CONCENTRATION OF ACID PHOSPHATASE, RIBONUCLEASE, DESOXYRIBONUCLEASE, β-GLUCURONIDASE, AND CATHEPSIN IN "DROPLETS" ISOLATED FROM THE KIDNEY CELLS OF NORMAL RATS*

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It was reported previously (1) that granules ("small droplets") with diameters from 0.5 to 1.5 μ, showing high acid phosphatase activity, could be isolated from the homogenate of the kidneys of normal rats.[1] They are concentrated in a thin, dark brown layer at the bottom of the mitochondrial sediment when mitochondria are prepared according to Hogeboom, Schneider, and Palade (2). It has been suggested (1) that these granules may be related to "secretory granules" and "Golgi bodies" and to fractions rich in acid phosphatase which had been isolated from the cells of the liver of the rat by Palade (3), Novikoff, Podber, Ryan, and Noe (4), and Berthet *et al.* (5). De Duve *et al.* (6) have shown recently that this liver fraction is distinct from the mitochondrial fraction and that acid phosphatase, β-glucuronidase, ribonuclease, desoxyribonuclease, and cathepsin are concentrated in it. It will be shown that the same enzymes are also highly concentrated in the granules isolated from the cells of the kidney of the rat. Since these "droplets" from the kidney cells were found to vary in size over a wide range, 3 fractions with granules of different diameters have been isolated and their enzyme content compared.

Materials and Methods

Male rats of the Sherman strain, 400 to 500 gm. in weight, were used. The kidneys were removed under ether anesthesia and were chilled immediately; cooling was applied in all further manipulations. The cortices, after removal of the capsules and medullae, were homogenized in 0.88 M sucrose solution (2) in a motor-driven homogenizer with teflon pestle, and the homogenate was filtered through 2 layers of cheese-cloth. The isolation of the droplet fractions I, II, and III proceeded as follows:—

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[1] "Large droplets" with diameters from 1 to 5 μ, showing similar enzymatic properties, were isolated from the kidneys of rats after intraperitoneal injection of egg white (1). In the present investigation, only untreated rats were used.

Droplet Fraction I.—The isolation of the largest granules, 1 to 5 μ in diameter, followed the procedure described previously for the isolation of large droplets appearing after injection of egg white (1). A higher centrifugal force, 20 minutes at 650 *g*, was applied for the sedimentation of these granules because the very large droplets are less numerous in the kidney cells of untreated animals than in those injected with egg white. Agglutinated nuclei, whole kidney cells, and blood cells were removed by short centrifugations for 5 minutes at 160 *g*, while most of the remaining nuclei which contaminated the large droplets were separated by filtration of the suspension through a column of non-absorbent cotton (1).

In detail, the following procedure was applied: The kidney homogenate was centrifuged for 5 minutes at 160 *g*, the sediment resuspended in 30 per cent sucrose solution, and centrifuged again for 5 minutes at 160 *g*. The combined supernatant fluids were centrifuged for 20 minutes at 650 *g* and the sediment was resuspended in 30 per cent sucrose solution. The same procedure, *i.e.* two centrifugations for 5 minutes at 160 *g* followed by centrifugation of the combined supernatant fluids for 20 minutes at 650 *g*, was repeated 2 to 3 times more. The resulting sediment was resuspended, the suspension filtered through cotton (1), and the filtrate spun down for 20 minutes at 1100 *g*. The sediment consisted of almost pure large droplets.

Droplet Fraction II.—The droplets of fraction II, 0.5 to 1.5 μ in diameter, were isolated according to the procedure described previously for the small droplets from the kidney cells of untreated rats ("shortened procedure") (1). The following details may be mentioned. After sedimentation of most of the nuclei and granules of fraction I as described in the preceding paragraph, the supernatant fluid was centrifuged for 1 minute at 15,000 *g* (in addition to the time required for the acceleration and deceleration of the centrifuge). The dark brown bottom sediment contained the droplets. Some clumped nuclear material and yellow-brown mitochondrial material, layered on top of it, was removed as well as possible by swirling with 30 per cent sucrose solution and decanting. The remaining dark brown sediment was resuspended in 30 per cent sucrose solution and the granules spun down again by centrifugation for 1 minute at 15,000 *g*. After resuspension of the brown sediment in sucrose solution, the droplets were sedimented finally for 20 minutes at 1100 *g*.

Droplet Fraction III.—The granules of fraction III, with an estimated diameter of 0.1 to 1.0 μ, were found concentrated at the bottom of the mitochondrial sediment and could be recognized by the dark brown color of the pellet. The sediment was obtained by centrifuging for 15 minutes at 15,000 *g* the supernatant fluid from the 1 minute's centrifugation at 15,000 *g*, described in the preceding paragraph. Since it was difficult to separate the thick mitochondrial sediment from the thin, brown bottom pellet, the following procedure was applied. After most of the yellow-brown mitochondrial sediment had been removed by resuspending it in 30 per cent sucrose solution and decanting, the 50 ml. cellulose nitrate tube was sliced just above the brown pellet with a razor blade. The light colored mitochondrial material still covering the brown pellet was removed as well as possible, using a small surgical forceps, the curved tips of which were surrounded with a little absorbent cotton wetted in 30 per cent sucrose solution. The remaining dark brown pellet was resuspended in 3 to 4 ml. of 30 per cent sucrose solution and the above described procedure repeated once more, and occasionally a second time. Small cellulose nitrate tubes (5 or 10 ml.) were used for the repeated sedimentations.

Mitochondria were prepared according to Hogeboom, Schneider, and Palade (2) and purified further by resuspending and resedimenting the main yellow-brown layer of the sediment; the "fluffy layer" was discarded and the dark brown bottom sediment was used for the isolation of the droplet fraction III described above. The high speed centrifugations were made in the Servall SS1 angle centrifuge.

N was determined according to the micro-Kjeldahl and Nessler methods, and P according to Fiske and SubbaRow (7).

Acid phosphatase was determined by measuring the amount of P split off by the enzyme during incubation for 15 minutes at 37° with β-glycerophosphate as substrate. It was observed that the development of the blue color with the molybdate reagent in the Fiske-SubbaRow assay is considerably slowed down in the presence of an excess of β-glycerophosphate. In the previous tests for acid phosphatase in which small samples of droplets were assayed in the presence of 0.032 M β-glycerophosphate (1), the color intensity which had developed after 10 minutes was only ½ to ⅓ of the color developed after 1 hour. Since this delay of the color development was not understood at that time and since the color intensity after 10 minutes was taken as the end-point, the values for acid phosphatase activity of the droplets indicated earlier (1) were much too low. In the present assays, the concentration of the substrate was reduced 5 times, resulting in normal color development within 10 minutes. The reaction mixture contained 0.0064 M sodium-β-glycerophosphate, 0.075 M acetate buffer, pH 5.0, test solution, and water in a total volume of 2.0 ml. The results were expressed in milligram of P split off in 15 minutes per milligram N.

Ribonuclease and desoxyribonuclease were determined by slight modifications of the method of Schneider and Hogeboom (8) by measuring the increase in optical densities at 260 mμ in the Beckman model DU spectrophotometer after incubation for 30 minutes at 37°. The reaction mixture for RNAase contained 5 mg. of RNA (Schwarz) purified by reprecipitation and dialysis, 0.09 M acetate buffer, pH 5.0, and test solution, in a total volume of 2.5 ml.; for DNAase, 0.4 mg. of DNA (Worthington), 0.06 M acetate buffer, pH 5.0, 0.01 M $MgSO_4$, and test solution, in a total volume of 2.5 ml. The reaction of RNAase was stopped by the addition of 1.0 ml. of a mixture containing 5 per cent TCA and 0.75 per cent uranylacetate, and of DNAase by the addition of 1.0 ml. of 5 per cent $HClO_4$. The optical measurements were made after 1:10 dilutions of the clear supernatants. The blanks deducted were identical with the samples except that the substrates were added after the acid. The results were expressed as increase in optical density at 260 mμ per 30 minutes per 0.1 mg. of N.

β-Glucuronidase was determined by incubation of the test solution with 0.0005 M phenolphthalein-glucuronic acid (9) (Sigma Chemical Company) for 30 minutes at 37° in 0.05 M acetate buffer, pH 4.6. After addition of glycine buffer, pH 10.4, the liberated phenolphthalein was measured in the Coleman Universal spectrophotometer at 540 mμ. Five μg. of phenolphthalein served as standard for 100 units of enzyme activity. Results were expressed in units per mg. N per 30 minutes.

Cathepsin was determined according to Anson's hemoglobin method (10). The samples were incubated for 30 minutes at 37° in acetate buffer, pH 4.0. Tyrosine served as reference for cathepsin units (10), and the results were expressed in milliequivalents of tyrosine per milligram N per minute × 10^4.

Cytochrome oxidase was determined colorimetrically by measuring the red pigment formed from dimethyl-*p*-phenylenediamine after incubation for 1 minute at 37° (11). The cytochrome *c* (Sigma Chemical Company) was purer than the cytochrome *c* used previously (11) and did not show any inhibition. With this preparation, approximately 30 per cent higher values for cytochrome oxidase activities of mitochondrial suspensions and total homogenates were found than those reported previously (11). Results were expressed in "quinonediimonium red" units per milligram N per minute.

Uricase was determined according to a slight modification of the method of Schneider and Hogeboom (12) by measuring the decrease in optical density at 290 mμ with lithium urate as substrate in borate buffer, pH 9.0.

As was shown by de Duve *et al.* (5, 13) in the case of the liver granules, the enzymes react only partially with the substrate as long as the granules remain intact. Preincubation, treatment with the Waring blendor, or dilution with distilled water is required to transform the "bound" enzymes into "free" enzymes. The same probably occurs with droplets from kidney cells. In the present and previous (1) experiments, the isolated droplets were always resuspended in distilled water and left overnight in the refrigerator. It is probable that the disintegration of the droplets taking place in distilled water had liberated most of the enzymes. In the following experiments, the suspensions of the isolated droplets in distilled water were further preincubated for 30 to 60 minutes at 37° in buffer solution, pH 4–5, to assure complete liberation of the enzymes.

ENZYME CONTENTS OF DROPLETS

A. Specific Enzymatic Activities.—In Table I, the specific enzymatic activities for acid phosphatase, β-glucuronidase, ribonuclease, desoxyribonuclease, cathepsin, and cytochrome oxidase in different preparations of droplet fractions I, II, and III are indicated and compared to the activities of the total homogenate and of the mitochondrial fraction.

As can be seen from Table I, the specific activities for acid phosphatase, β-glucuronidase, ribonuclease, desoxyribonuclease, and cathepsin are more than 10 times higher for the droplets than for the total homogenate and for the mitochondrial fraction. Droplet preparation Id was partially, and all the other preparations highly purified. Since only a few milligrams of highly purified droplets could be obtained from the kidneys of 10 rats, most enzyme determinations were not made in duplicate. Thus, possible small analytical errors could not be corrected and may account for some of the fluctuations of the figures in Table I.

The β-glucuronidase activity of preparation IIIm (Table I) was much lower than that of most other preparations. Similar low activities were occasionally observed in other preparations. De Duve *et al.* (6) report that in liver fractions an inhibitor of β-glucuronidase may arise from mitochondrial fragments. Since low β-glucuronidase activities were only found in droplet preparations in which the test for cytochrome oxidase had shown the presence of relatively high mitochondrial contamination, it is probable that the low β-glucuronidase activities of these preparations were also caused by an inhibitor of the enzyme.

No uricase activity could be detected in the isolated droplets and not even in the total homogenate of the kidney. This enzyme was tested because it was found by other investigators (6, 14–16) to be associated in liver cells with granules of similar size as those containing acid phosphatase.

B. Intracellular Distribution of the Enzymes.—Whereas the droplets shown in Table I had been highly purified without regard to loss during the pro-

cedure of purification, it was not possible to attain a high degree of homogeneity of the isolated fractions and a good recovery of the enzymes, at the same time. In the experiments, intended for maximal recovery of the enzymes (Table II), the purity of the fractions was therefore low.

TABLE I

*Specific Activites of Enzymes in 3 Droplets Fractions Containing Granules of Different Size**

Fraction and no. of preparations	Estimated particle size	Acid phosphatase	β-Glucuronidase	RNAase	DNAase	Cathepsin	Cytochrome oxidase
Homogenate	—	0.095	1,070	0.74	0.25	0.22	12.4
Mitochondria	—	0.060	430	0.45	0.16	0.19	44.4
Droplets Ia	1–5 μ	1.27	—	—	—	—	2.9
" Ib	"	1.08	11,850	—	—	—	2.7
" Ic	"	0.95	14,500	9.3	4.3	—	4.5
" Id‡	"	0.70	6,750	—	—	1.80	—
" IIe	0.5–1.5 μ	1.37	14,500	—	—	—	4.2
" IIf	"	1.35	—	12.9	—	—	3.1
" IIg	"	1.05	10,300	7.7	3.0	—	4.3
" IIh	"	1.29	13,000	9.1	4.0	—	3.8
" IIi	"	1.50	—	13.7	—	3.16	—
" IIIk	0.1–1.0 μ	1.37	13,800	—	—	—	3.9
" IIIm	"	1.05	2,400	9.4	3.2	—	12.2
" IIIn	"	1.65	18,100	9.6	—	—	9.0
" IIIo	"	1.62	11,200	9.3	—	—	16.1
" IIIp	"	1.05	—	8.2	—	2.36	—

* The enzymatic activities were expressed as indicated under methods.

‡ Partially purified preparation.

Thus, the nuclear fraction (NDrI), obtained by centrifugation of the homogenate for 20 minutes at 650 *g*, contained also the droplet fraction I and was contaminated with whole kidney cells, blood cells, and many mitochondria. The droplet fraction II (DrII), obtained by centrifuging the supernatant fluid of fraction NDrI for 1 minute at 15,000 *g*, was contaminated with some nuclei and mitochondria. The droplet fraction III (DrIII) consisting of the dark brown bottom portion of the mitochondrial sediment, was contaminated with many mitochondria. The mitochondrial sediment (M), prepared by centrifugation of the supernatant fluid of fraction DrII for 15 minutes at 15,000 *g*, included the fluffy layer. The microsomal sediment (P), prepared by centrifugation of the supernatant fluid of fraction M for 90 minutes at 18,000 *g*, consisted of an opaque lower layer and a transparent upper layer.[2] The final supernatant fluid (S) contained a portion of the microsomes which could not be completely sedimented with the centrifuge available.

[2] The opaque lower layer was estimated from its cytochrome oxidase activity to contain approximately 50 per cent of mitochrondrial material. Thus, the centrifugation for 15 minutes at 15,000 *g* had not been sufficient for complete sedimentation of the mitochondria.

In Experiment A, the fractions were spun down only once. In Experiment B, the sediments, except the microsomal sediment, were resuspended in 30 per cent sucrose solution and resedimented, and the wash fluids were added to the following fractions. Since the droplets seem to disintegrate partially during the homogenization of the tissue, the homogenization was not carried to completion, and a homogenizer with a loosely fitted teflon pestle was used. Approximately 20 per cent of the tissue remained unhomogenized and was disregarded. The results of the experiments were probably not much altered by the incomplete homogenization since the enzymatic activities of the residue were similar to those of the total homogenate.

TABLE II

Distribution of Enzymes in Fractions of Rat Kidney

Percentage values; total homogenate = 100 per cent; NDrI, nuclear fraction + droplets fraction I; DrII, droplets fraction II; DrIII, droplets fraction III; M, mitochondrial fraction; P, microsomal fraction; S, supernatant fluid. Experiment A, fractions sedimented once; Experiment B, sediments, except microsomal sediment, washed and resedimented.

	NDrI	DrII	DrIII	M	P	S	Recovery
Experiment A							
Nitrogen	17.9	2.7	4.5	11.6	8.3	51.5	96.5
Acid phosphatase	18.3	9.1	12.2	10.6	10.0	32.2	92.4
RNAase	23.6	10.7	12.3	8.0	7.6	35.8	98.0
DNAase	24.4	10.4	14.0	8.4	7.7	31.0	95.9
Cathepsin	18.2	9.5	10.7	11.1	15.8	26.0	91.3
β-Glucuronidase	16.1	3.2*	13.8	10.1	9.6	37.1	89.9
Experiment B							
Nitrogen	15.0	0.4	1.9	13.5	12.1	55.4	98.3
Acid phosphatase	11.2	3.6	6.2	10.1	11.2	59.4	101.7
RNAase	13.9	3.4	6.1	10.0	9.5	54.4	97.3
DNAase	15.0	4.2	5.2	7.2	7.8	51.5	90.9
Cytochrome oxidase	13.2	0.3	5.4	44.8	25.5	4.3	93.5

* Enzyme probably inhibited.

As can be seen from Table II, the distribution patterns of acid phosphatase, β-glucuronidase, ribonuclease, desoxyribonuclease, and cathepsin were roughly similar in the crude fractions of the kidney homogenate and very different from that of cytochrome oxidase. In Experiment A, 40 to 50 per cent of the 5 enzymes were found in fractions NDrI, DrII, and DrIII containing most of the droplets, and 30 to 40 per cent in the supernatant fluid. In Experiment B, in which the sediments were washed and resedimented, only 20 to 25 per cent of the enzymes were found in fractions NDrI, DrII, and DrIII, and 50 to 60 per cent in the supernatant fluid. These observations seem to indicate that a part of the droplets had been broken up during the homogenization of the tissue and during the vigorous shaking required for the resuspension

of the droplet sediment, and had then released their enzymes into the supernatant fluid. As was indicated earlier (1), the sedimented droplets form clumps from which they are difficult to resuspend.

The low β-glucuronidase activity of fraction DrII was probably caused by the inhibitor discussed above. The β-glucuronidase activity of fraction DrIII increased from 780 to 4400 units after the suspension had been left for 3 days in the refrigerator, and the latter figure was used in the table.

DISCUSSION

A comparison of the isolated fractions I, II, and III (Table I) shows that the enzyme content of the droplets of different sizes is similar and that the concentrations of the 5 enzymes in the droplets over those of the total homogenate are of the same order of magnitude. It may be concluded that acid phosphatase, β-glucuronidase, ribonuclease, desoxyribonuclease, and cathepsin are associated in kidney cells with the same type of granules. This interpretation is supported by the recovery experiment (Table II) showing a similar distribution pattern of the 5 enzymes in the isolated fractions of the kidney homogenate. Although only about 50 per cent of the 5 enzymes was found in those fractions containing the droplets, a much higher proportion of the enzymes may be localized in the droplets in the intact cells. It seems that after destruction of the cells, the droplets are broken up by the subsequent manipulation and so induced to release their enzymes.

The concentration of acid phosphatase, β-glucuronidase, ribonuclease, desoxyribonuclease, and cathepsin in the droplets of kidney cells as well as in the granules isolated by de Duve *et al.* (6) from liver cells indicates the close relationship between the granules from both organs. These granules were probably also present in fractions from liver cells rich in acid phosphatase, described by Palade (3) and Novikoff *et al.* (4), as well as in subfractions rich in desoxyribonuclease described by Kuff and Schneider (14). Most of the acid phosphatase has been estimated by Kuff, Hogeboom, and Dalton (15) to be present in liver cells in granules of about 0.12 μ diameter, while a smaller amount of acid phosphatase was associated with larger and smaller granules. Thomson and Moss (16), too, found the diameter of the acid phosphatase containing granules around 0.2 μ. The droplets from kidney cells, on the other hand, seem to be much more polydisperse. Whereas fraction III, described above, contained granules with diameters comparable to those described for liver granules (6, 15, 16), the droplets in fractions I and II were considerably larger.

The activities of cytochrome oxidase were low in droplet fractions I and II but higher in fraction III. This activity was probably caused by mitochondrial contamination. Whereas most of the mitochondria could be removed from fractions I and II by low speed centrifugation, as could be verified by micro-

scopic examination, complete separation of the mitochondria from the droplets could not be achieved after high speed sedimentation of fraction III. It should be expected that the mitochondrial contamination of fraction III would cause the activities of the other enzymes to be reduced more than was observed in some of the preparations (IIIm and IIIo, Table I). The following interpretations are possible. Either the concentration of the 5 enzymes was higher in preparations IIIm and IIIo than in the other droplet preparations, or the concentration of cytochrome oxidase was higher in the mitochondria present in preparations IIIm and IIIo than in the bulk of the mitochondrial sediment. As a third possibility, it may be considered that there exist transitional forms of granules between mitochondria and droplets as suggested by the work of Oliver *et al.* (17), Kretchmer and Dickerman (18), Zollinger (19), and Rhodin (20). Because of their small size, the droplets and mitochondria of fraction III could not be distinguished by optical microscopy. Electron microscopy would be required to estimate the homogeneity of this fraction. Since little cytochrome oxidase was found in the droplet fractions I and II and since those enzymes which were highly concentrated in the droplets were not concentrated in the mitochondrial fraction, it appears that the droplets represent a distinct class of cytoplasmic granules rather than a subtype of the mitochondria. A similar conclusion was reached for the granules of liver cells by de Duve *et al.* (6). If this interpretation is correct, it may be assumed that the large droplets arise from the small ones, rather than from the mitochondria.

Since it is important to correlate the analysis of isolated cell constituents with cytological and histochemical observations, the work of other investigators bearing on this problem, should be briefly cited. Davies (21) has studied the droplets of kidney cells in different species and age groups and has pointed out that their strong periodic acid–Schiff reaction is indicative of a high glycoprotein content. Huber (22) has described the staining reactions of the droplets in the kidney cells of rabbits, and Holt (23) has demonstrated histochemically granules containing acid phosphatase in kidney cells of rats. Oliver, MacDowell, and Lee (17) have described the histological and histochemical properties of droplets appearing after injection of proteins, and Lee (24) after injection of amino acids. Rhodin (20) has shown by electron microscopy that the kidney cells of mice contain "big granules" with "inner granules" which are perhaps related or identical with the droplets.

Experiments are planned to test changes in size, number, and enzyme content of the droplets under different physiological and pathological conditions. The discussion of the function of the droplets will be deferred until these experiments are completed.

SUMMARY

1. Three fractions of "droplets" having diameters of 1 to 5 μ (fraction I), 0.5 to 1.5 μ (fraction II), and 0.1 to 1.0 μ (fraction III) were isolated from the kidney cells of normal rats.

2. All three "droplet" fractions showed 10 to 15 times higher activities of acid phosphatase, β-glucuronidase, ribonuclease, desoxyribonuclease, and cathepsin than the total homogenate and the mitochondrial fraction.

3. After a rough fractionation of the total homogenate, approximately 50 per cent of the 5 enzymes was found in the fractions which contained the "droplets" and approximately 30 per cent in the supernatant fluid.

4. The similarities between the enzymatic properties of the "droplets" from kidney cells and of the fractions isolated from liver cells by other investigators have been discussed.

BIBLIOGRAPHY

1. Straus, W., *J. Biol. Chem.*, 1954, **207,** 745.
2. Hogeboom, G. H., Schneider, W. C., and Palade, G. E., *J. Biol. Chem.*, 1948, **172,** 619.
3. Palade, G. E., *Arch. Biochem.*, 1951, **30,** 144.
4. Novikoff, A. B., Podber, E., Ryan, R., and Noe, E., *J. Histochem. and Cytochem.*, 1953, **1,** 27.
5. Berthet, J., and de Duve, C., *Biochem. J.*, 1951, **50,** 174. Berthet, J., Berthet, L., Appelmans, F., and de Duve, C., *Biochem. J.*, 1951, **50,** 182.
6. de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., and Appelmans, F., *Biochem. J.*, 1955, **60,** 604.
7. Fiske, C. H., and SubbaRow, Y., *J. Biol. Chem.*, 1925, **66,** 375.
8. Schneider, W. C., and Hogeboom, G. H., *J. Biol. Chem.*, 1952, **198,** 155.
9. Talalay, P., Fishman, W. H., and Huggins, C., *J. Biol. Chem.*, 1946, **166,** 757.
10. Anson, M. L., *J. Gen. Physiol.*, 1937, **20,** 565.
11. Straus, W., *Biochim. et Biophysica Acta*, 1956, **19,** 58.
12. Schneider, W. C., and Hogeboom, G. H., *J. Biol. Chem.*, 1952, **195,** 161.
13. Gianetto, R., and de Duve, C., *Biochem. J.*, 1955, **59,** 433.
14. Kuff, E. L., and Schneider, W. C., *J. Biol. Chem.*, 1954, **206,** 677.
15. Kuff, E. L., Hogeboom, G. H., and Dalton, A. J., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 33.
16. Thomson, J. F., and Moss, E. M., *Arch. Biochem. and Biophysics*, 1956, **61,** 456.
17. Oliver, J., MacDowell, M., and Lee, Y. C., *J. Exp. Med.*, 1954, **99,** 589.
18. Kretchmer, N., and Dickerman, H. W., *J. Exp. Med.*, 1954, **99,** 629.
19. Zollinger, H. U., *Schweiz. Z. Path. Bakt.*, 1950, **13,** 146.
20. Rhodin, J., Correlation of Ultrastructural Organisation and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney, Karolinska Institutet, Stockholm, Aktiebolaget Godvil, 1954.
21. Davies, J., *Am. J. Anat.*, 1954, **94,** 45.
22. Huber, P., *Helv. Physiol. et Pharmacol. Acta*, 1953, **11,** C41.
23. Holt, S. J., *Proc. Roy. Soc. London, Series B.*, 1954, **142,** 160.
24. Lee, Y. C., *J. Exp. Med.*, 1954, **99,** 621.