periments are quite different from the first two series of experiments. A check on possible factors producing this has not revealed any explanation for this variation.

If the *in vivo* results are actually produced by enzymatic degradation of the tubular cells, the high degree of correlation between the *in vivo* and *in vitro* experiments supports the hypothesis that the bound sodium of the phospholipids is not an artifact of the extraction process but indeed represents some function of the molecule in the intact kidney. This function could be a role in sodium transport.

Summary. Phospholipase C and pancreatic lipase reduce the reabsorption of fluid droplets infused into rat proximal tubules. Phospholipase D, chymotrypsin, hyaluronidase, collagenase and $CaCl_2$ appeared to have no effect on reabsorption rate. Ribonuclease also reduced fluid reabsorption. When kidney homogenates are treated with the same enzymes, phospholipase C and chymotrypsin reduce the ability of extractable phospholipids to bind sodium and potassium. The data suggest that phospholipase D and ribonuclease may enhance cation binding. It is suggested that phospholipids play a critical role in salt transport by the proximal tubule.

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Acid Phosphatases of Rat Polymorphonuclear Leucocytes.* (31321)

BYUNG PAL YU,† FRED A. KUMMEROW, AND TOSHIRO NISHIDA
Burnsides Research Laboratory, University of Illinois, Urbana

Since lysosomes were first proven to be a liver cell organelle rich in a variety of hydrolytic enzymes(1), numerous studies have been directed toward detection and separation of lysosomes or similar particles from other mammalian tissues. Cohn and Hirsch showed that granules isolated from rabbit polymorphonuclear (PMN) leucocytes possess properties and functions very similar to those of hepatic lysosomes(2). Other investigators have reported similar results(3). At present, granules in PMN leucocytes are considered to be "primary lysosomes," or storage sacs of hydrolytic enzymes(4).

Among the hydrolytic enzymes present in lysosomes, acid phosphatase has frequently been used as a marker enzyme to detect lysosomes by histochemical methods. As much as 70% to 80% of acid phosphatase was found in the lysosomal fraction upon fractionation of rat liver homogenate(5), and approximately 70% was recovered from the granule fraction of rabbit PMN leucocytes(2). Although the heterogeneous nature of acid phosphatase associated with lysosomal and soluble fractions of rat liver was indicated by Shibko and Tappel(6), the properties of this enzyme in subcellular fractions of PMN leucocytes have not been clarified.

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† Present address: Dept. of Physiology, Woman's Medical College of Pennsylvania, Philadelphia.

The present study is an attempt at determining the distribution and some of the properties of acid phosphatase in rat PMN leucocytes.

Materials and methods. One-year-old Albino rats weighing approximately 400 g were injected intraperitoneally with 50 ml of sterile saline (0.9%) containing one mg glycogen/ml. Eighteen hours after the injection the animals were sacrificed and the peritoneal cavities washed 3 times with ice-cold physiological saline. Stained smears and wet mount differential counts of leucocytes in the exudate revealed the population of PMN leucocytes to be 98% or more. The processing of the cells and the subsequent separation of granular, supernatant, and nuclear fractions were performed essentially according to the method of Cohn and Hirsch(2).

To compare the distribution of acid phosphatase, alkaline phosphatase, and β-glucuronidase in PMN leucocytes, the total homogenate and the nuclear, granular, and supernatant fractions in 0.34 M sucrose were subjected to repeated freezing and thawing in the presence of 0.1% Triton X-100. Acid and alkaline phosphatases were assayed according to the procedure of Andersch(7) with disodium p-nitrophenyl phosphate as the substrate in 0.09 M citrate buffer of pH 5.5 and 0.1 M glycine buffer of pH 10.5, respectively. β-glucuronidase was assayed with phenolphthalein-β-glucuronide as the substrate utilizing the method of Folette *et al*(8). Each enzyme assay was conducted under conditions that would yield linear responses with the enzyme concentration.

To determine the relative stability of acid phosphatase, each subcellular fraction was treated with 0.1% Triton X-100 in 0.34 M sucrose at 0°C for 45 minutes, then centrifuged for 20 minutes at 16,000 × *g* in a Spinco Model L centrifuge under refrigeration. The supernatants thus obtained were divided into 2 parts and incubated at 0°C and 38°C, and aliquots were removed at 30 and 60 minutes for assay of acid phosphatase.

The pH optima of acid phosphatase in leucocyte subcellular fractions were determined by conducting the enzyme assay in 0.09 M citrate buffer with the pH ranging from 4.0 to 6.5. The inhibitory effects of alloxan, (+)—tartaric acid, sodium fluoride, and oxalic acid on the activity of acid phosphatase were determined with alloxan at 5×10^{-2} M concentration and the other 3 inhibitors at the concentration of 1×10^{-2} M.

TABLE I. Inactivation of Acid Phosphatase at 37°C from Each PMN Leucocyte Subcellular Fraction.*

Fractions	Incubation period 30 min	60 min
Whole leucocytes	61.1%	70.5%
Nuclear fraction	51.5	67.7
Granular fraction	22.2	29.7
Supernatant fraction	68.2	75.2

* Values are mean percentages of reduction in the initial activity of acid phosphatase in 5 experiments.

Results. Although more than 70% of alkaline phosphatase and 90% β-glucuronidase in PMN leucocytes were associated with the granule fraction, only about 45% acid phosphatase activity was detected in the same fraction (Fig. 1). The nuclear and supernatant fractions contained 21% and 34% of acid phosphatase, respectively.

When supernatants obtained upon treatment of leucocyte subcellular fractions with 0.1% Triton X-100 were incubated at 37°C, over 65% of the acid phosphatase from the original nuclear and supernatant fractions became inactivated during a 60-minute incubation period (Table I), while supernatant from the granule fraction showed a decrease in activity of only 30% during the same incubation period. In spite of the lability of acid phosphatase at 37°C, this enzyme was very stable at 0°C, where no sign of inactivation appeared in the supernatant from any fraction.

The granule acid phosphatase exhibited a well-defined pH optimum of 5.3, while the values for the nuclear and supernatant fractions were approximately 5.7 and 6.0, respectively (Fig. 2).

Although 1×10^{-2} M sodium fluoride inhibited 7% and 15% of the enzyme activity in the nuclear and the supernatant fractions, respectively, it inhibited as much as 33% of acid phosphatase in the granule fraction

(Fig. 3). In all fractions, both 5×10^{-2} M alloxan and 1×10^{-2} M (+)—tartaric acid suppressed over 90% of acid phosphatase activity, while 1×10^{-2} M oxalic acid exerted less than 40% inhibition.

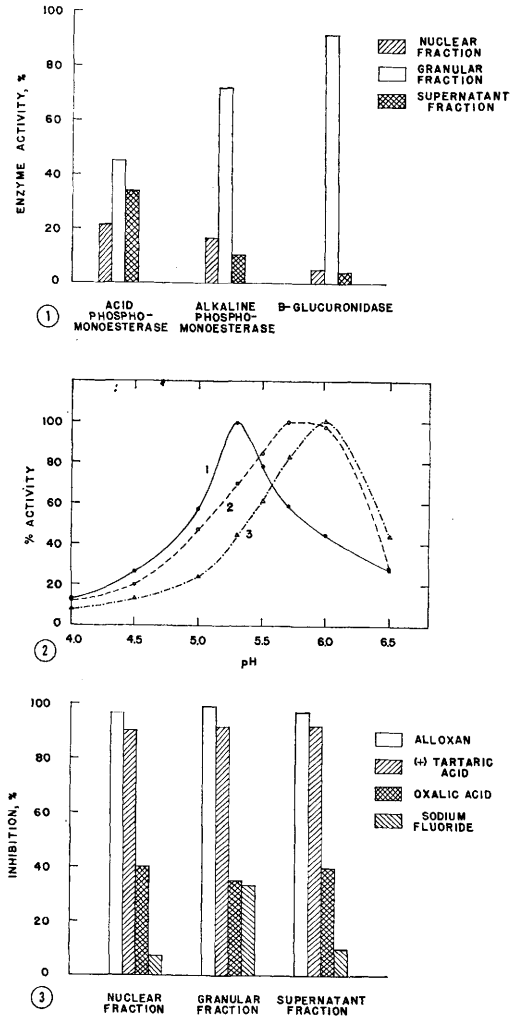

FIG. 1. Distribution of PMN leucocyte enzymes in each subcellular fraction. Recovery of enzyme from each fraction was expressed as percentage of enzyme activity in total homogenate.

FIG. 2. pH optimum curves for acid phosphatase in PMN leucocyte subcellular fractions. Curves 1, 2, and 3 represent the percentage activity at various pH values in granular, nuclear, and supernatant fractions, respectively, in comparison with the activity of the enzyme at optimum pH values.

FIG. 3. Effect of inhibitors on acid phosphatase in each PMN leucocyte subcellular fraction. Final concentration of alloxan was 5×10^{-2} M, and 1×10^{-2} M for the other 3 inhibitors. Enzyme assay was conducted at optimum pH for each fraction: 5.3 for granular fraction; 5.7 for nuclear fraction; and 6.0 for supernatant fraction.

Discussion. The granule fraction obtained from rat PMN leucocytes exhibits an abundant distribution of β-glucuronidase and alkaline phosphatase, which can be classified as granular enzymes, but the relatively low acid phosphatase content in this fraction raises the question of whether acid phosphatase can likewise be termed a granular enzyme. The conflicting observation by Cohn and Hirsch that in rabbit PMN leucocytes 70% of the total acid phosphatase was localized in the granular fraction(2) may be due to marked species differences in the distribution of acid phosphatase.

There also exists the possibility that acid phosphatase in the soluble fraction might have been derived from the rupturing of granules during the isolation process and the enzyme in the nuclear fraction from contamination by granule particles. However, the higher degree of stability of acid phosphatase released from granules as compared to that from nuclear and supernatant fractions implies that a distinct difference exists between the enzyme present in granules and the one associated with the nuclear and supernatant fractions. The instability of acid phosphatase in these latter fractions may be a result of an inherent lability in its structure.

The apparent heterogeneity of PMN leucocyte acid phosphatase became more evident in the present study when the pH optima of acid phosphatase were found to be 5.3 for the granular fraction, 5.7 for the nuclear fraction, and 6.0 for the supernatant fraction. Considerable overlapping of the pH curves of the latter two fractions may indicate that a different enzyme does not necessarily exist in each of these two fractions. If the enzymes in these fractions are the same, contamination of the nuclear fraction by granules may have been responsible for the lowering of the pH optimum for the nuclear fraction.

Of the compounds tested, alloxan, (+)—tartaric acid, and oxalic acid showed no selective inhibition of acid phosphatase in the leucocyte subcellular fractions. Sodium fluoride, however, was found to exert preferential

inhibition of the enzyme in the granule fraction and little inhibition in the nuclear and supernatant fractions. This result together with higher stability and lower pH optimum of acid phosphatase in granule fraction clearly indicates that the properties of the enzyme in this fraction are distinctly different from those of the enzyme in the nuclear and soluble fractions.

The morphological heterogeneity of PMN leucocyte granules has been shown by electron microscopy(9,10). The decision as to whether heterogeneous granules contain acid phosphatase of identical properties cannot be made prior to the development of a method for differential fractionation of the granules.

Summary. The present study has revealed that 45% of acid phosphatase in rat polymorphonuclear leucocytes can be recovered from the granular fraction, 21% from the nuclear fraction, and 34% from the supernatant fraction. Acid phosphatase in the granule fraction appeared to have properties distinctly different from those of the enzyme in the nuclear and soluble fractions. The enzyme in the granule fraction was more stable, possessed a lower pH optimum, and was preferentially inhibited by sodium fluoride.

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Electrical Stimulation and Metabolism of a Phosphoinositide Complex in the Salivary Gland.* (31322)

Z. N. GAUT, C. STEFFEK, AND C. G. HUGGINS (Introduced by A. G. C. White)
Department of Biochemistry, Tulane University School of Medicine, New Orleans, La.

Burford and Huggins(3,4) reported that supersensitive submaxillary glands of cats secreted more water, sodium, and mucin than did normal glands when both were stimulated with doses of either acetylcholine or epinephrine found to be the threshold for the normal gland. Subcutaneous injection of radiophosphorus followed by a 2-hour experimental period of secretory stimulation resulted in an increased specific activity of the phosphorus in the phosphoinositide complex (polyphosphoinositides) and in phospholipid fractions obtained from supersensitive glands when compared with normal glands. When atropine or dihydroergotamine was used to inhibit salivary secretion, the specific activities of the phosphoinositide complex and phospholipid fractions did not increase in the supersensitive gland.

Many theories concerning the basis for developing supersensitivity in submaxillary glands have been reported(5,6,9,25). To an-

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