The new enzyme has been obtained in aqueous solution, free of the cellular structure, by killing the cells with cold acetone, or with toluol and chloroform.

This enzyme appears to exhibit a high degree of specificity for creatine, since it does not convert the closely related compound glycocyamine into glycocyamidine, nor does it change hydantoic acid into its anhydride, hydantoin. The enzyme does not produce creatine or creatinine from arginine, a possible precursor of creatine in the animal body. Methyl guanidine and acetic acid, component parts of creatine, are not synthesized into the latter compound by the enzyme.

The enzyme is not produced when the bacterial cells are grown in a medium which does not contain creatine; it seems, therefore, to belong to the group of "adaptive enzymes" which are produced only in the presence of their specific substrate.

The conversion of creatine into creatinine offers an opportunity for the study *in vitro* of (a) the enzymatic production of a biologically important cyclic compound from an aliphatic one, and (b) the enzymatic combination of an amino and a carboxyl group to form the important CO-NH linkage.

10094

Changes in the Ant. Pituitary Gland of Rats with Experimental Goiter.

ROSE COHEN NELSON AND JOSEF WARKANY. (Introduced by Glenn E. Cullen.)

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The relationship of the thyroid to the pituitary gland has been explored by various methods. Earliest mention of this relationship is found in the description of the hypophysis[1] in pathologic conditions of the thyroid gland. Large, pale cells with nuclei poor in chromatin were noted in the anterior hypophyses in cases of thyroid deficiency.[2] Similar observations were made in dogs with spontaneous goiter.[3] Following thyroidectomy, the disappearance of

[1] Niepce, B., *Traite due goitre et du cretinisme*, Paris, J. B. Bailliere, 1851.

[2] (a) MacCallum, W. G., and Fabyan, M., *Bull. Johns Hopkins Hosp.*, 1907, **18**, 341; (b) Schilder, P., *Virchows Arch. f. path. Anat.*, 1911, **203**, 246; for review see (c) Brauchli, H., *Frankfurt Ztschr. f. Path.*, 1925, **31**, 450.

[3] Romeis, B., *Virchows Arch. f. path. Anat.*, 1924, **251**, 237.

acidophils and appearance of large chromophobes or basophils—frequently designated as thyroidectomy cells—have been described.[4] Marine, Rosen and Spark[5] recently found an increase in the size of all the glandular cells and a decrease of the acidophilic granules in the anterior pituitary of rabbits maintained on a goitrogenic diet. Addition of iodine or desiccated thyroid to the diet of these animals prevented such changes or restored the appearance of the anterior pituitary gland to normal.

In rats reared on a goitrogenic diet we have found changes in the anterior pituitary gland which appear similar to those described in pathological human glands and following thyroidectomy. The Steenbock and Black[6] rachitogenic diet No. 2965 was found to be goitrogenic by Krause and Monroe.[7] In order to protect the rats against rickets, irradiated ergosterol in olive oil was added to their diet. Every tenth day an amount equivalent to 60 International Units was fed by pipette. The addition of vitamin D did not interfere with the regular production of hypertrophy and hyperplasia of the thyroid gland[8] and made it possible to keep the animals on the diet for a long time. Twenty-one males and 7 females were put on the diet after weaning; they were sacrificed after 2, 3, 4, 5, or 12 months. The growth of the rats was considerably retarded and they did not reproduce. Control animals were kept on Bill's modification of the Steenbock stock diet.[9]

In every experimental animal the thyroid showed hypertrophy and hyperplasia. Fig. 1, A and B, shows the obvious difference between the thyroid of an experimental animal and a control. The pituitary changes were not marked in animals which had been on the diet for 2 months or less. After this period changes were observed which were much more definite in the males than in the females. In sections stained with hematoxylin and eosin, the presence of groups of large and pale cells gives the anterior lobe a spongy appearance visible under low power magnification (Fig. 2). These groups are usually seen in the center of the gland, rarely in the lateral

[4] (a) Rogowitsch, N., *Beitr. z. path. Anat. u. z. allg. Path.*, 1888-89, **4**, 453; for review see (b) Zeekwer, I. T., Davison, L. W., Keller, T. B., and Livingood, C. S., Jr., *Am. J. M. Sc.*, 1935, **190**, 145.

[5] Marine, D., Rosen, S. H., and Spark, C., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 803.

[6] Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, **64**, 263.

[7] Krauss, W. E., and Monroe, C. F., *J. Biol. Chem.*, 1930, **89**, 581.

[8] (a) Thompson, J., *J. Nutrition*, 1932, **5**, 359; (b) Levine, H., Remington, R. E., and von Kolnitz, H., *J. Nutrition*, 1933, **6**, 325.

[9] Bills, C. E., Honeywell, E. M., and MacNair, W. A., *J. Biol. Chem.*, 1928, **76**, 251.

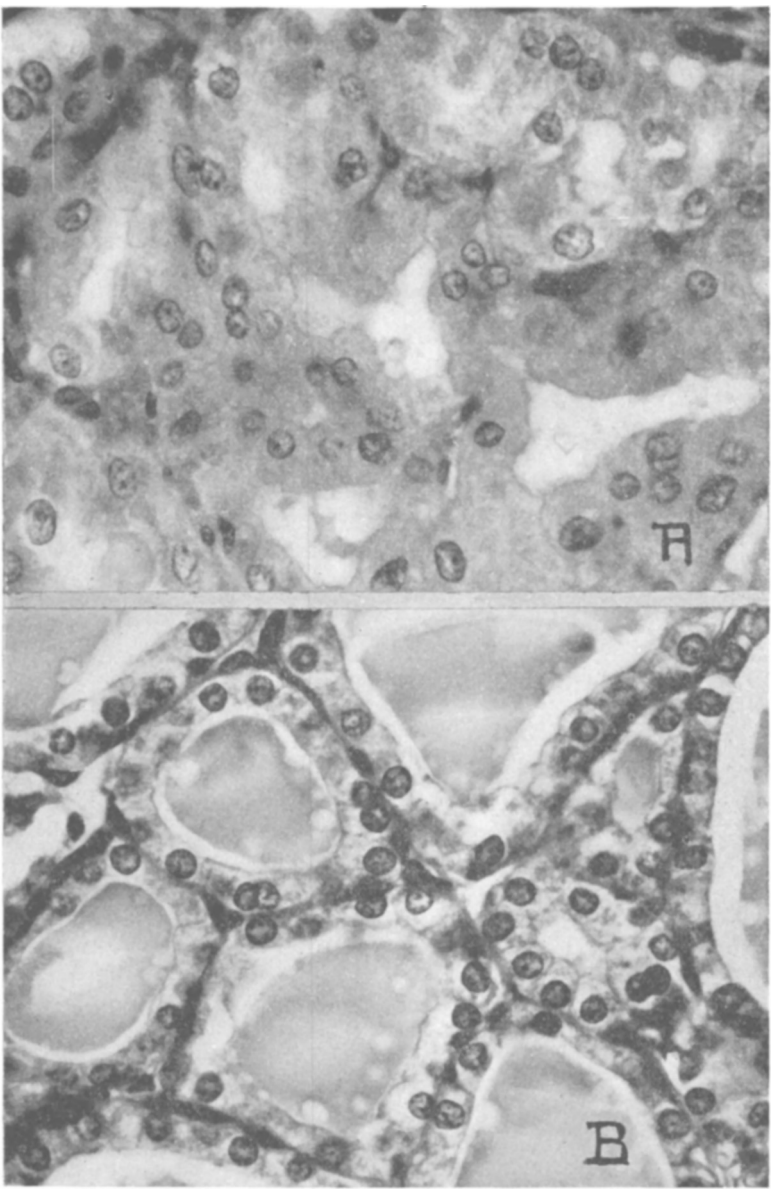

FIG. 1.

A. Hyperplastic thyroid gland of male rat 6Y after 4 months on goitrogenic diet. (800×)

B. Normal thyroid gland of control male rat 8YC after 4 months on standard diet. (800×)

parts of the anterior lobe. The cells appear in nests of 5 to 20 and show well-defined cell boundaries in many cases. Under higher magnification (Fig. 3 A), it becomes obvious that the abnormal size

of the cell is due to an increase of the amount of cytoplasm, which appears finely granular about the nucleus and vacuolated about the periphery. The nucleus is not markedly changed in size but to some extent is lacking in chromatin. This description tallies closely with that of thyroidectomy cells or goiter cells. The relationship of these

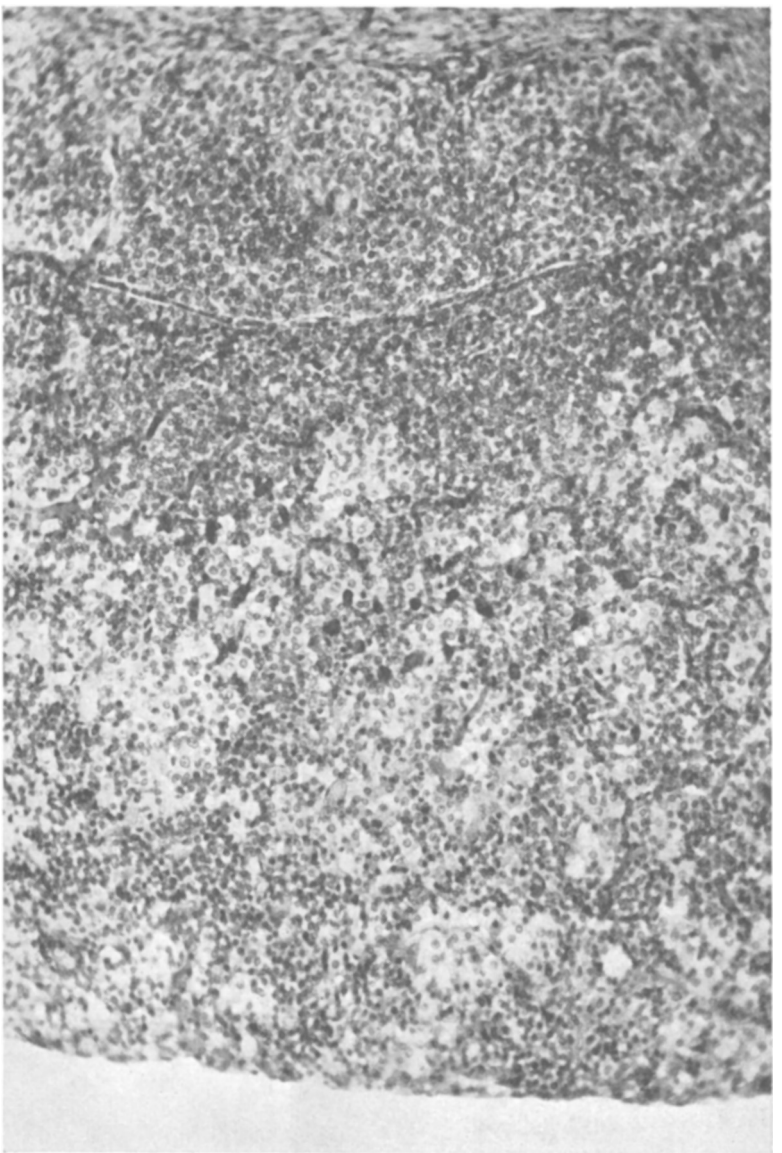

FIG. 2.
Anterior and median lobe of the pituitary gland of rat 6Y showing nests of large cells. (200×)

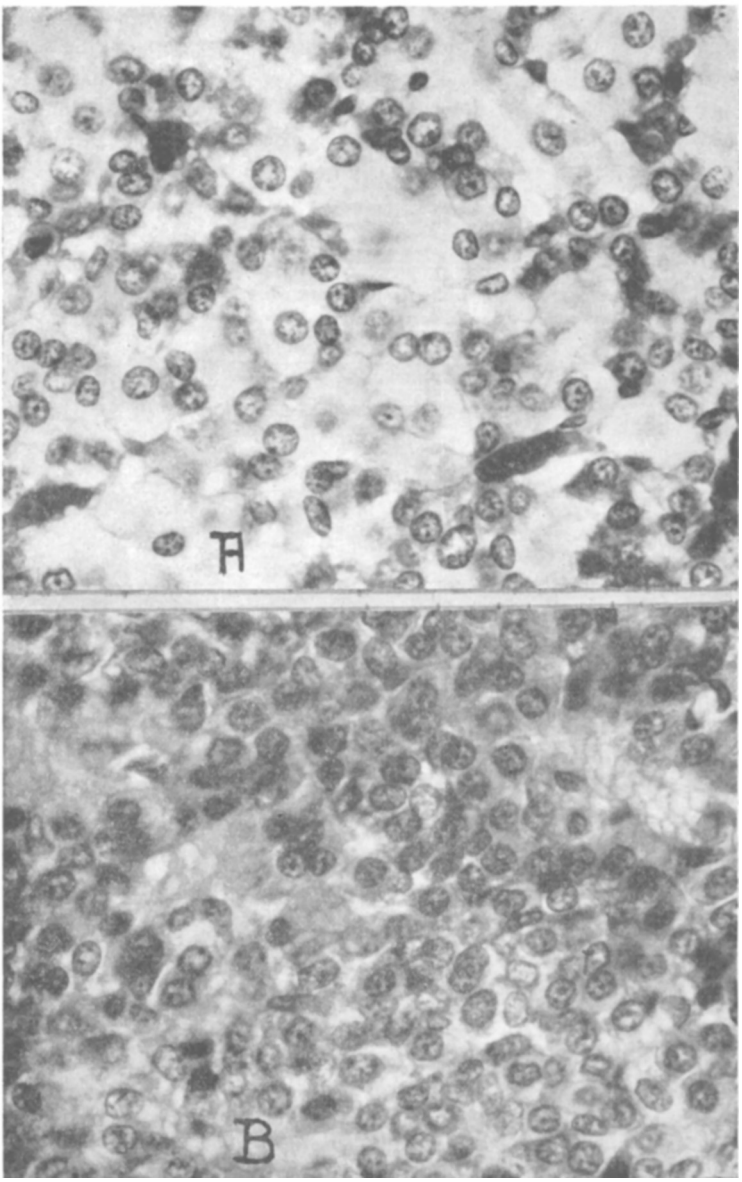

FIG. 3.
A. Section of anterior pituitary shown in Fig. 2. (800×)
B. Section of anterior pituitary of control male rat 8YC. (800×)

cells to castration or pregnancy cells is still under discussion and it would appear premature to make any statement as to their functional significance. Among 12 males which were on the diet longer than 2 months, 11 showed definite changes while only 2 of the 7 females

presented similar findings under the same condition. In an additional group of 9 females put on the diet after maturation, only 2 showed the large cells described above. The reproductive system was examined in all animals. The rats which were 2½ to 3 months of age and had been on the diet 2 months or less showed a retardation of the reproductive organs. Among the males who remained on the diet for a longer period, 11 showed only slight retardation on histologic examination; one appeared definitely retarded. The females reared on the diet showed delayed maturity at the age of 5 or 6 months, while those put on the diet after maturation presented normal reproductive organs. In order to rule out the absence or insufficiency of vitamins E and A as the cause for the changes found, wheat germ oil and carotene oil were added to the diet of some of the experimental animals. These additions had no effect upon the results.

10095

The Standardization of Diluted Tuberculin.

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In developing the method for standardization of tuberculin described in this paper, we were seeking one superior to any hitherto reported, in two respects: (1) a method that would be accurate within at least 20% instead of the 40 and 60% accuracy hitherto available; (2) an accurate method so sensitive that it could be used to standardize tuberculin ready diluted 1-10,000. We required such a method so that tuberculin ready diluted in the diluent previously described[1] could be tested with precision after dilution, and just before distribution, so that the material used by the physician in the field could be of known potency.

We had been comparing tuberculin of our manufacture with the International Standard tuberculin of the League of Nations for a number of years by injecting a 1-10,000 dilution of the standard and the unknown tuberculin in each arm of 100 individuals. Variations in the results indicated that this was far from an accurate method of comparison. Also carefully run experiments in which a 1-10,000 dilution, and 70, 80, or 90% of this concentration of a

[1] Gottschall, R., and Bunney, W. E., *J. Immunol.*, 1938, **34**, 103.