Bull. N. Y. Med. Coll., Flower and Fifth Ave. Hosps., 1950, v13, 15.

5. Dische, Z., and Osnos, M., Personal communication.

6. Tiselius, A., *Nova Acta Regiae Soc. Sci., Upsala,* 1930, vIV, 7(4).

7. Tiselius, A., Henschen, G. I., Svensson, H., *Biochem. J.,* 1938, v32, 1814.

8. Grossberg, A. L., Komarov, S. A., and Shay, H., *Am. J. Physiol.,* 1951, v165, 1.

9. Wolf, S., and Wolff, H. G., *Human Gastric Function,* Oxford Univ. Press, N. Y., 2nd Ed., 1947.

10. Northrop, J. H., Kunitz, M., and Herriott, R. M., *Crystalline Enzymes.* 2nd Ed., Columbia Univ. Press, 1948.

11. Longsworth, L. G., *Ann. N. Y. Acad. Sci.,* 1939, v39, 187; *Ind. and Eng. Chem.,* Analytical Edition, 1946, v18, 219; *J. A. C. S.,* 1939, v61, 529.

12. Glass, G. B. J., Boyd, L. J., Rubinstein, M. A., and Svigals, C. S., *Science,* 1952, v115, 101.

13. Mack, M., Stern, K., and Wolf, S., *Electrophoretic Studies of Whole Human Gastric Juice,* in preparation.

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

M. WACHSTEIN AND E. MEISEL.

From the Department of Pathology, St. Catherine's Hospital, Brooklyn, N. Y.

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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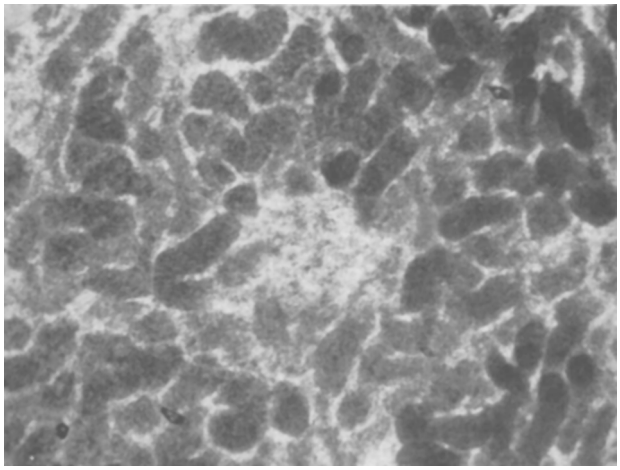

FIG. 1. Kidney cortex of human. Fresh frozen section. Gomori's method for esterase activity. Marked esterase activity is seen in the proximal and distal convoluted tubules. A glomerulus in the center is almost devoid of the lead sulphide deposits which indicate enzymatic activity. × 100.

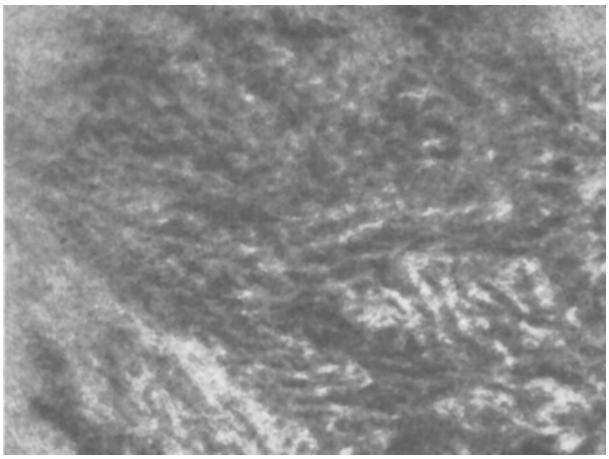

FIG. 2. Renal carcinoma of papillary type. Fresh frozen section. Gomori's method for esterase. Considerable esterase activity is seen in the cells composing the tumor. × 100.

cytoplasmic activity (Fig. 2) in 5 of the 7 carcinomas. Esterase activity was seen in paraffin sections of only a few of many examined carcinomas by Gomori(2) and in several bronchus carcinomas by Menk and Hyer(8).

Comment. The recorded observations make it quite obvious that the process of dehydrating and embedding destroys most of the histochemically demonstrable esterase activity in the human kidney. The latter on chemical examination contains a lower average amount of esterase than the kidney of mouse, rat, guinea pig, rabbit, and dog(9). Since in paraffin sections, however, remnants of esterase activity can be demonstrated with beta naphtyl acetate as substrate, the azo dye method appears somewhat more sensitive than Gomori's technic. It is thus apparent that an important source of error in the histochemical esterase technic is caused by the loss of enzyme during the process of histological preparation. Some of the previously reported negative findings and inconsistencies(5) using paraffin embedded material must be re-evaluated with frozen sections. Formalin fixation, especially for longer periods of time(10) should probably be avoided especially if only small amounts of esterase are present since it

also leads to considerable inactivation of the enzymes(11). Barnett and Seligman(12) have recently introduced 2 more substrates, Indoxyl acetate and Indoxyl butyrate, for the histochemical demonstration of tissue esterase which do not diffuse into lipoids as the azo dye will do in fresh frozen sections. These authors stressed the usefulness of the Coons modification(13) of the Linderstrøm-Lang and Mogensen technic(14) for the preparation of thin frozen sections of various organs well suited for histochemical enzyme studies.

Summary. Esterase activity in the human kidney is not visualized in paraffin sections of acetone fixed tissues with Gomori's technic and only very irregularly with the azo dye method. It can be regularly demonstrated with both methods in fresh frozen sections. In 5 of 7 renal carcinomas esterase was found in the cytoplasm of tumor cells if fresh frozen sections were used but not in paraffin fixed material.

1. Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1945, v58, 362.
2. ———, *Arch. Path.*, 1946, v41, 121.
3. Wachstein, M., *J. Exp. Med.*, 1946, v84, 25.
4. ———, *Am. J. Path.*, 1946, v22, 658.
5. Sneath, P. H. A., *Nature*, 1950, v166, 699.
6. Nachlas, M. M., and Seligman, A. M., *J. Nat. Cancer Inst.*, 1949, v9, 415.
7. ———, *Anat. Rec.*, 1948, v105, 677.
8. Menk, F. T., and Hyer, H., *Arch. Path.*, 1949, v48, 305.
9. Nachlas, M. M., and Seligman, A. M., *J. Biol. Chem.*, 1949, v181, 343.
10. Mark, D. D., *Arch. Path.*, 1950, v49, 545.
11. Seligman, A. M., Chauncey, H. H., and Nachlas, M. M., *Stain Technol.*, 1951, v26, 19.
12. Barnett, R. J., and Seligman, A. M., *Science*, 1951, v114, 579.
13. Coons, A. H., Leduc, E. H., and Kaplan, M. H., *J. Exp. Med.*, 1951, v93, 173.
14. Linderstrøm-Lang, J., and Mogensen, K. R., *Compt.-rend. trav. Lab. Carlsberg, serie chim.*, 1938, v23, 27.

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Calcification VIII. Glycolytic Enzymes and Phosphorylated Intermediates In Preosseous Cartilage.* (19485)

HARRY G. ALBAUM, ALTA HIRSHFELD, AND ALBERT EDWARD SOBEL.

From the Biology Research Laboratory, Brooklyn College, and the Department of Biochemistry, The Jewish Hospital of Brooklyn, Brooklyn, N. Y.

At present there is direct evidence for the existence of 4 components of the glycolytic cycle in preosseous cartilage, namely, glycogen (1,2), phosphorylase(3), lactic acid(4), and adenosine triphosphate(5). Additional evidence of the glycolytic system in preosseous cartilage comes from the use of glycolytic inhibitors(6). These agents inhibit *in vitro* calcification(6,7), which is restored by phosphorylated intermediates beyond the locus of action of the enzymes inhibited. The suggestion was made from these studies(3,7) that a glycolytic system is present in preosseous cartilage. However, while the use of inhibitors is suggestive, it is not conclusive evidence, since the degree of specificity of the inhibitors is not well established(8).

The purpose of this investigation, therefore, was to seek more direct evidence for the existence of other components of the glycolytic system in preosseous cartilage.

Procedure. Most of the enzymes to be described in cartilage have been demonstrated using the procedures of Racker(9). These depend upon the enzymatic oxidation of reduced diphosphopyridine (DPN) in the spectrophotometer. For this purpose, aqueous extracts of rachitic bone cartilage were employed. 250 to 400 mg of cartilage obtained from the metaphyses of the tibiae of rachitic

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