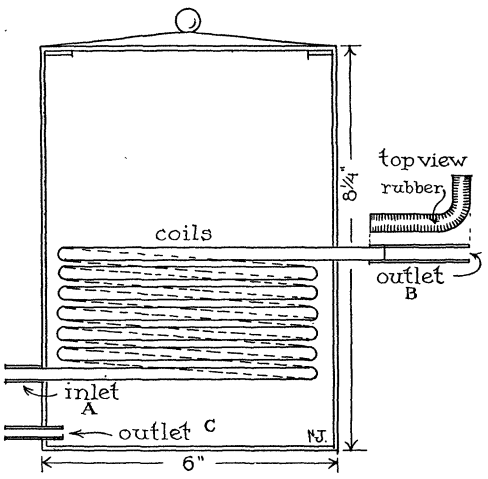


FIG. 1

air throughout the cooling chamber. The inner surface of the latter is painted with black asphaltum, while the exterior is overlaid with asbestos. The protruding portion of the copper tubing forming the inlet A is connected by rubber tubing to a calcium chloride tube for the purpose of dehydration, and in turn to a compressed air supply. The copper tubing, insulated with rubber, passing through the wall of the cooling chamber to form the outlet B, is bent at right angles horizontally, thus permitting direction of air at the surface of the block, and yet allowing for placing the cooler to one side and in front of the microtome. The height of the tubing at this point is variable with the type of microtome employed, but should be so placed as to allow full utilization of the cold air supply. If desirable, an outlet C, as a drain, may be inserted.

In actual operation, the ice chamber is filled with cracked ice, ice and salt or other freezing mixtures. For purposes of this laboratory, the former gave a temperature range sufficiently cool for cutting during the summer months. The microtome and cooler are so oriented that the cold air emitted at the outlet B will play directly on the cutting surface of the preparation. The distance of the former from the latter may be determined by the extent of cooling desired. A few sections are cut without turning on the compressed air. Having thus obtained the basis for a ribbon, a gentle stream of air is directed at the block and cutting is resumed.

Contrary to an opinion that may occur to the reader, the air draft created does not hinder manipulation. During a period of seven months' use, no difficulties were experienced with electrification of the paraffin ribbon.

GERMAIN CROSSMON

# SPECIAL ARTICLES

## CRYSTALLINE CARBOXYPOLYPEPTIDASE

CARBOXYPOLYPEPTIDASE splits the amide linkages of certain amino-acid compounds, such as chloracetyl tyrosine, tyrosyl tyrosine and leucyl glycyl tyrosine, with the liberation in each case of an amino-acid which in the intact compound has a free carboxyl group.[1] I have isolated from bovine pancreas a crystalline water-insoluble protein which attacks chloracetyl tyrosine. Peptic digests are also attacked, even in the presence of formaldehyde. Other substrates have not been tested, so it is not yet certain that all the supposed substrates of carboxypolypeptidase are digested by a single enzyme. It may be that what has hitherto been called carboxypolypeptidase is a group of different enzymes.

Recrystallization of the globulin does not change its carboxypolypeptidase activity but frees it of proteinase. Heating a solution of the crystalline globulin until half the protein is coagulated results in destruction of half the solution's activity. These facts are strong but not conclusive evidence that the crystalline protein is identical with the enzyme whose activity has been measured. A solution of the crystalline globulin diluted to attack chloracetyl tyrosine at the same rate as a given crude extract of pancreas likewise attacks a formolized peptic digest at the same rate as the crude extract. This fact is strong evidence that the enzyme in the crude extract which attacks chloracetyl tyrosine is likewise responsible for the digestion of the formolized peptic digest. Finally, the fact that the crystalline globulin digests a peptic digest even in the presence of formaldehyde proves that the presence of the free amino groups of neither enzyme nor substrate is essential for carboxypolypeptidase activity. No proteolytic enzyme of the pancreas other than carboxypolypeptidase is known to be active in the presence of formaldehyde.

In outline the preparation of the crystals is as follows. To the spontaneously activated turbid fluid which exudes when frozen pancreas is allowed to stand overnight at 5° C., 5 N acetic acid is added until the solution is green to brom cresol green. The acid solution is kept at 37° C. for two hours and the clotted suspended matter is filtered off. The filtrate is diluted with ten times its volume of water. The resulting precipitate is allowed to settle, the supernatant solution is rejected and the suspension is filtered. Water is added to the precipitate to give a suspension twice as active as the original turbid fluid and then 0.2 M

[1] E. Waldschmidt-Leitz, *Physiol. Rev.*, 11: 358, 1931; M. Bergmann, SCIENCE, 79: 439, 1934.

$Ba(OH)_2$ is added until the suspension is pink to phenolphthalein. Whereas NaOH would dissolve all the dilution precipitate under these conditions, $Ba(OH)_2$ dissolves only a part of the protein but all the carboxypolypeptidase. After removal by centrifugation of the undissolved protein 1 N acetic acid is added to the supernatant solution until the solution is orange to phenol red. The globulin crystals thereupon appear, promptly if the solution is seeded, slowly if it is not. The protein can be dissolved with NaOH and recrystallized by neutralization.

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## THE EFFECTS OF PITUITARY IMPLANTS AND EXTRACTS ON THE GENITAL SYSTEM OF THE LIZARD

OVULATION has been induced in a serpent, *Xenodon merrimi,* six days after five homoplastic whole pituitaries were implanted.[1] Hypertrophy of the genital system has been produced in Lacerta[2] and in young alligators[3] by means of mammalian pituitary extracts. Removal of the pituitary causes atresia in the testes of the garter snake (*Thamnophilis sirtalis* and *Thamnophilis radix*) followed by a partial restoration to normal when pituitaries are implanted.[4]

In a series of experiments carried on between October 30, 1933, and April 4, 1934, fifty-five females and seventy-nine males of *Anolis carolinensis* received injections of Antuitrin S (human pregnancy urine extract, Parke Davis), while twenty-five females and twenty-five males received injections of sheep pituitary (whole pituitary extract of Parke Davis). Approximately fifty animals were kept as controls. A single dose with either extract was not more than .02 cc diluted with two or three volumes of cold-blooded Ringer. This proved to be the maximum dose that was safe to use.

The males responded very completely to both extracts and could be very easily distinguished from controls in the following particulars: (1) The dorsal crest along the neck and back was raised, often to the height of an eighth of an inch. (2) The hemipenes could be everted. (3) The testes were often enlarged to two or three times the size of those of controls. (4) The epididymis and vas deferens were always greatly enlarged. In an extreme case of hypertrophy a single loop of the epididymis was found to be at least fourteen times the diameter of that of a control which was killed at the same time. (5) Spermatozoa were found in the epididymis after the fourth daily injection. (6) The vas eferens was slightly enlarged. (7) Courtship and fighting were a common activity on every sunny day during winter and early spring.

In regard to the females, hypertrophy of the ovaries and oviducts was produced with both Antuitrin S and sheep pituitary, but actual egg-laying resulted only with the latter extract. Some females which were injected with sheep pituitary retained mature ova within the ovaries. These eggs were slowly resorbed during the ensuing three months. Neither the ovaries nor oviducts enlarged as much with Antuitrin S as with the sheep extract. After twelve injections of sheep pituitary, two eggs were laid on March 23 and three more the next day. A sixth egg was laid on April 11. The first egg to be laid by any of the controls was on April 18 and a second egg on May 8. No more eggs were laid by controls until June, July and August.

The metabolism of injected animals was greater than that of controls as was shown by an increase both in appetite and in the amount of food eaten. Also, food was required oftener. General activity and speed of movement were undoubtedly greater. Moulting occurred more frequently.

The after-effects were noticeable. Four months after the last injection found many of the treated lizards to be persistently thin, although on an average they ate more than the controls. A few died apparently of starvation while controls, which had received approximately the same amount of food, lived in a perfectly healthy condition.

Complete details concerning these experiments with mammalian pituitary extracts are to be reported later.

It may be of further interest to state that in connection with some experiments with pituitary implantations in *Anolis carolinensis* (December 21, 1933, to April 14, 1934), one female which received four whole pituitaries (taken from males of the same species) failed to ovulate, but the genital system was approximately twice the size of that of controls. Out of three females which received five similar homoplastic implants, two died before ovulation (autopsy showed hypertrophy of the genital system) but the remaining female actually laid two eggs, one on March 24 and another four days later. Another female, which received three frog pituitaries, laid an egg on April 12.

When the two females last mentioned were killed (April 14) they showed mature ova just ready to leave the ovary. Only one ovary in each female, however, contained a mature ovum, although the oviducts

[1] B. A. Houssay, *Compt. Rend. Soc. Biol.*, 106: 377–378, 1931.
[2] M. Herlant, *Arch. de Biol.*, 44: 347, 1933.
[3] T. R. Forbes, *Proc. Soc. Exp. Biol. Med.*, 31: 1129, 1934.
[4] W. H. Schaefer, *Proc. Soc. Exp. Biol. Med.*, 30: 1363–1365, 1933.