16204

Enzyme Studies on the "Endocrine Kidney."

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Selye[1] reported a technic for the transformation of the kidney into a purely endocrine organ in the rat. This technic consists in partially constricting the aorta between the origins of the 2 renal arteries by means of a ligature adjusted so that the blood pressure in the kidney below the constriction (in the rat the renal arteries leave the aorta at different levels) decreases to a point where no filtration occurs. To further insure against filtration taking place in that kidney the ureter is tied off and cut. The circulation of blood remains adequate, however, for the nutrition of the organ. Rats so treated have been shown to develop a very acute form of malignant hypertension.[2] The present experiment deals with the determination of the arginase and phosphatase content of these kidneys, which are non-functional as regards urine formation.

Procedure. Twelve female albino rats weighing between 150 to 160 g were used in our experiment. The operation as described by Selye and Stone[2] was performed under ether anesthesia. Seven days later the animals were sacrificed and the kidneys removed, weighed and placed in separate vials containing 5 ml of distilled water; the right (normal) kidneys served as controls. Five samples were then sent fresh by air mail to Rochester where they were analyzed for the various enzyme activities by the previously described procedures.[3-6]

Results. There is a marked, indeed almost a complete, disappearance of both arginase and "alkaline" phosphatase from the "endocrine" kidney (Table I). The "alkaline" phosphatase was determined also with the addition of magnesium sulfate[7] to the substrate mixture in order to rule out the possibility of lack of activator instead of a decrease in amount of this enzyme. The increase in activity was identical for the 2 sets of tissues, 47.7 and 46.7% for the "endocrine" and normal kidneys respectively. The change in "alkaline" phosphatase is in agreement with the observations by Wilmer[8] on the kidneys of rabbits and rats after ligature of the ureters.

TABLE I.

		Arginase		Phosphatases "Alkaline"		Phosphatases "Acid"	
Kidney	Wt, mg	Total U	U/g	Total U	U/g	Total U	U/g
Normal	811	64	99	109	141	15.0	18.2
Endocrine	357	6	17	5.5	16	6.0	16.6
% diff.	—56	—91	—83	—95	—89	—60	—8.8

U = Units.

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It is of special interest that the "acid" phosphatase, in contrast to the other enzymes, decreased in total amount but not in concentration (units per gram of tissue). It would seem, therefore, that this enzyme is necessary for the integrity of the cell and perhaps its endocrine function while the "alkaline" phosphatase and arginase are necessary only in connection with urine formation.

Summary. In "endocrine kidneys" there is a marked indeed almost a complete disappearance of both arginase and "alkaline" phosphatase. On the other hand the "acid" phosphatase decreases in total amount but not in concentration.

16205

Activation of Hypertensin and Tyrosine by Subthreshold Amounts of Epinephrine.*

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Fractions of hog kidney containing renin do not produce constriction of the vessels of the rabbit's ear until subthreshold amounts of epinephrine are added to the perfusate.[1] Similar vaso-activation of liver fractions by epinephrine has been noted when they are combined with subthreshold amounts of epinephrine.[2] Fresh plasma is also capable of activating liver fractions.[3] This last observation is of interest because of the report that vaso-inactive renin-containing kidney fractions become vasoconstrictive when plasma is added to the ear perfusate.[4] This was ascribed to the formation of hypertensin rather than to the activation of renin by plasma. Since a similar constriction occurs following the addition of plasma to liver fractions under circumstances that would seem to be incompatible with the formation of hypertensin, a reinvestigation of the action of hypertensin on the vessels of the rabbit's ear was undertaken.

The experiments that follow show that hypertensin, a protein fraction, constricts the vessels of the rabbit's ear only when the perfusate is supplemented with traces of epinephrine or fresh plasma. This suggested the possibility that parts of the protein molecules, *e. g.*, peptides or even amino-acids, are responsible for the observed effect. Accordingly, the studies were extended to encompass a large majority of the known amino-acids. Special attention was directed to tyrosine, tyrosine-containing peptides, and a tyrosinamide in accord with Cruz-Coke's[5] hypothesis that tyrosine molecules are necessary for the *in vivo* action of hypertensin.

Materials and Methods. Hypertensin was prepared from purified hog renin with the bovine globulin Fraction IV-1 (Armour) serving as substrate. Three hundred cc of a 1% solution of substrate were buffered at pH 7.5 and incubated for 10 minutes with 2-3 Swingle units of renin. N1 HCl was then added to bring the pH to 5.2 and the mixture immersed in boiling water for 10 minutes. The coagulated proteins were then removed by filtration and the almost water-clear filtrate lyophilized. The lyophilized material was taken up in 16 cc of distilled water and the small residuum of undissolved particles was

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