Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 91 APRIL, 1956 No. 4

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Cytoplasmic Fractionation of Kidney Proteins.* (22315)

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The kidney, like the liver, the heart, and certain other organs, has the capacity to hypertrophy and to atrophy under suitable experimental conditions. When one kidney is removed from the rat, the remaining kidney immediately enlarges, and at the end of 6 days, its protein content has increased by approximately 35%(1). If the rat is starved for a period of 7 days, the kidneys may loose 20% of their structural protein(2). It is not known whether the protein added to or lost from the kidney involves certain cellular structures or whether proteins from all cellular components are equally labile. In order to provide an answer to these questions, the distribution of total protein in the various cytoplasmic fractions of normal, hypertrophied, and atrophied kidneys are determined after homogenization and differential centrifugation.

Methods. Female albino rats of the Slonaker-Addis strain weighing 175-185 g were used in all experiments. Renal hypertrophy was produced by removing right kidney while animal was under ether anesthesia and allow-

* This investigation was supported by a research grant from the National Heart Institute of the National Institutes of Health, Public Health Service.

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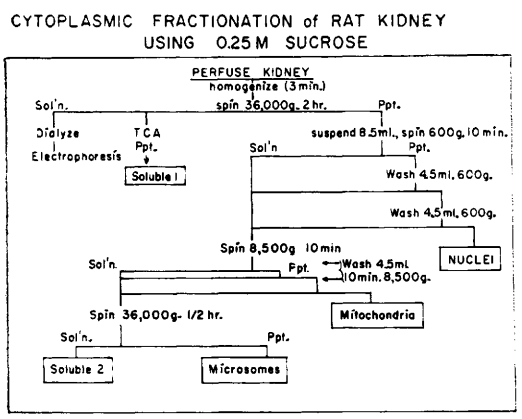

FIG. 1. Flow diagram of fractionation procedure.

ing remaining left kidney to enlarge for 6 days. Renal atrophy was produced by withdrawing all food for 7 days. During this time, the animals subsisted on water only. Rats were anesthetized with ether and their kidneys perfused *in situ* with 0.89% sodium chloride solution as described previously(3). The capsule was removed and the kidneys were blotted dry and transferred to cold room (2°C.) All subsequent steps were carried out at this temperature. Homogenization was carried out in a solution of 0.25 M sucrose containing 0.2% Versene. The homogenate usually contained approximately 3 g of kidney suspended in equal volume of sucrose-Versene solution. The centrifugal method of Hogeboom, Schneider and Pallade(4) was modified slightly as shown in Fig. 1. The particulate fractions were resuspended in distilled water using a pestle designed to fit into the plastic centrifuge tubes. Protein from each fraction was precipitated with 100 ml of 7% trichloracetic acid, washed once with fresh 7% trichloracetic acid, twice with a mixture of equal parts of acetone and ether, once with acetone, and finally once with anhydrous ether. After final centrifugation, the last traces of ether were removed by placing the tubes first in warm water bath and then in oven at 90°C for one hour. The tared tubes containing washed, dried protein were then weighed.

Electrophoresis Procedure. An aliquot of the Soluble 1 fraction was dialysed against boric acid buffer, pH 8.6, for 20 hours at 2°C. Electrophoresis was performed on paper between silicone coated glass plates(5). The paper strips were dried under infra-red lamps and stained with brom phenol blue. The stained paper was cut into strips 0.5 cm wide. The color from each strip was extracted in a solution of 10 parts of concentrated NH_4OH and 90 parts of acetone. Color density of extraction fluid was determined in a Coleman Jr. spectrophotometer at wave length of 610 mμ. The diagrams as shown in Fig. 2 were obtained by plotting per cent absorption on ordinate against distance of migration in centimeters along abscissa.

Results. The results are given in Table I. The data are expressed as means $\pm$ standard deviation from the mean. The significance of the differences observed was determined by the method of Fisher(6) for small numbers of observation. P values of <0.01 are taken to indicate a significant difference between the means compared.

It can be seen that the per cent distribution of the total kidney protein in various cytoplasmic fractions studied in normal rats and in starved rats are not significantly different. After 6 days of hypertrophy, the nuclear fraction is slightly, but significantly less, while the mitochondrial fraction is somewhat in-

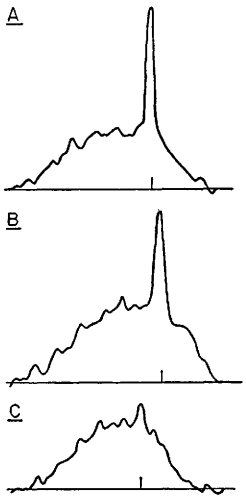

FIG. 2. Electrophoresis diagrams of Soluble 1 fractions. Mobility to the left is toward cathode, mobility to right is toward anode. Arrow indicates point of origin. (A) Normal kidney. (B) Kidney after 6 days hypertrophy. (C) Normal kidney with top fatty layer eliminated.

TABLE I. Percent of Total Protein in Rat Kidney.

Exp. cond.	Control	Neph. 6 days	Starv. 7 days	P value
No. exp.	22	8	8	
Nuclei	19.3 ± 2.7*	14.9 ± 3.3	20.7 ± 1.5	.01 to .001
Mitochondria	21.5 ± 1.5	23.8 ± 2.3	21.3 ± 1.4	.01 to .001
Microsomes	15.8 ± 1.3	15.5 ± 1.4	15.1 ± 0.6	
Soluble 1	27.8 ± 1.4	28.6 ± 1.4	25.5 ± 0.5	
" 2	16.0 ± 1.6	17.1 ± 2.0	17.5 ± 0.8	
" 1 + 2	43.8 ± 2.0	45.7 ± 2.4	43.0 ± 0.8	

* Stand. dev. $= \pm\sqrt{\frac{\Sigma d^2}{n-1}}$.

creased over what is found in kidneys of normal control rats.

Proteins in Soluble 1 fraction of normal and hypertrophied kidneys were subjected to filter paper electrophoresis as described above. Representative patterns are shown in Fig. 2. When the Soluble 1 fraction was withdrawn with a pipette from below a thin fatty surface layer, most of the peak seen at the origin could be eliminated (Fig. 2C.)

Approximately 14 separate peaks can be identified in the patterns from both normal and hypertrophied kidneys. It has not been possible to distinguish electrophoresis patterns of the Soluble 1 fraction as coming from normal kidneys or from hypertrophied kidneys. Differences exist, but they are small and inconsistent.

Discussion. We have been unable to find reports in the literature dealing with this type of analysis of kidney or other visceral organs undergoing hypertrophy or atrophy. While the flux of tissue protein of liver and kidney during regeneration and atrophy may not be strictly analogous, it will be pertinent to refer to certain experiments performed on liver proteins under these circumstances. Thus, Soroff, Claus and Cohen(7) studied proteins of rat liver by the technic of free electrophoresis. They state that throughout an 8 day period of liver regeneration following 70% partial hepatectomy and throughout a 9 day fast, electrophoretic analysis of soluble rat liver proteins showed no significant difference from those of normal rat liver extracts. de Lamirande, Allard and Cantero(8) report that no difference exists in the soluble proteins of cell fractions from regenerating and normal liver by the technic of electrophoresis. However, a decrease in certain components of the mitochondrial fractions of regenerating liver was observed. Luck(9) found that various fractions of liver protein increase equiproportionally with increased protein intake.

It is clear from the present study that from a loss of 20% of the kidney tissue protein during a fasting period through a gain of approximately 35% during a period of compensatory hypertrophy, the relative protein content of the various cytoplasmic fractions of the kidney remains essentially unchanged. This would indicate that under the experimental conditions outlined above, protein is lost from or added to all cellular fractions of the kidney in a nearly equiproportional manner. The results are inconsistent with the view that a

certain fraction of the cellular protein of the kidney is more labile than other fractions under conditions of atrophy and hypertrophy.

Summary. 1. Distribution of kidney proteins in various cytoplasmic fractions has been determined in normal, hypertrophied, and atrophied rat kidney. The Soluble I fraction from normal and hypertrophied kidneys has been subjected to paper electrophoresis. 2. Despite a gain in total protein of approximately 35% in the hypertrophied kidney, and a loss of approximately 20% in total protein in the atrophied kidney, the distribution of proteins in the various cytoplasmic fractions shows only minor deviations from the normal. 3. No consistent differences could be seen in electrophoresis patterns of the Soluble I fraction between normal and hypertrophied kidney. 4. The significance of these findings is discussed.

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Received January 26, 1956. P.S.E.B.M., 1956, v91.

Structures Responsible for the Characteristic Purple Color Development in Diphenylamine Reaction with Serum.* (22316)

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Numerous workers(1-4) have reported that the intensity of purple color which resulted when serum was heated with Dische reagent (5) was increased in a variety of diseases. The reaction has been associated with the serum proteins containing carbohydrate moieties: mucoproteins or glycoproteins found in the alpha globulin fraction of serum(2,6,7). Mucoproteins isolated from bovine tonsils and from human urine also gave the color reaction (8,9). The color reaction has been attributed to the presence of sialic acid(10) in the mucoprotein molecule(7). A number of polyhydroxylic acids, neuraminic(11,12), hemataminic(13), and lactaminic(14), have been isolated and appear to be related to sialic acid(15).

It is the purpose of this report to point out that the structure for sialic acid proposed by Gottschalk(16,20) is consistent with structures known to give the characteristic color development when heated with the Dische reagent.

Absorption Studies. Purple color which results when serum or mucoproteins are heated with the reagent is due to 2 absorption regions in the visible spectrum (Fig. 1). One absorption maximum occurs at about 520 mμ while the second occurs at 650 mμ. The absorption maximum at about 520 mμ is the significant one. The second absorbing region at 650 mμ can be attributed to conversion of common hexoses present in mucoproteins to 5-hydroxymethylfurfural when heated in an acid solution(17,18). Absorption maxima in the vicinity of 520 mμ, as will be shown below, can probably be attributed to a deoxyhexose struc-

* Supported in part by grant from U. S. Atomic Energy Commission Contract AT (11-1) 366 with The Rheumatic Fever Research Institute.

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