Brief Notes

Segregation of an Intravenously Injected Protein by "Droplets" of the Cells of Rat Kidneys. By Werner Straus.* *(From the Department of Pathology State University of New York, Downstate Medical Center, New York)*‡

The ability of the "droplets" of kidney cells to segregate proteins is suggested by the work of Gerard and Cordier (1), Smetana and Johnson (2), Oliver, MacDowell, and Lee (3), Rather (4), and others. The question arises as to how the segregating ability of the droplets is related to the function of the enzymes (acid phosphatase, ribonuclease, desoxyribonuclease, cathepsin, and β-glucuronidase) which have been found to be highly concentrated in the droplets (5, 6), and how it is related to the changes occurring after intraperitoneal injection of egg white. As reported in a preceding paper (7) it was observed that the injection of egg white caused the development of large droplets (1 to 5 μ diameter) from small droplets (0.1 to 1.0 μ diameter) and the concomitant release of acid phosphatase, cathepsin, ribonuclease, and desoxyribonuclease from the droplets. The increased content of cathepsin, acid ribonuclease, and deoxyribonuclease in the total homogenate suggested a resynthesis of these enzymes after their release by the droplets (7).

The uptake of fluid and solid particles (so called pinocytosis and athrocytosis) is probably a more general ability of cells than has been suspected previously; it seems to be related to phagocytosis and to be localized in granules derived from the Golgi region of the cells (7–9). Recently, Rose (10) has investigated by phase contrast cinematography granules which take part in pinocytosis in tissue cultures of HeLa cells and has found several interesting analogies to the droplets of kidney cells and to the "lysosomes" of liver cells (de Duve *et al.*, 11).

The ability of the droplets to segregate proteins required definite proof. Straus and Oliver (12) observed that droplets isolated after injection of egg white contained several times as much serologically reacting egg white as the other fractions of the kidney homogenate. However, some uncertainty remained, since the serological method used in these experiments was only semiquantitative. The uncertainty increased when Mayersbach and Pearse (13) could not confirm the concentration of egg white within the droplets by histochemical techniques employing fluorescent antibodies (17). Therefore, a simple, quantitative method was worked out with the aim of settling the question.

The method consists in injecting a small amount of a plant enzyme, horseradish peroxidase, as a tracer, and in analyzing the distribution of this enzyme in isolated tissue fractions by a simple colorimetric test. It should also be possible to trace the injected enzyme histochemically by conventional histo-

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chemical methods for peroxidase. It is important to note that most animal tissues do not contain peroxidase, and that no toxic effects of the injected enzyme were observed. Since the molecular weight may have an influence on permeability and segregation in biological systems, it may be mentioned that the molecular weight of horseradish peroxidase is approximately 44,000 (14), and that this relatively low molecular weight if an excess of the appropriate substrates has been added. This property was utilized previously in the microdetermination of cytochrome *c* oxidase by adding cytochrome *c* to the cell material. Polyphenol-oxidase was also tested by adding 3,4-dihydroxyphenylalanine to a potato extract (15). By using H_2O_2 as substrate and a commercial peroxidase preparation (Nutritional Biochemicals Corporation, Cleveland, Ohio) as the enzyme source, it

TABLE I

Segregation of Intravenously Injected Horseradish Peroxidase by Droplets from Rat Kidney Cells

H, homogenate; DrI, droplet fraction I; DrII, droplet fraction II; DrIII, droplet fraction III; M, mitochondrial fraction; P, microsomal fraction; S, final supernatant fluid. Specific activities of peroxidase and cytochrome oxidase expressed as units of red pigment formed from dimethyl-*p*-phenylenediamine per mg. N per minute (15); specific activity of acid phosphatase expressed as mg. P liberated (from β-glycerophosphate) per mg. N per 15 minutes.

	H	DrI	DrII	DrIII	M	P	S
Experiment A							
Peroxidase	2.2	12.1	11.1		1.8	0.8	1.7
Acid phosphatase	0.072	0.405	0.395		0.067	0.085	0.046
Cytochrome oxidase	19.0	9.1	26.7		42.6	10.6	—
Experiment B							
Peroxidase	0.6	4.8	3.1	3.4	0.6	0.2	0.5
Acid phosphatase	0.078	0.850	0.695	0.710	0.060	0.090	0.048
Cytochrome oxidase	17.8	2.2	11.3	28.6	45.7	4.9	—

may be favorable for the present application.

The colorimetric method for peroxidase is a modification of the assay for cytochrome *c* oxidase worked out previously (15). It makes use of the transformation, by oxidative enzymes, of dimethyl-*p*-phenylenediamine into a red pigment of semiquinone nature. Microgram amounts of cell material, resuspended or diluted with distilled water, develop only little color during the first few minutes of incubation. They will give strong color development, proportional to the amount of enzyme and to the time of incubation, was found that the color developed from dimethyl-*p*-phenylenediamine was proportional to peroxidase in the range of 0.2 to 4.0 μg. of the commercial preparation, and was also proportional to the time of incubation during the first 40 to 60 seconds. Only little color was formed in blanks containing the reagents alone (without tissue samples), or in blanks containing kidney fractions of peroxidase-injected rats in the absence of H_2O_2, or kidney fractions of non-injected rats in the presence of H_2O_2. A more detailed description of the method, together with some applications showing the distribu-

tion of peroxidase in homogenates of different tissues or in subcellular fractions after intravenous injection, will be given later.

Table I shows the application of the method to partially purified fractions from rat kidney cells. In the first experiment, A, 15 mg. of the commercial peroxidase preparation (Nutritional Biochemicals Corporation), dissolved in saline, were injected into the femoral vein of a rat, and the kidneys were removed 3 hours later. They were homogenized in 30 per cent sucrose solution (16) and fractionated into 5 crude fractions by single sedimentations in the centrifuge. Partially purified suspensions of droplet fraction I (DrI) and combined droplet fractions II and III (DrII + DrIII) were obtained as described previously (5–7) by resuspending the dark brown lowest layers which separate at the bottom of the nuclear and mitochondrial sediments. Mitochondria (M) and microsomes (P) were prepared by slight modifications of the method of Hogeboom, Schneider, and Palade (16)[1]. In the second experiment, B, peroxidase in saline was injected into 5 rats, 7.5 mg. into each animal, and the kidneys were removed 2 hours later and pooled. Since more tissue was available than in the first experiment, the sediments could be purified by one resuspension and resedimentation. Six fractions were prepared: droplet fraction I (1 to 5 μ diameter), droplet fraction II (0.5 to 1.5 μ diameter), droplet fraction III (approximately 0.1 to 1.0 μ diameter), mitochondria, microsomes, and a final supernatant fluid. In both experiments, the homogenates and the cytoplasmic fractions were assayed for peroxidase, acid phosphase, cytochrome oxidase, and nitrogen, and the specific enzymatic activities (activity per mg. N) were determined.

As can be seen from Table I, the specific activities of peroxidase were 5 to 8 times higher in the droplet fractions than in the other fractions of the homogenate. It can be estimated that the droplet fractions shown in Table I were not more than 35 to 50 per cent pure, since highly purified droplets had activities of acid phosphatase of approximately 1.6 mg. P per mg. N per 15 minutes (6), and since the activities of cytochrome oxidase of the droplet fractions shown in Table I indicate relatively high mitochondrial contamination. Thus, a still more pronounced segregation of the injected peroxidase by the droplets will probably be detected if the droplets are purified more extensively.

The method is now being applied to analyze the role of the kidney droplets in proteinuria and protein reabsorption in greater detail. We are also investigating whether fractions rich in acid phosphatase from tissues other than the kidney have the ability to segregate the injected protein and whether this can be correlated with the histochemical localization of the injected peroxidase.

[1] The crude nuclear fractions were not tested since they were contaminated with many large droplets. Purified nuclei, free from most droplets, will be analyzed later. This may be of interest since Coons, Leduc, and Kapland (17) by applying their fluorescent antibody method, observed the concentration of injected antigens in the nuclei.

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