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### Genetically Determined Differences in the Amylase Activity of Mouse Submandibular Glands.\* (32418)

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Lacassagne in 1940 demonstrated the sexual dimorphism of the submandibular glands in mice and other rodents. Since that time numerous investigators have examined the effect of various endocrine manipulations such as castration, thyroidectomy, castration and thyroidectomy and hypophysectomy upon rodent salivary glands. Much of this work has been reviewed by Shafer and Muhler(1). Several reported studies have been concerned specifically with the influence of the sex hormones on the amylase content of rat or mouse submandibular glands(2,3,4). Schneyer (5) has shown that the concentration of amylase in the submandibular-sublingual gland complex varied significantly in males from 4 different strains of mice. She concluded that the enzyme level was genetically determined. Our research supported her findings. Further, we suspected that the influence of sex hormones on salivary gland amylase was not the same for all strains of mice.

The purpose of this study was 2-fold: first, to examine the normal amylase levels in submandibular glands from both sexes of

several inbred strains and 2 hybrids; second, to observe the effect of castration and ovariectomy on submandibular gland amylase levels in these strains.

*Methods.* Adult male and female mice from 10 inbred strains (C3H/HeJ, AKR/J, CBA/J, C57BL/6J, A/WySn, CE/J, DE/J, DBA/2J, BALB/cJ, LP/J) and 2 hybrids (DED2F<sub>1</sub> and D2DEF<sub>1</sub>, D2CEF<sub>1</sub> and CED2F<sub>1</sub>) were obtained from The Jackson Laboratory, Bar Harbor, Maine. All the mice used were between 7 and 9 months of age. At that time 10 to 12 individuals from each sex, strain and hybrid were gonadectomized. Thirty-five days later the animals were killed by cervical dislocation, the submandibular glands removed and dissected free from the closely adhering major sublingual glands, frozen at dry ice temperature, and freeze-dried. The lyophilized glands were used for both dry weight and amylase determinations. It has been shown(4) that freeze-drying the tissues does not change the amylase levels from those of fresh material.

To serve as controls, 10 to 12 intact males and females from each of the strains and hybrids were sacrificed and their submandibular glands removed, weighed and lyophilized. Amylase activities were determined spectrophotometrically using Van Loon's(6)

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adaptation of the methods of Huggins and Russell, and Smith and Roe. Activity is expressed in units per 100 g of tissue.

The submandibular glands from 2 males and females from each strain and from the experimental and control groups were removed, fixed in buffered formalin, sectioned, and stained with hematoxylin and eosin. The sections were examined histologically for changes induced by castration or ovariectomy.

The data were compared by an analysis of variance test, and in all instances where it is stated in the body of the paper that the weight or amylase activity differs significantly from another, the value for P is ( $<.001$ ).

**Results.** The amylase activity found in the submandibular glands of the intact males and females from all strains and hybrids studied is presented in Table I. The strains have been

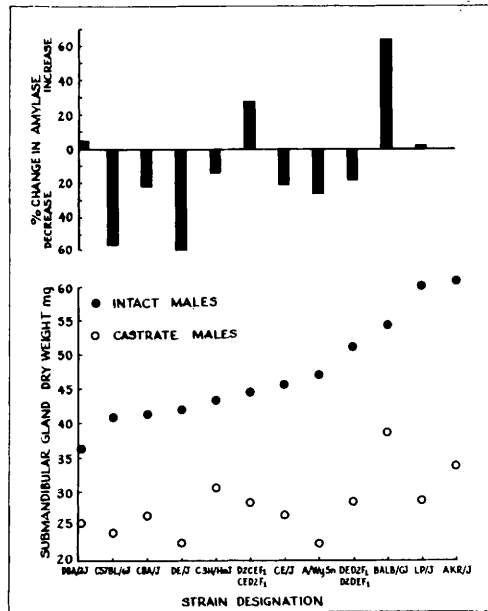
TABLE I. Amylase Activity in the Submandibular Glands from Normal Intact Adult Male and Female Mice of Various Strains and Hybrids.

Strain designation	Amylase activity*	
	Intact males	Intact females
C3H/HeJ	34,159	31,682
AKR/J	—	31,600
CBA/J	29,251	19,722
L-M (hybrid)	26,864	24,845
C57BL/6J	26,400	7,200
A/WySn	26,039	23,503
CE/J	22,869	19,510
J-K (hybrid)	21,874	27,624
DE/J	21,668	14,278
DBA/2J	21,112	19,202
BALB/cJ	18,239	26,346
LP/J	15,998	23,608

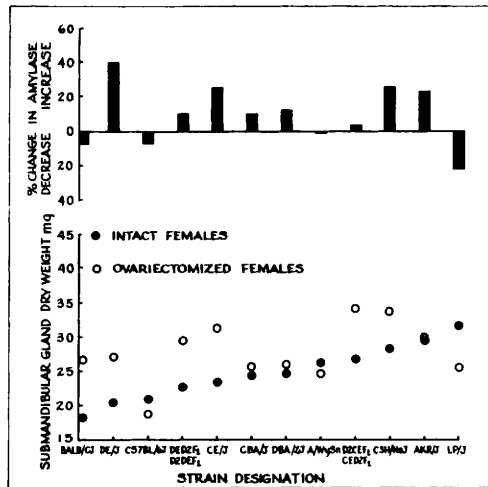
\* Amylase in U/100 g lyophilized gland—mean value from 6-8 individuals.

arranged in descending order according to levels of activity in the male glands. Strain LP/J exhibited the lowest activity, slightly less than one-half that observed in the strain with the highest value.

The data obtained for the females indicate that they do not follow the same rank order as their corresponding males, and further, the lowest level for the females is less than one-fourth the value observed in the highest strain. In 2 of the strains, BALB/cJ, and LP/J, and in hybrid D2CEF<sub>1</sub> and CED2F<sub>1</sub>, the level of amylase activity exceeds that for the males of the corresponding strains. In all other instances the level of amylase ac-



1



2

FIG. 1. Effect of castration on weight and amylase activity of submandibular glands of male mice from 10 strains and 2 hybrids. The mean value for amylase activity from intact normal animals of each strain is represented by the zero per cent line. The direction and percentage change induced by castration is shown as a histogram bar above or below this line.

FIG. 2. Effect of ovariectomy on weight and amylase activity of submandibular glands of female mice from 10 strains and 2 hybrids. The mean value for amylase activity from intact normal animals of each strain is represented by the zero per cent line. The direction and percentage change induced by ovariectomy is shown as a histogram bar above or below this line.

tivity is higher in the males than in the females of the same strain.

In Fig. 1 the mean value of the lyophilized dry weight of the submandibular gland for the males of each strain and hybrid is plotted on the abscissa beginning with the value for the smallest glands and progressing to that having the largest. The same figure shows the values for the mean weight of the submandibular glands from castrate individuals. In all instances the mean weights of the glands from the castrates are significantly lower than the value for intact members of the same strain.

The effect of castration on the amylase activity in the males appears across the top portion of Fig. 1 and is represented as per cent increase or decrease in activity of the enzyme over the mean value obtained from the intact animals for each strain and hybrid. The mean value for the intact animals is represented by the zero per cent line.

Castration resulted in a significant decrease in the amylase activity in the C57BL/6J, CBA/J, CE/J, DE/J, C3H/HeJ, A/WySn strains, and the DED2F<sub>1</sub> and D2DEF<sub>1</sub> hybrid. In 2 strains, DBA/2J and LP/J, no significant change from the intact levels was noted. In contrast, strain BALB/cJ and the hybrid D2CEF<sub>1</sub> and CED2F<sub>1</sub> both showed a significant increase in amylase activity following castration. No data are available for the amylase activity from strain AKR/J. The comparable data for the females are presented in Fig. 2. Again the strains of mice are arranged along the abscissa in order of increasing mass of the lyophilized submandibular glands from the intact individuals. As with the amylase values, there is no apparent relationship between the sequence of females and males of the various strains and hybrids when ranked according to the absolute weights of their submandibular glands.

No significant change in weight of the submandibular glands followed ovariectomy in the C57BL/6J, CBA/J, DBA/2J, A/WySn and AKR/J strains. In contrast the BALB/cJ, DE/J, CE/J, and C3H/HeJ strains and the DED2F<sub>1</sub> and D2DEF<sub>1</sub>, D2CEF<sub>1</sub> and CED2F<sub>1</sub> hybrids showed a significant increase in submandibular gland mass

induced by removal of the ovaries. In strain LP/J a decrease in mass was noted.

At the top of Fig. 2 the per cent change in amylase from the intact levels following ovariectomy is indicated. Enzyme activity increased significantly in the DE/J, DED2F and D2DEF, CE/J, CBA/J, DBA/2J, C3H/HeJ, and AKR/J or remained the same as in the BALB/cJ, C57BL/6J, A/WySn, D2CEF and CED2F, or decreased significantly in the LP/J strain.

*Discussion.* It is apparent that the endocrinological imbalance caused by castration or ovariectomy can markedly alter the submandibular gland mass and amylase activity in various strains of mice. An explanation of the exact mechanism of how genetic control of amylase activity in the submandibular gland is exerted remains speculative. We have found, as did Schneyer(5), that the genetic effect probably does not operate through differences in stromal-parenchymal ratios, since microscopic examination revealed no readily discernible differences among strains.

Previous work in our laboratories has shown that exogenous testosterone and estradiol benzoate can restore amylase levels to normal in castrated male C57BL/6J mice, while only testosterone is stimulatory in the females of this strain(4). Woolley(7,8) has shown that in certain strains of mice, changes leading to adrenal cortical carcinoma are accompanied by histological changes in the accessory reproductive organs, which suggests that steroid hormones are being produced. It is conceivable in those strains showing increased enzyme activity that gonadectomy in some manner initiates increased production of one or both of the sex hormones from the adrenal cortex; they in turn alter enzyme activity, possibly by influencing the synthesis and/or storage of the amylase in cells of the submandibular glands.

In the case of males, the weight of glands was depressed even though the D2CEF<sub>1</sub> and CED2F<sub>1</sub> hybrid and BALB/cJ strain showed marked increases in enzyme activity. In females, increase in enzyme activity was often accompanied by an increase in mass of the submandibular gland. Since the amylase activity was measured in units of enzyme ac-

tivity per 100 g of tissue, the per cent changes reflect increases in activity which cannot be explained solely on the basis of hyperplasia of the gland.

**Summary.** Examination of 10 different inbred strains and 2 hybrids of mice indicated that the normal levels of amylase activity vary from one strain to another. Further, the activity of the enzyme in the salivary gland tissue is markedly altered by gonadectomy. The effect of such endocrinological imbalancing varies with each strain. In some strains enzyme activity remains unchanged. In some, activity increases while in others it decreases. The pattern of change was not always the same for females as for males of the same strain. In other words, we are dealing with an enzyme system which is related to the genotype, but which may be influenced in an undetermined fashion by endocrine manipulation.

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### Influence of the Jensen Sarcoma, Walker 256 Carcinoma, and Endotoxin on Plasma- and Hepatic-Bound Carbohydrates In the Rat. (32419)

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We have previously reported(1) that concentrations of serum-bound carbohydrates were not elevated in animals bearing intramuscular transplants of the Jensen sarcoma and Walker 256 carcinoma. This was contrary to the general findings in tumor hosts (2). When a bacterial infection was present in the tumor or a bacterial endotoxin was injected into normal animals, however, the serum-bound carbohydrate concentration was elevated. These studies have now been extended by determining the extent of glucosamine-C<sup>14</sup> incorporation into serum and hepatic proteins of control, tumor-bearing, and endotoxin-treated rats.

**Materials and methods.** The bacteria-free tumors(1) were carried as intramuscular transplants in the *rectus femoris* muscle of female Holtzman rats for 7 days. Control

and experimental rats were selected of the same age and lot and were fed *ad libitum*. *E. coli* endotoxin (Difco), suspended to contain 100 µg/1.0 ml, was injected intraperitoneally 24 hours prior to experimentation. Glucosamine-1-C<sup>14</sup> (50 µc/mg, Isotopes, Inc.) at a level of 5.0 µc/0.2 ml was injected into the femoral vein 30, 60, and 120 minutes prior to sacrifice. The animals were anesthetized with Na secenal and bled by cardiac puncture. One aliquot of blood (2.5 ml) was mixed with an equal volume of saline containing 0.01 M EDTA, and the mixture centrifuged at 2500 rpm in a clinical centrifuge for 20 minutes to obtain a red blood cell volume and a plasma fraction. The percent plasma volume of whole blood was calculated from these values. The diluted plasma was fractionated by precipitation with TCA, defatted, and dried using the