Electrophoretic Studies of the Mucin Fractions from the Human Gastric Juice.* (19483)

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Glass and Boyd have separated from the mucin fraction of human gastric juice two substances which they call mucoproteose and glandular mucoprotein. They have published evidence that mucoproteose derives from the surface epithelial cells, perhaps as a breakdown product of visible mucus, while mucoprotein appears to be a product of the neck glands of the fundus and corpus(1-4). Precipitated with acetone, mucoproteose forms brownish, slightly hydrated resin-like clumps which adhere to the walls of the test tube, while mucoprotein appears as a light flocculent opal colored, highly hydrated precipitate. After drying with acetone, mucoproteose forms a chalk-white amorphous substance, whereas mucoprotein forms greyish crystal-like particles. Mucoproteose remains soluble while mucoprotein is insoluble below pH 4.0 after previous precipitation with acetone. On alkaline hydrolysis mucoproteose yields only 3.9-4.2 mg % tyrosine, while mucoprotein yields 7.5 $\pm$ 0.65. The nitrogen content of the mucoproteose is much lower than that of mucoprotein (5.7-7.3% as compared to 12.6 $\pm$ 0.44% respectively). On the other hand, the content of reducing substances in mucoproteose fraction is much larger than that in glandular mucoprotein. Recently Dische and Osnos examined the polysaccharide composition of these two fractions(5). They found that mucoproteose is very rich in galactose and mannose and contains very little hexuronic acid, while mucoprotein contains hexosamine and hexuronic acid, but does not contain galactose or mannose.

The electrophoretic technic of Tiselius(6) offers further promise for the separation and identification of various mucin fractions. To date, electrophoretic studies of gastric secretory products have been reported only on pepsin of animal origin(7) and on proteins of canine gastric juice(8). The present study is concerned with the identification of the electrophoretic properties of the two mucous substances separated from the human gastric juice by the technic of Glass and Boyd(1).

Materials used for electrophoretic analysis. I. *Gastric mucoproteose from human gastric juice.* Samples of gastric juice were recovered directly from the stomach of Tom, a fistulous subject who has been described in detail elsewhere(9). Other samples were obtained from pooled gastric juices of normal individuals and patients with gastric hyperfunction and duodenal ulcer. After mucoprotein had been precipitated and removed by the technic of Glass and Boyd(1) mucoproteose was prepared from the supernatant liquid either by a) acetone precipitation, followed by washing several times with acetone-water and acetone, and drying in the fresh air at room temperature, or b) by lyophilization of the supernatant fluid, after it had been submitted to dialysis against cold running tap water for 24 hours, and distilled water for another 24 hours at 6°C. II. *Glandular mucoprotein from human gastric juice* was recovered from the pooled specimens because Tom's gastric juice was uniformly poor in mucoprotein.

The mucoprotein precipitate was either a) dried with acetone after washing several times with distilled water, or b) lyophilized by the dry ice technic after washing several times with dilute HCl and distilled water, or c) directly dissolved in the buffer for electrophoresis after being washed several times with distilled water. The gastric juices used for separation of mucoproteose and mucoprotein fractions were placed in the refrigerator immediately after collection, and processed as soon as possible afterwards. The lyophilized mucoprotein and mucoproteose were all fairly soluble in phosphate and veronal buffers.

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However, it was necessary to first dissolve the acetone-dried proteins in a small amount of dilute alkali before adding the buffer. III. *Commercial porcine mucin* from two sources (Winthrop-Stearns granular mucin and Wilson powdered mucin†) was also analyzed for purposes of comparison. These powdered preparations were ground in a mortar with 1/5 normal NaOH so that they could be brought into solution before adding the buffer. An attempt was made also to separate the "glandular mucoprotein" fraction from the commercial mucin by processing it by the technic mentioned above. IV. *Crystalline pepsin (Armour),* prepared by the method of Northrop(10), lot 80802, was used for electrophoresis without further processing. It was fairly soluble in phosphate and acetate buffers. V. *Canine gastric juice* was collected after sham feeding in dogs with gastric fistulae‡ and dialyzed for 48 hours against running tap and distilled water at 6°C, then lyophilized by the dry-ice technic. *Technic of electrophoresis.* The electrophoretic technic used was the moving boundary method(6), and the apparatus used for this study was a machine built to the specifications of L. G. Longsworth(11). The light source was the standard mercury lamp with a green filter, and photographic plates used were of the Panchromatic type. The light source was found to be unsatisfactory for use with solutions of high concentrations (above 2%) because of turbidity. It is hoped that this difficulty will be eliminated in future studies by the use of a source of infra-red emission and infra-red sensitive photographic plates or film. The average variation in temperature during the course of any single experiment was 1°C, being less in most experiments (from 0.4 to 1.0°C) although in a few it varied up to 1.8°C. The current varied at the most 0.7 milliamperes. In all but three experiments (done on Armour pepsin) an ionic strength of 0.1 was used. In these three experiments

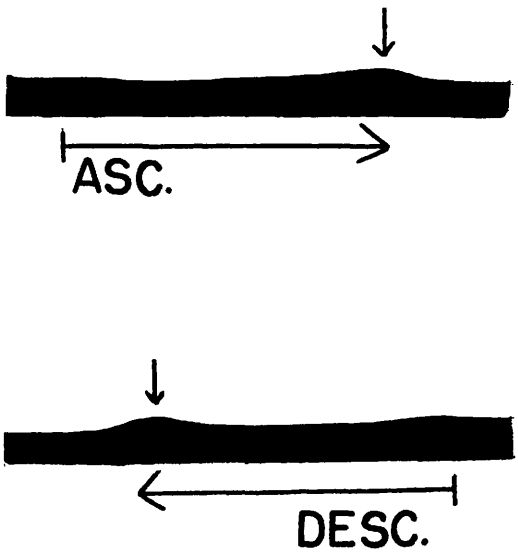

FIG. 1. Prepared mucoproteose from non-dialyzed human gastric juice run at pH 6.1. Note the broad ill-defined peak with a negative mobility of 5.51 × 10^{-5}, indicated by the vertical arrow. The center of the stationary boundary is indicated by the tail of the horizontal arrow in this and subsequent figures.

the ionic strength used was 0.05. Buffers used were acetate, phosphate and barbiturate (veronal), ranging from pH 3.7 to 8.5. The concentrations of pepsin used were from 0.6 to 1.0%, of glandular mucoprotein from 0.33 to 1.0% (usually 0.4-0.5), and for mucoproteose from 0.6 to 1.0%. The porcine mucins were run at the concentration of 1.0%, and the lyophilized canine gastric juice at the concentration from 1.0 to 1.5%. The potential gradient was from 4.37 to 8.57 volts/cm, and the time of run was in most instances from 2 to 5 hours with the exception of 5 shorter runs. The electrophoretic mobility of each component was calculated from its velocity, *i.e.,* the distance moved by the peak divided by the time, and the potential gradient, *i.e.,* the amperage divided by the average cross-sectional area of the cell and the electrical conductivity of the protein solution and buffer. In calculations of mobility in this study the distance moved by the peak (Δx) was measured from the descending limb pictures, with the exception of three runs in which it was necessary to make calculations from the ascending limb.

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‡ We gratefully acknowledge the assistance of Dr. W. L. Mersheimer, Department of Surgery, New York Medical College, who prepared the dogs with gastric fistulae.

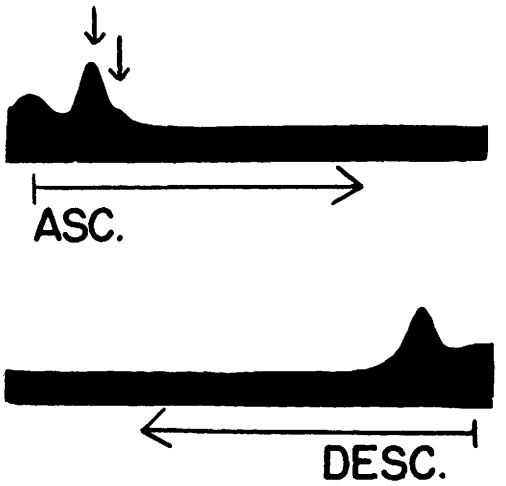

FIG. 2. Prepared mucoproteose, dialyzed and lyophilized, from human gastric juice run at pH 6.1. Note the fairly well defined peak of mobility $-.6 \times 10^{-5}$, indicated by vertical arrow, and one or more poorly defined ones.

Results. Dissolved gastric mucoproteose from human gastric juice. Two preparations obtained from dried acetone precipitate and run at 0.6 and 1.0% concentration and pH 6.0 and 6.1 showed a very broad ill-defined peak (Fig. 1) with a negative mobility of 5.51 and 6.13 x 10^{-5} cm^2 $volt^{-1}$ sec^{-1}. Two other fractions of mucoproteose prepared by dialysis and lyophilization, and run at 0.6 to 1.0% concentration and at pH 6.1 and 8.5 showed one fairly well-defined, and one other poorly defined peak with negative mobility (Fig. 2). The well-defined peak of the dialyzed, lyophilized fraction at pH 6.1 was much slower (mobility -0.6 x 10^{-5}) than the peak seen at the same pH and concentration in the non-dialyzed, acetone dried preparations. *Comment.* The marked difference in the electrophoretic pattern between the acetone dried preparation of mucoproteose and that prepared by dialysis and lyophilization may be attributable to denaturing of the material by the drying technic or to the loss of some of the components through dialysis.

Glandular mucoprotein from human gastric juice. Six samples from a single pool (A) were prepared by the acetone drying technic. The runs were done at a constant concentration of 0.4%, except in one case where a concentration of 0.33% had to be used, because of lack of material. The pH range varied in separate determinations from 4.6 to 8.6 in acetate, phosphate and veronal buffers. Three other mucoprotein samples prepared by the same technic (Pools B, C, and D) were run at a concentration 0.5-1.0% and at pH from 5.0 to 6.4. Another one (No. 2) was prepared by lyophilization at a concentration of 0.5% and pH 5.0, and 2 other samples (from Pools E and F) were prepared by technic (c) and run at pH 6.0 and 8.5 at concentration 0.5 and 0.4% respectively. In 11 out of the 12 samples of glandular mucoprotein tested, obtained from various pools and run over a pH range from 4.6 to 8.6, a single peak was obtained. This appeared fairly symmetrical and negatively charged throughout the range of pH tested. In one of the preparations (No. 2) which was run at pH 5.0 a small additional peak with a negative mobility was observed. This was much slower moving than the larger peak, and may have been due to contamination of the preparation by other protein from gastric juice (mucoproteose?). One of the typical electrophoretic patterns of glandular mucoprotein obtained is shown in Fig. 3. The mobility of glandular mucoprotein was found

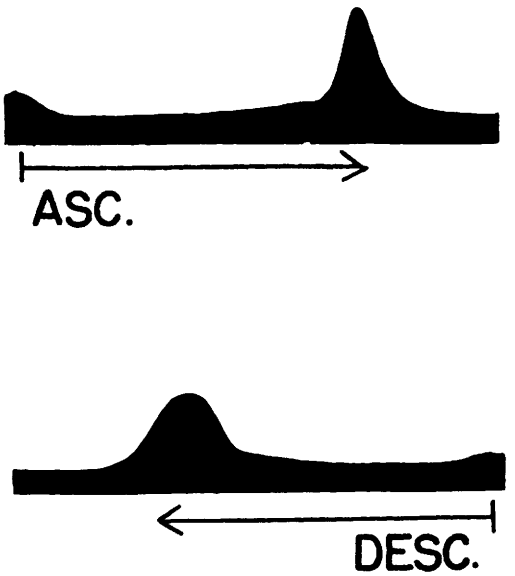

FIG. 3. Prepared glandular mucoprotein from human gastric juice run at pH 6.4. A single peak appeared with mobility of -7.2×10^{-5}.

TABLE I.

No.	Source	Preparation	Concentration, %	pH	Buffer	Elect. field strength	Time of run, sec	Electrophoretic mobility × 10^{-5}	Peak I	Peak II	Peak III	Peak IV
					Human mucoproteose							
1.	Pool A	Method a	.6	6		5.51	17100	−5.1				
2.	P. A. pat.	a	1	6.1	Phosphate	6.13	11940	−6				
3.	Pool B	b	.6	6.1		6.52	13020	− .6				
4.	B	b	1	8.5	Veronal	6.64	17880	− .6				
					Human mucoprotein							
1.	Pool A	a	.4	4.6		5.29	10500	−4.1				
2.	Ulcer pat.	b	.6	5	Acetate	5.26	9420	−7.3				
3.	Pool B	a	.5	5		5.21	11970	−6.7				
4.	C	a	1	6		6.67	4590	−8.5				
5.	A	a	.4	6		6.48	4140	−8.6				
6.	F	c	.55	6	Phosphate	6.00	9300	−9.8‡				
7.	D	a	1	6.4		6.74	9480	−7.2				
8.	A	a	.4	6.4		6.88	10260	−8.4				
9.	A	a	.4	7.3		7.29	9330	−8.7				
10.	E	c	.4	8.5		6.15	12360	−7				
11.	A	a	.33	8.5	Veronal	4.37	10590	−8.4				
12.	A	a	.4	8.6		5.78	11250	−7.3				
13.	Mucoprotein added to Tom's gastric juice (total conc. 1.33%)		.33	6	Phosphate	5.44	9345	−9.2				
					Porcine mucin							
1.	Gran.		1	7.9		7.35	8040	−1.4				
2.	Wilson		1	6	Phosphate	6.40	2910	−13.5	−7.3	−3		−.9
3.	Gran. mucoprotein		1	8		5.05	5490	−18 ‡				
					Whole canine gastric juice							
1.	Pool A		1.5	6	Phosphate	6.58	8220	−10	−7.6	−5.3		−.6
2.	B		1	8.5	Veronal	6.04	3600	†	−8.3	−4.6		−.2
					Pepsin							
1.	Armour's com'l lot 80802		.6	3.7	Acetate	8.24	17580	−3.3*				
2.			.6	4.1		7.45	12960	−6.1*‡				
3.			.6	4.1		8.32	12180	−5.5*				
4.			1	5.5		6.12	10500	−8.9				
5.			1	6.1		6.96	6780	−10.6				
6.			1	6.1	Phosphate	5.73	8430	−11.6				
7.			1	6.4		6.92	7200	−10.9				
8.			.6	6.7		8.57	4380	−8				
9.			1	6.8		8.54	2460	−7.9				

* Ionic strength = .05. † No measurement possible. ‡ Calculated from asc. pattern.

to be very fast, so that in phosphate buffers at pH 6.0 and concentration 0.4-1.0% it was about −8.5 x 10^{-5} cm^2 $volt^{-1}$ sec^{-1}. The range of mobility of mucoprotein in various samples studied and run at a concentration from 0.4 to 1.0% was from −7.2 to −8.6 x 10^{-5} cm^2 $volt^{-1}$ sec^{-1} over the range of pH from 6.0 to 7.3. The mobility of mucoprotein in veronal buffer at pH 8.5-8.6 was surprisingly similar to that observed in phosphate buffers over the range of pH 6.0 to 7.3. To ascertain whether the peak of glandular mucoprotein could be recognized on electrophoresis of a sample of whole gastric juice, the following experiment was done. Tom's whole gastric juice which did not contain any mucoprotein was lyophilized after dialysis, and submitted to electrophoresis at 1.3% concentration in phosphate buffer of pH 6.0. Several small peaks with negative mobilities were evident, but all of them were much slower than those of mucoprotein. Thereafter, glandular muco-

protein from one of the pools was added at a concentration of 0.33% to the same (Tom's) gastric juice preparation and the run was repeated at the same pH. A new peak appeared, the mobility of which was 9.2 x 10^{-5} cm^2 $volt^{-1}$ sec^{-1}, and which therefore fell in the range observed in mucoprotein preparations. Detailed data are listed in Table I.

Attempted identification of pepsin peak. Since the technic used in separating mucoprotein from gastric juice by precipitation at a pH of 2.0 leaves pepsin in the supernatant fluid an attempt was made to prepare the mucoprotein fraction in another way so that it could contain pepsin as a contaminant. This was done on two samples of gastric juice rich in pepsin, by precipitating mucoprotein at a pH of 3.5 (at which pepsin appears in the protein precipitate). Electrophoretic study of these mucoprotein fractions, however, showed again only one single peak whose mobility at pH 8.5 in veronal buffer was similar to that of other mucoprotein samples, but which in phosphate buffer at pH 6.0 (calculated from the ascending limb) seemed to be higher than that of remaining mucoprotein samples.

Porcine mucin. The electrophoretic pattern of Wilson powdered commercial hog mucin was determined at pH 6.0 and concentration 1%. One very fast moving peak of a mobility –13.5 x 10^{-5} and 3 slower moving peaks (–7.3, –3.0 and –0.9 x 10^{-5} cm^2 $volt^{-1}$ sec^{-1}) were found. The slowest peak was the largest one. The electrophoretic pattern of the Winthrop-Stearns granular hog mucin was studied at pH 7.9 and in 1.0% concentration. One slow moving peak with a negative mobility of –1.2 x 10^{-5} was found, but it was strongly suspected that another fast moving peak was also present, but was "lost" when the experiment was left unobserved for two hours. From the same mucin a "glandular mucoprotein fraction" was prepared by the above-mentioned technic(1). The electrophoresis was done at pH 8.0 and in concentration of 1.0%. A single very fast moving peak was found with a very high negative mobility of –18.0 x 10^{-5} (if calculated from the ascending limb).

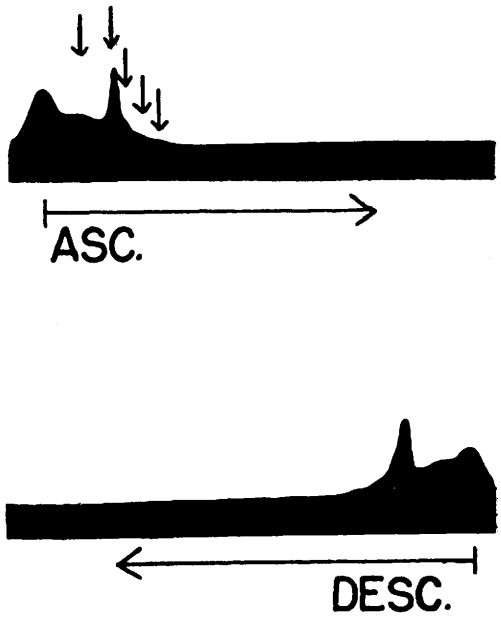

FIG. 4. Whole canine gastric juice, dialyzed and lyophilized, at pH 8.5. There are approximately 5 peaks of mobility ranging from –8.3 to –.2 × 10^{-5}.

Canine gastric juice. Four or more peaks were observed in the electrophoretic pattern of the canine gastric juice which was dialyzed and lyophilized and run at a concentration of 1.0-1.5% and a pH 6.0 and 8.5. In Fig. 4 one of these electrophoretic patterns is shown. The small first and second peaks are somewhat faster than peaks observed by Grossberg *et al.*(8), the third and the fourth (largest) peaks exhibit mobility corresponding exactly to F-1 and F-2 fractions as described by these authors. However, the fifth peak is slower than the slowest peak observed by these authors.

Crystalline Northrop pepsin from porcine origin (Armour). Nine electrophoretic runs were done on this material at a concentration from 0.6 to 1.0% and over the range of pH from 3.7 to 6.8 in acetate and phosphate buffers. All electrophoretic patterns showed one single large peak with a negative mobility, which being calculated at pH 5.5 was –8.9 x 10^{-5}, and at pH 6.1 was from 10.6 to 11.6 x 10^{-5} cm^2 $volt^{-1}$ sec^{-1}. This wide variation in speed is unexplained but may be the result of using the crude preparation without further

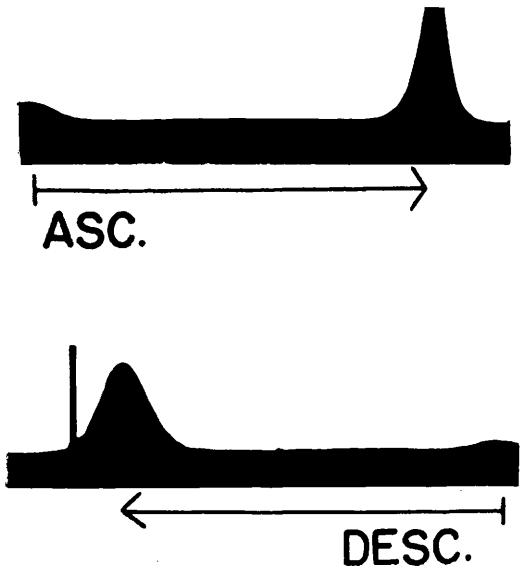

FIG. 5. Crystalline pepsin (Northrop) of porcine origin run at pH 5.5. One single large peak is seen with mobility of -8.9×10^{-5}.

purification. These mobilities are only slightly higher than those observed in similar preparations by Grossberg *et al.*(8). They are, however, definitely higher than the mobility of the glandular mucoprotein from human sources. The electrophoretic pattern of porcine pepsin is shown in Fig. 5.

Discussion. These electrophoretic data appear to confirm the earlier inferences of Glass and Boyd that mucoproteose and glandular mucoprotein are two quite separate components of the gastric juice and that mucoprotein is a relatively homogeneous substance. The various peaks of the mucoproteose fraction move more slowly than that of mucoprotein. The very fast moving peak observed in the specimens of Wilson porcine mucin may correspond to human glandular mucoprotein while the granular hog mucin preparation which is mainly surface mucus was characterized by a much slower peak which may correspond to human mucoproteose.

The electrophoretic method cannot settle definitely the question as to whether or not glandular mucoprotein is a single chemical substance. Although the appearance of the peak on electrophoresis is fairly compatible with homogeneity, it is not possible to exclude contamination of this component with other substances present in small concentrations. This is an important consideration since recent evidence indicates that glandular mucoprotein is the main carrier of Castle's intrinsic factor or even may be identical with the latter(12).

The failure to identify a peak of pepsin in human gastric juice is in keeping with the findings of Grossberg *et al.*(8) who also were unable to obtain a separate pepsin peak in their samples of canine gastric juice. Whether pepsin is adsorbed in one or more of the mucin fractions, or whether it is bound mainly to glandular mucoprotein, or has as yet an unidentified peak of its own is currently being studied, as are the electrophoretic patterns of the whole human gastric juice(12).

Summary. 1. Electrophoretic analysis was performed on 2 prepared constituents of dissolved mucin from human gastric juice, *i.e.*, glandular mucoprotein from gastric glands and dissolved mucoproteose from surface epithelium as separated by the technic of Glass and Boyd. The patterns obtained from human sources were compared with those obtained on electrophoresis of crystalline porcine pepsin, commercially available hog mucin, and lyophilized and dialyzed canine gastric juice. The runs were done over a wide range of pH and various preparations of these materials were studied with the moving boundary technic. 2. It was found that the electrophoretic pattern of mucoproteose with peaks of slow negative mobility was distinct from that of glandular mucoprotein which displayed a slightly asymmetrical single peak of fast negative mobility. It seems reasonable to conclude that these two substances represent separate constituents of the gastric mucus. Their possible functions will be the subject of later study.

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Histochemical Demonstration of Esterase Activity in the Normal Human Kidney and in Renal Carcinoma.* (19484)

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With the method of Gomori(1) esterase activity could be demonstrated in paraffin sections of various animals(2,3) but not in the human kidney(2,4,5). Some irregular staining is occasionally seen, however, in paraffin sections in which esterase activity is demonstrated by the technic of Nachlas and Seligman(6) using beta naphtyl acetate as substrate(7). It is the purpose of this communication to point out the fact that with both methods esterase activity can be regularly visualized in the normal human kidney as well as in some renal carcinomas if one works with frozen sections instead of paraffin sections.

Method. The material used consisted of 20 kidneys obtained at autopsy and 10 surgically removed specimens. The age of the subjects from which the kidneys had been obtained varied from a few hours to 70 years. Seven renal carcinomas were also studied. Frozen sections of unfixed tissues were cut at 10 to 15 μ. Frozen sections may also be prepared from tissues that have been fixed in acetone at 4°C for 24 hours or in 10% formalin for 3 hours at room temperature. Esterase activity in these sections can then be demonstrated with either the technic of Gomori or the dye method of Nachlas and Seligman. According to Gomori's method sections were incubated for 18 to 20 hours with Tween 40 as substrate. After developing the sites of enzymatic activity by treatment with diluted hydrogen sulphide, the sections were kept for several hours in 10% formalin and mounted in glycerogel. These preparations could be preserved for several months. Sections stained with the dye method had to be studied rapidly since the color fades within a few minutes. All attempted modifications did not alter this disadvantageous condition.

Results. The localization of esterase activity in frozen sections of human kidney not involved by disease with both methods was similar, although the dye method gave more uniform results. Within the cortex esterase activity was present in the cytoplasm of both the proximal and distal convoluted tubules (Fig. 1). Slight granular deposits were also noted in glomeruli but their significance appears doubtful. Some tubules in the medulla also showed esterase activity. From their location and caliber they are presumed to be straight portions of the proximal convoluted tubules and ascending portions of Henle's loop. The renal tumors studied were carcinomas of the clear cell and papillary types. No esterase activity was demonstrable in paraffin sections. In frozen sections, however, neoplastic cells showed a varying degree of

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