plexus, the ciliary epithelium and the submaxillary salivary gland[4]. They are also a very prominent constituent of the convoluted tubule cells. Furthermore, a number of the tumour cells exhibit a free apical surface provided with microvilli (Fig. 2) which are identical to the embryonic form of brush borders.

Here then are ultrastructural constituents of the clear cells in renal carcinomas which indicate clearly that these tumours arise from the epithelial cells of the convoluted tubule.

CH. OBERLING
M. RIVIÈRE
FR. HAGUENAU

Institut de Recherches sur le Cancer,
Villejuif, Seine.

[1] Stoerk, O., *Beitr. Anat. und Allg. Path.*, **43**, 393 (1908).
[2] Apitz, K., *Virch. Arch.*, **311**, 285 (1943).
[3] Feyrter, F., "Über diffuse endokrine epitheliale Organe" (J. A. Barth, Leipzig, 1938).
[4] Pease, D. C., *J. Biophys. Biochem. Cytol.*, **2** (Supp.), 203 (1956).

## BIOLOGY

### Collagenase Maceration of Kidney for Microdissection

THE traditional method of preparing kidney for microdissection is by maceration of formalin-fixed tissue with concentrated hydrochloric acid[1]. This method gives an excellent preparation of loosened nephrons and collecting tubules that can be disentangled and studied by microdissection. However, it suffers from the disadvantage that the acid treatment changes the nuclei so that they cannot be demonstrated in dissected preparations by the usual staining methods. A method of maceration that would allow nuclear staining after dissection would considerably widen the scope of the technique.

A variety of alternative macerating agents has been tried without success[2]. The present communication records the successful use of the collagenase of *Clostridium histolyticum* for maceration, permitting subsequent nuclear staining, of alcohol–acetic acid-fixed kidney.

In the course of the investigation, collagenase has been tested on fresh unfixed tissue and on tissue fixed with either alcohol, alcohol–acetic acid, acetone, acetone–acetic acid, saturated mercuric chloride, saturated picric acid, mercuric-dichromate (Zenker stock) or formol saline. Phosphate and borate buffers have been compared as diluents for collagenase; formol saline and 1 per cent acetic acid have each been tried as final fixatives for the macerated tissue; and the pretreatment of tissue slices with 6 *M* urea[3] has been tested. It has been confirmed that it is not possible to use collagenase for formalin-fixed tissue. Formalin alters the collagen substrate so that it is no longer susceptible to collagenase[4,5]. Alcohol-fixation does not inhibit the activity of collagenase, but the nephrons and collecting tubules are too brittle for dissection although good nuclear staining is possible. Acetone-fixation does not inhibit collagenase, the nephrons and collecting tubules are much easier to dissect and nuclear staining is not impaired. The addition of 25 per cent glacial acetic acid to either alcohol or acetone for fixation further improves the macerated tissue for microdissection.

Slices of alcohol–acetic acid-fixed kidney[6], about 1 mm. thick, are washed for 4 hr. in running water and are then immersed in phosphate buffer at *p*H 7 containing 0·25 mgm./ml. collagenase (Collagenase *ABC* Form II obtained from Agricultural Biochemicals Corporation, New York) and 0·1 mgm./ml. chloramphenicol. After 24 hr. at 37° C. the macerated tissue is transferred to formol saline. After microdissection, portions of nephron or collecting tubule that require staining are mounted on a slide under a coverslip and stained by drawing Mayer's hæmatoxylin through with blotting paper. After a few minutes the stain is rinsed away with Scott's tapwater and the nuclei are clearly stained in pale cytoplasm.

Although collagenase-macerated material is not so easy to dissect as acid-macerated tissue, the method, by allowing nuclear staining, yields valuable information not otherwise obtainable.

NANCY J. HAYWARD
Department of Pathology,
University of Melbourne.

[1] Oliver, J., MacDowell, M., and Tracy, A., *J. Clin. Invest.*, **30**, 1307 (1951).
[2] Darmady, E. M., and Stranack, F., *Brit. Med. Bull.*, **13**, 21 (1957).
[3] Nouman, R. E., and Tytell, A. A., *Proc. Soc. Exp. Biol. N.Y.*, **73**, 409 (1950).
[4] Robb-Smith, A. H. T., "Nature and Structure of Collagen", edit. by Randall, J. T., 21 (Butterworths, London, 1953).
[5] Keech, M. K., *J. Path. Bact.*, **77**, 351 (1959).
[6] Clarke, J. L., *Phil. Trans. Roy. Soc.*, B, **141**, 607 (1851).

### Culture of Human Leucocytes on the Chorio-allantoic Membrane of the Chick

A NEW technique has been developed for the culture of human leucocytes and other cells.

Suspensions of cells of the 'buffy coat' of heparinized human blood were introduced under sterile conditions into diffusion chambers consisting of two sheets of membrane filter ('Oxoid'), 2 cm. in diameter, cemented to a polystyrene ring. The inoculated chamber was sealed and dropped on to the chorio-allantoic membrane of the seven-day chick embryo through a slot in the egg-shell. Eggs were incubated at about 39° C. Cultures were examined at intervals to determine the state of the cells. After removal from the egg the filter disks were cut free from the polystyrene ring, placed on slides and stained with acetic orcein.

After two days of incubation many macrophage-like cells were present on the inside of both upper and lower disks of the chamber, but primarily on the upper disk. The fluid in the chamber contained mostly small lymphocytes and polymorphonuclear leucocytes which were also found on the bottom disk but apparently not attached. Present on the bottom disk was also a considerable number of large mononuclear cells which appeared to be attached to the membrane filter.

Cell divisions were first observed after four or five days of incubation and only in cells which seemed to be of the large mononuclear class. Divisions were observed in isolated cells (Fig. 1*a*) as well as in cells which were part of a group. Dividing cells were found only on the inside of the bottom disk of the chamber, that is, on the membrane filter which was in direct contact with the chorio-allantoic membrane of the chick. At five days most of the cells on the upper disk of the chamber were dead while the fluid in the chamber still contained healthy looking small lymphocytes and polymorphonuclear leucocytes. No human erythrocytes were left by this time. Chick cells were effectively excluded from the inside of the chamber.