

pathway of the rabbit would explain the progestational action. Amphenone has been reported to inhibit the synthesis of corticosterone, hydrocortisone, and cortisone in calf adrenal perfusion experiments(10). A block in the biosynthetic pathway between progesterone and corticosterone could result in excess secretion of a potent progestogen in amounts sufficient to induce progestational proliferation of the rabbit endometrium. Adrenocortical progesterone is probably the active progestogen since comparison with the principal adrenal corticosteroids in the Clauberg assay indicated that progesterone was much more potent than any of the active compounds.

Summary. Progestational proliferation of the rabbit endometrium in response to amphenone has been shown to be dependent on the adrenals. The progestational effect of amphenone was prevented by adrenalectomy as well as by cortisone treatment. Large doses of ACTH failed to produce progestational changes in the rabbit endometrium. It was suggested that amphenone exerts its progesta-

tional effect on the rabbit uterus by inhibition of specific enzyme systems in the adrenocortical biosynthetic pathway resulting in excess progestogen secretion.

The invaluable assistance of Mr. Donald L. Barber and Mr. Howard L. Erwin is gratefully acknowledged.

1. Allen, M., Hertz, R., Tullner, W. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v74, 632.
2. Bencze, W. L., Allen, M. J., *J. Org. Chem.*, 1957, v22, 352.
3. Korman, J., Olsen, E. C., *ibid.*, 1957, v22, 870.
4. McPhail, K., *J. Physiol.*, 1934, v83, 145.
5. Hertz, R., Tullner, W. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1956, v91, 76.
6. Corner, G. W., Allen, W. M., *Am. J. Physiol.*, 1929, v88, 326.
7. Englehart, E., *Klin. Wchnschr.*, 1930, v9, 2114.
8. Beall, D., Reichstein, T., *Nature*, 1938, v142, 479.
9. Lyons, W. R., Li, C. H., Johnson, R. E., Cole, R. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1953, v84, 356.
10. Rosenfeld, G., Bascom, W. D., *J. Biol. Chem.*, 1956, v222, 565.

Received March 10, 1958. P.S.E.B.M., 1958, v98.

Genetic Control of Amylase Levels of Mouse Submaxillary Glands.* (23974)

CHARLOTTE A. SCHNEYER (Introduced by L. H. Schneyer)

University of Alabama School of Dentistry, Birmingham

The amylase level of the submaxillary gland of certain rodents has been shown to be, at least in part, under hormonal control (1). Recent work has suggested that the autonomic nervous system may also play a role in regulating formation of amylase in rodents(2). The extent to which genetic control is involved in regulation of amylase levels is only generally suggested by data which show significant species differences in amylase levels of the submaxillary glands of rabbits, guinea pigs, rats and mice (unpublished data); such differences, however, may be an indirect reflection of genetic control of structural variation in glands of the several species.

It was, therefore, considered of importance to determine the role that hereditary mechanisms may have in controlling quantitative differences in amylase level. Furthermore, although proof of a genetic control of biochemical reactions has been demonstrated in certain microorganisms(3,4), little data of such a nature are available concerning such control in higher animals(5). Accordingly, to delineate the genetic role in amylase production, inbred lines of mice, where genetic uniformity is maximum, and where quantitative differences are therefore readily demonstrable (6), were used.

Methods. Adult male mice, 2-4 months old, were used. The animals were fasted for 48 hours, and, under Nembutal anesthesia, the submaxillary-sublingual glands were re-

* This investigation was supported by research grants from the Nat. Inst. of Health.

TABLE I. Amylase Levels of Mouse Submaxillary-Sublingual Glands.

Strain	No. of animals	Amylase level
CFW	22	.15 $\pm$.012*
C57BL/6	14	.14 $\pm$.006
C3H	35	.19 $\pm$.008
DBA/1	41	.10 $\pm$.003
F ₁ (C3H-DBA/1)	15	.15 $\pm$.008

* Mean $\pm$ stand. error of mean.

moved, weighed rapidly on a torsion balance, and frozen for future amylase analysis. For analysis, the glands were homogenized and the amylase content of appropriately diluted homogenate was then determined by the method of Myers, Free and Rosinski(7). Amylase activity is expressed in terms of milligrams of reducing sugar formed/mg wet weight of gland. Inbred mice of strains C57BL/6, C3H and DBA/1 (Jackson Memorial Laboratory) and CFW were used. C3H females were mated with DBA/1 males, and the submaxillary-sublingual glands from the resulting hybrid animals were analyzed for amylase content. Representative gland samples from each strain and from the C3H-DBA/1 hybrid were fixed in Bouin's solution, sectioned and stained, either with haematoxylin and eosin, or with Mallory's stain and examined for relative distribution of connective tissue elements.

Results. From Table I, it is apparent that statistically significant differences in amylase level of submaxillary-sublingual glands exist among the several inbred strains indicated. It is also clear that mating of the strain with highest amylase level (C3H, .19 mg reducing sugar/mg wet tissue) with that strain having the lowest amylase level (DBA/1, .10 mg) produced an F₁ hybrid generation with submaxillary gland amylase content intermediate in value (.15 mg). The difference in amylase level between the F₁ and the parent C3H and DBA/1 animals is statistically significant, with P values in all cases less than .0001. A significant difference also exists between mean values in the parent groups ($P < .0001$).

The C3H-DBA/1 hybrids, all of which resemble the parent C3H in hair color and body size, were mated among themselves and a wide range of hair color and amylase level was found in these F₂ animals. The mean amy-

lase levels of the 36 F₂ males obtained may be grouped as follows: 14/36, $M \pm SE = .15 \pm .008$; 9/36, $M \pm SE = .18 \pm .009$; 5/36, $M \pm SE = .33 \pm .012$; and 8/36, $M \pm SE = .07 \pm .009$.

The characteristic strain difference in amylase level is probably not the result of genetic differences in amount of connective tissue or number of ducts and acini in glands of the several strains. Histological examination of glands from each mouse strain showed no significant differences in relative distribution of these elements. It may be concluded, therefore, that the amylase level characteristic of each strain, under the experimental conditions selected, is genetically determined. The precise mechanism of this control, however, can only be suggested, since the number of animals employed was limited. The data, however, are consistent with a gene control that is multiple and additive, possibly with each dominant allele producing an amylase quantity in the approximate range .05-.07 mg reducing sugar/mg wet gland. On this basis, the parental genotypes for amylase may be represented as AABbCc for C3H (amylase level = .19 or approximately 4 x .05), aabbCC for DBA/1 (amylase level = .10 or approximately 2 x .05), and AaBbCc for the hybrid (amylase level = .15 or approximately 3 x .05).

Summary. Under uniform conditions of age, sex and environment, the concentration of the specific enzyme, amylase, present in mouse submaxillary-sublingual glands varies with the strain, the amounts being (amylase activity expressed as mg of reducing sugar/mg wet tissue) CFW, .15 mg; C3H, .19 mg; C57BL/6, .14 mg; and DBA/1, .10 mg. The characteristic amylase level is probably not the result of genetic differences in amount of connective tissue or number of ducts and acini in glands of the several strains as histological examination of representative tissues of each mouse strain shows no significant differences. Thus, the submaxillary gland amylase level for each strain is genetically determined.

2. Schneyer, L. H., Schneyer, C. A., *Am. J. Physiol.*, 1956, v187, 403.
3. Bonner, D., *Cold Spr. Harb. Symp. on Quant. Biol.*, 1946, v11, 14.
4. Tatum, E. L., Lederberg, J., *J. Bact.*, 1947, v53, 673.
5. Stutts, E. C., Johnson, W., Briles, W. E., Kunkel, H. O., *Proc. Soc. Exp. Biol. and Med.*, 1956, v91, 60.
6. Budds, O. C., Russell, E. S., Abrams, G. E., *ibid.*, 1953, v84, 176.
7. Myers, V. C., Free, A. H., Rosinski, E. E., *J. Biol. Chem.*, 1944, v154, 39.

Received March 10, 1958. P.S.E.B.M., 1958, v98.

Influence of Steroids Upon Osmotic Pressure Changes in Isolated Frog Skin Pouches.*† (23975)

MATTHEW TAUBENHAUS AND JOHN V. MORTON

Department of Metabolic and Endocrine Research, Michael Reese Hospital, Chicago, Ill.

It was reported(1) that various steroids, added to the interior of pouches prepared from skin of frog legs, suspended in and filled with frog Ringer's solution, were capable of raising the osmotic pressure on the inside of the pouch in experiments lasting up to 16 hours. Similar pouch preparations from the opposite leg of the same animal to which no steroid or just the solvent was added served as controls. The ability of surviving frog skin to transfer sodium across the membrane had been demonstrated before(2,3,4). It was assumed, from our experiments, that the further increase of osmotic pressure after addition of appropriate steroids was an expression of a direct action of such steroids upon electrolyte transfer at the cellular level. No measurements of electrolytes were performed in our previous investigations. Steroids which exhibited the most pronounced effect were: desoxycorticosterone, 9 α -fluorohydrocortisone, hydrocortisone and, to a much lesser degree, estriol. Testosterone showed no effect. No significant changes in fluid volume inside the skin pouch were observed, despite the change of osmotic pressure.

Methods. In experiments to be reported here, the action of other steroids under similar

experimental conditions was tested. All experiments were again performed on skin pouches prepared from hind legs of frogs (*Rana pipiens*), the epithelial layer remaining on the outside. Steroids in various concentrations were added to preparations from one leg, the other leg serving as control. Methods of dissolving steroids, determining freezing point depression in a Fiske osmometer etc., remained the same, as previously published(1). Most experiments were terminated after 8 hours. Sodium and potassium measurements were performed with the flame photometer. Data were computed in relation to total surface of skin pouch, rather than per cm². This is permissible, since in a sequence of 50 unselected preparations, the mean surface of the actively exposed skin was 16.37 cm² with standard deviation of only 0.58 and standard error of mean of 0.081. The steroids tested were: dl-aldosterone, 2-methyl-9 α -fluorohydrocortone, prednisone and prednisolone. The first 2 were chosen because of their known strong effects upon electrolyte shifts, the latter because of their relative inertness.

Results. Table I illustrates the effects of these substances upon osmotic pressure. Dl-aldosterone and 2-methyl-9 α -fluorohydrocortone both exhibited a striking effect resulting in a considerable increase in osmolarity of the fluid in the treated pouch as compared with control preparation. In general, the higher the steroid concentration, the more potent was the effect, with the exception of the ex-

* In part supported by Michael Reese Research Fn.

† We are grateful to Ciba Corp., the Upjohn Co., and Schering Corp. for generous supply of steroids. Dr. Martin B. Mathews, University of Chicago, was very helpful in testing material for uronic acid and glucosamines. We are also indebted to Dr. Helen Heath for statistical evaluation of our results.