal prelactating mammary tissue in its metabolic activity, as judged by P^{32} uptake, and is significantly less active than mammary tumor tissue. That the tumor tissue should show a high P^{32} uptake is not surprising (*cf.* 2). Kenney(3) has shown a differential absorption of P^{32} in human mammary carcinoma, which was about 5 times that of normal breast tissue, and Tuba *et al.*(4) demonstrated a higher Qo_2 of tumorous than of normal mammary tissue in C3H mice.

The fat extracted by the ether-ethanol is of two principal kinds: (1) that forming the bulk of adipose tissue and (2) that found in the intracellular vacuoles and secretion mass which characterize hyperplastic, prelactating, and lactating mammary epithelium. As indicated in Table I, the bulk of normal tissue samples (except in the lactating mouse where mammary adipose tissue is largely replaced by mammary epithelium) and probably the bulk of nodule samples, consist of adipose tissue from which the parenchyma of the mammary gland cannot be separated by dissection. Extracted adipose tissue samples show a high P^{32} uptake, often as high as extracted mammary tumor samples. Hence, the relative P^{32} uptake of normal, hyperplastic, and tumorous tissues is not a reflection of their content of adipose tissue. In fact, the presence of adipose tissue biases the data against the findings reported in Table I. The differences between the P^{32} uptake of the three mammary tissues are highly significant and demonstrate that these tissues are metabolically, as well as morphologically, different.

The several endocrine treatments were designed to alter the levels of steroid hormones known to affect the immature mammary gland. However, the data do not indicate appreciable metabolic changes resulting therefrom in the adult multiparous C3H mouse and are in partial agreement with our unpublished morphologic observations. Nevertheless, the P^{32} uptake of normal and precancerous mammary tissues is altered by the major changes in the endocrine state of the host involved in pregnancy and lactation.

Summary. The hyperplastic nodule of the nonpregnant C3H/He mouse mammary gland shows a radiophosphate (P^{32}) uptake intermediate between that of normal and tumorous mammary tissue. The relative P^{32} incorporation (tumor $>$ hyperplastic nodule $>$ normal) was not appreciably affected by any of a series of endocrine manipulations (ovariectomy; androgen, estrogen, or cortisol administration). However, during the second half of pregnancy, the uptake by both normal and hyperplastic tissues increases.

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Hydrolysis of Arginine Esters by Male Accessory Sexual Tissues. (22384)

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During the course of studies on coagulation of rodent semen(1) it was observed that extracts of the coagulating gland of the guinea pig hydrolyzed tosyl-L-arginine methyl ester (TAMe) at very rapid rates. This TAMe hydrolyzing enzyme could be separated from vesiculase, the enzyme responsible for the coagulation of seminal vesicle proteins, by fractionation with ammonium sulfate and with acetone. In this paper it will be shown that certain esters of L-arginine, but not those of a number of other amino acids, are hydrolyzed by extracts of the prostate gland of some species. Factors affecting the activity of this arginine ester hydrolyzing enzyme, and its intracellular and tissue distribution are discussed.

Methods. The esters of free or substituted amino acids were of commercial origin except for L-lysine ethyl ester, which was synthesized according to Werbin and Palm(2). The melting points of all these substances agreed well with those in the literature. Five times recrystallized soy bean trypsin inhibitor was obtained from the Nutritional Biochemical Corporation. Manometric measurements were made with conventional Warburg flasks fitted with one sidearm. Protein was determined spectrophotometrically(3). Dry weights were determined by heating to constant weight at 100°. The hydrolysis of casein and of hemoglobin at pH 7.5 was measured by the methods of Kunitz(4) and Anson(5) respectively. Canine prostatic fluid was obtained from two dogs which had undergone the prostatic isolation operation of Huggins *et al.*(6). The animals were castrated and had received 50 mg of testosterone propionate per day for some months. The fluid was obtained by the administration of pilocarpine(6), centrifuged to remove small amounts of debris, and stored in the frozen state until used. The accessory sexual organs of various rodents were dissected carefully in order to avoid contamination by secretions of neighboring tissues. We are grateful to Dr. Evelina Ortiz for dissection of the accessories of the mouse and the hamster. In all animals the secretion of the seminal vesicles was removed by expression. The hydrolysis of free or substituted amino acid esters was followed by manometric determination of the carbon dioxide liberated from 0.026 M sodium bicarbonate buffer in equilibrium with 95% nitrogen-5% carbon dioxide at 38°. The reaction was initiated by the addition of the substrate from the sidearm after a temperature equilibration of 10 minutes. In every case suitable blank vessels were set up to correct for any nonenzymatic hydrolysis of the substrate, and also for any acid formation by the tissue extract. All values are given in terms of micromoles of carbon dioxide liberated by the hydrolysis of a given substrate. With crude saline extracts of the coagulating gland of the guinea pig, the rate of hydrolysis of TAMe was strictly proportional to the amount of extract added and to the time of incubation provided that the activity was not greater than 30 μmoles per hour.

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