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Received December 5, 1966. P.S.E.B.M., 1967, v124.

Renal Acid Deoxyribonuclease: Observations on Distribution and Cell Relationship. (31883)

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Acid deoxyribonuclease is a recognized member of the lysosomal enzyme group(1). It has been extensively investigated in the liver(1,2,3), but has received less study in kidney tissue(4,5). Straus(6,7) utilizing kidney cortical homogenates, isolated 3 fractions sedimenting between 650 and 15,000 $\times$ *g*, rich in acid deoxyribonuclease, and similar in enzyme content to deDuve's liver cell lysosomes(1). Interestingly, the acid deoxyribonuclease activity in these fractions responded differently to activation by osmotic effects. This report presents further evidence in this regard and illustrates the striking regional distribution of acid deoxyribonuclease in the rat kidney.

Materials and methods. Male Wistar rats weighing between 350 and 500 g were sacrificed by decapitation and exsanguination. The kidneys were quickly removed, washed in saline, and their capsules stripped. A 5.5 mm cork borer was inserted midway between the renal poles in such a manner as to completely include papilla. The core thus obtained was divided into 4 segments: outer cortex (O.C.), inner cortex (I.C.), outer medulla (O.M.), and inner medulla (papilla, I.M.). In a previous study(8), this method for separating the renal segments is described in detail. In each experiment, segments from 10 kidneys were combined. The tissue was homogenized in 0.25 M sucrose (1:20 w/v) using 5 uniform passes with a teflon pestle (Potter-Elvehjem Homogenizer) at 2000 RPM. A portion was centrifuged for 30 minutes at 37,000 $\times$ *g* in a refrigerated centrifuge (Servall Superspeed RC-2 with an S-34 angle head). The remainder of the homogenate was frozen and thawed 10 times for optimal enzyme activation(9). Such a procedure did not alter the activity of the supernatant.

For the distribution experiment, 300 μliters of homogenate (after freeze-thaw activation) or supernatant were added to an equal volume of incubation media containing 1 mg DNA/ml (calf thymus, highly polymerized, Mann Research Laboratory, New York), 0.2 M acetate buffer, pH 5.0, and 20 mM $MgCl_2$(2,10). The samples were incubated for 10 minutes at 37°C. The reaction was stopped by addition of 600 μliters of 10% $HClO_4$. The tubes were then centrifuged, the supernatant removed, and absorption at 260 mμ was determined. Appropriate controls were done. The effects of molarity were examined by decreasing the homogenate volume to 200 μliters and adding 100 μl of either water or 1.0 M sucrose, resulting in a final sucrose molarity in the digestion mixture of 0.08 M or 0.25 M, respectively. At the same time, the effect of freeze-thaw techniques was also investigated. Supernatant and homogenate protein was determined by the method of Lowry(11). Enzyme activity was calculated both as specific (millimicromoles/minute/mg protein) and total (millimicromoles/minute/ml of 5% homogenate) activity. The units were in terms of liberated mononucleotides (2,12) assuming an average extinction coefficient of 8.5 $\times$ 10^6 cm^2 $mole^{-1}$. The results were calculated as the mean and standard deviation of 10 experiments; when appropriate, the Student's t-test (paired) was employed.

Results and discussion. When calculated as total activity (Fig. 1) the O.C. had the

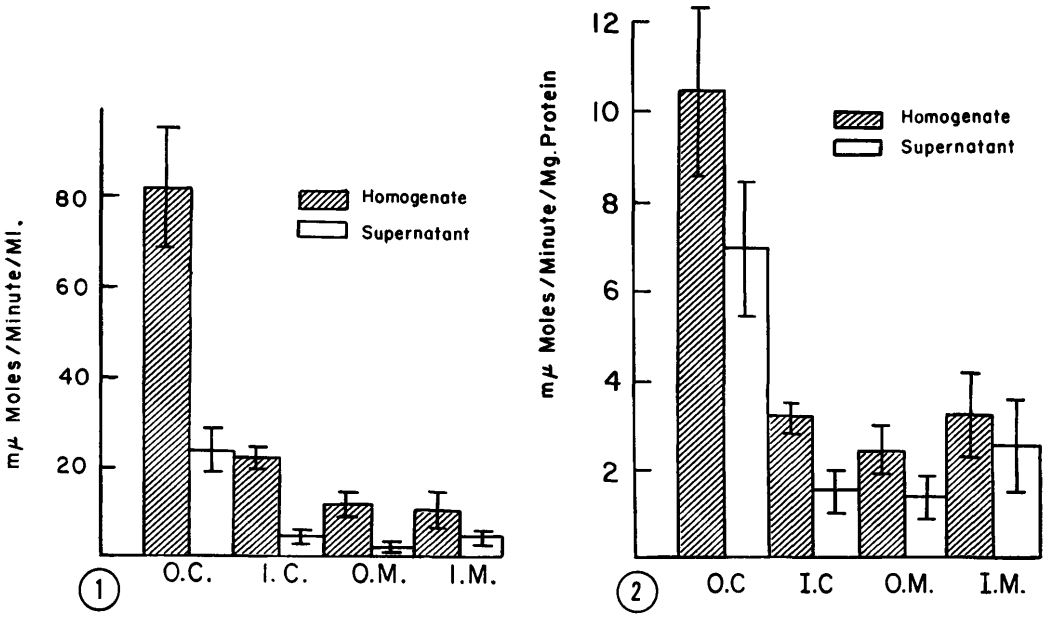

Fig. 1. Acid deoxyribonuclease distribution. Total activity. O.C., outer cortex; I.C., inner cortex; O.M., outer medulla; I. M., inner medulla.
Fig. 2. Acid deoxyribonuclease distribution. Specific activity.

highest enzyme content. The activity of the I.C. was considerably less and the medullary segments had the lowest values. The average ratio of supernatant total activity/homogenate total activity was highest in the I.M., 0.41, intermediate in the O.C., 0.29, the lowest in the I.C. and O.M., 0.21 and 0.20 respectively. In terms of specific activity, the O.C. (Fig. 2) was much higher than the other 3 segments which were about equal.

As expected, the highest values of enzyme activity equal to those in the aforementioned distribution experiments were obtained when the freeze-thaw procedure was utilized (Fig. 3). Varying the sucrose molarity of the incubation media had no effect. When freeze-thaw techniques were omitted, all segments except the I.M. showed a marked decrease in activity. If the sucrose molarity of the incubation media was increased to 0.25 M, a further loss of activity occurred only in the cortical segments. This was most apparent when values within individual experiments were compared. These findings were highly significant in the O.C. ($P = 0.001$) but less so in the I.C. ($P = 0.05$). Free activity (lowest mean total homogenate activity X100/highest mean total freeze-thaw homogenate activity in the various segments) was as follows: O.C. = 49.4%, I.C. = 28.5%, O.M. = 33.9%, and I.M. = 90.0%.

The striking regional distribution of renal acid deoxyribonuclease as found in this study is consistent with the distribution of another lysosomal enzyme, acid phosphatase(8), and the lysosomal distribution found in histochemical preparations(13). The results also reveal that the pattern of enzyme activation is different among the various renal segments. Such observations are consonant with the different tubular populations of each renal segment and suggest that the relationship of the enzyme to the cell may vary. The finding that osmotic activation occurs only in the cortical rather than the medullary segments implies structural differences in lysosomal membranes binding the enzyme. Under all circumstances of this study the papillary acid deoxyribonuclease was almost completely available; this observation raises the possibility that the enzyme may not even be related to the comparatively few lysosomes indigenous to this area(14).

Since homogenates were used, conclusions concerning lysosomal behavior based on this study must be approached with caution.

However it should be noted that the levels of free activity were comparable to those found by Straus(7) in partially purified lysosomal preparations. Finally in studies with acid phosphatase using osmotic and triton activation, we have noted a similar segmental

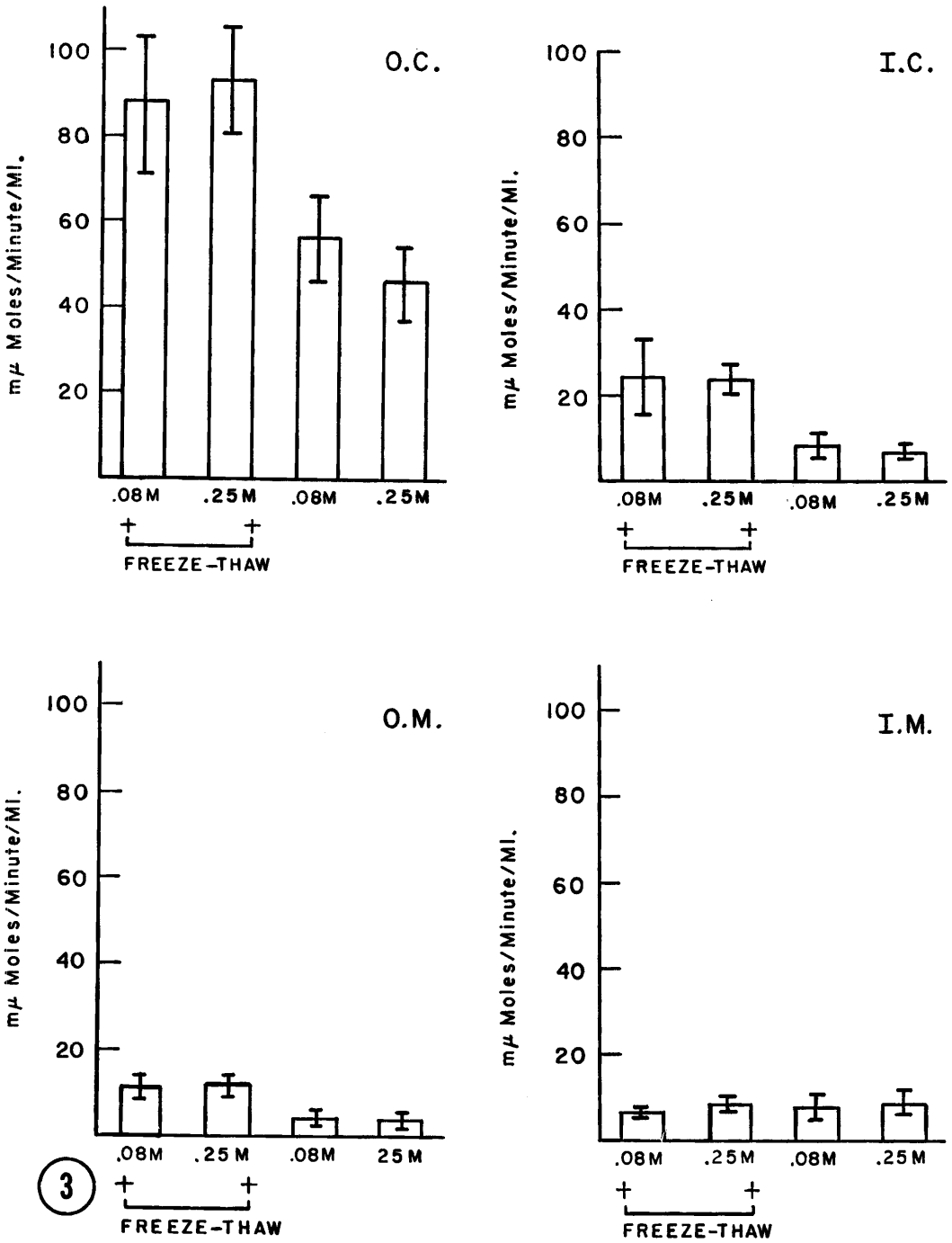

Fig. 3. Effects of incubation molarity and freeze-thaw on homogenate total activity. The molarity of the incubation media is expressed in terms of sucrose concentration.

pattern of enzyme availability(8).

Summary. Acid deoxyribonuclease activity was measured in rat kidney tissue divided into the various renal segments: outer cortex (O.C.), inner cortex (I.C.), outer medulla (O.M.) and inner medulla (I.M., papilla). In terms of total activity, the O.C. had the highest enzyme content. The activity of the I.C. was considerably less and the medullary segments had the lowest values. If freeze-thaw procedures were not utilized, all segments except the papilla markedly decreased in enzyme activity. The enzyme values of the cortical segments were increased by lowering the sucrose content of the homogenate media. Those findings suggest that in addition to regional variation of activity, the relationship of acid deoxyribonuclease to the cell is different among the renal segments.

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Received November 9, 1966. P.S.E.B.M., 1967, v124.

An *in vitro* Preparation of Dog Parotid Gland.* (31884)

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An *in vitro* preparation of an exocrine gland might serve as a convenient model for the study of exocrine gland electrolyte transport. Burg and Orloff have recently produced isolated renal tubuli by the treatment of kidney tissue with collagenase(1). With some modifications their procedure has been applied to the production of seemingly intact acinar and tubular elements from the parotid gland of the dog. This paper presents the method of preparation and an investigation of the viability of this system.

Methods. Parotid glands excised from young pentobarbital-anesthetized dogs were diced and washed in Krebs Ringer bicarbonate solution containing 200 mg% glucose (KRBG) at pH 7.4. The tissue was placed in an Erlenmeyer flask containing fresh KRBG and 400 mg% collagenase.‡ It was shaken in an Eberbach reciprocating shaker at 227 cycles/min with oscillations of approximately 4 cm at 25-27°C for 2 hours. At the end of this time a high percentage of tissue had been reduced to minute fragments. Unreduced tissue was discarded. The yield was washed with 4 volumes of fresh KRBG and centrifuged at 50 × *g* and 0°C for 5 minutes. The washing procedure was repeated 3 times.

As an index of viability of the preparation, its capacity to concentrate iodide was investigated. This parameter was chosen since

* Supported by USPHS Training Grant 5 T1 DE 3.

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