An in vitro System for Studying Mucus Secretion and Other Physiological Activities in Human Intestinal Mucosa

'Organ Culture'[1] is an in vitro technique which aims to maintain the architecture of a given piece of tissue and to preserve the delicate balance between the constituent cells and other tissue elements so that 'physiological equilibrium' is achieved. It offers, therefore, unique opportunities for investigating the effect of various factors, e.g. hormones, on a specific cellular activity, under conditions which aim to simulate the in vivo situation; however, it has as yet been used little in studies on intestinal mucosa. Human rectal mucosa is readily obtained by suction biopsy[2] and is rich in mucous cells; we therefore selected this tissue for our in vitro studies on mucous secretion in cystic fibrosis, a human hereditary disease characterized by excessively viscous mucous secretions and electrolyte abnormalities in serous secretions for which there is at present no adequate explanation[3].

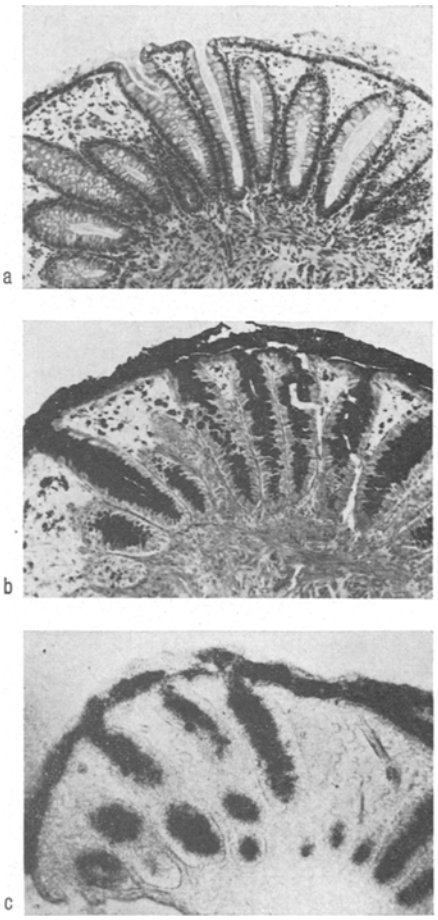
Materials and methods. Rectal biopsies were taken 6–10 cm from the anal orifice and placed immediately in a solution of Hank's B.S.S.-10% foetal calf serum containing antibiotics[4]; using fine cataract knives, any mucus, loose tissue or muscle was carefully removed and the specimen cut into explants approximately 2–3 cm square, which were placed mucosal surface uppermost on small squares of rayon net and transferred to the culture vessel. Over 500 cultures have been established and, of several organ culture methods examined in detail (e.g. plasma clot, roller tube, etc.), Trowell's technique[5] has proved to be the most satisfactory. The media, based on 199 or Trowell's T8, contained 4 mg/ml glucose, and usually 10% foetal calf serum was included. The cultures were incubated in an humidified atmosphere containing 5% CO_2 in oxygen and were transferred to fresh media after 48 h in vitro.

Results and discussion. In this study, the criterion for satisfactory survival has been based on histological examination only – orderly alignment of cells at the base of the crypts, mitoses in the crypt cells, mucous-filled goblet cells, the absence of necrosis in the lamina propria, and maintenance of the gross architecture of the tissue. Various additives to the media were tested; for example hydrocortisone (Solu-Cortef, Upjohn Co., 5–10 μg/ml) usually appeared to be advantageous to the cultures and is now always included in the media; insulin, on the other hand, caused excessive epithelial cell proliferation and tissue disorder and should therefore be omitted from Trowell's T8 media. Antibiotics were always added, and at the concentrations listed in the Table appear to have no toxic effects on the cells. In media containing 10% foetal calf serum plus hydrocortisone, this tissue can be maintained successfully for at least 90 h in vitro (Figure).

Maximum levels of antibiotics included in tissue culture media, all of which were found not to have toxic effects on the explants.

Penicillin	50 units/ml
Streptomycin sulphate	50 μg/ml
Neomycin sulphate	100 μg/ml
Polymyxin B	25 units/ml
Ampicillin	25 μg/ml
Chloramphenicol	20 μg/ml
Gentamycin	2 μg/ml
Fungizone	5 μg/ml

Two principal problems were encountered in this work: (1) rectal mucosa is usually heavily contaminated with bacteria, which often cannot be held in check by the antibiotics in the media, and (2) the quality of the biopsy is very important – damaged tissue or excessive lymphoid masses in the specimen results in poor survival; furthermore, the explants must be cut small enough to ensure adequate oxygenation of all cells, but without damaging too great a proportion of the delicate tissue irreversibly during the cutting procedure.



Rectal mucosa maintained in vitro for 90 h. Media: 10% foetal calf serum in medium 199 containing 4 mg/ml glucose, 5 μg/ml hydrocortisone succinate and 20 μg/ml chloramphenicol. Incubated at 37 °C in 5% CO_2 in oxygen. (a) Haematoxylin-eosin (× 190); (b) colloidal iron-PAS-haematoxylin (× 190) (mucus stain); (c) autoradiograph, demonstrating the incorporation of $^{35}SO_4^{=}$ added to the liquid medium after 24 h into the goblet cell mucus.

[1] A. MOSCONA, O. A. TROWELL and E. N. WILLMER, in *Cells and Tissues in Culture, Methods, Biology and Physiology* (Ed. E. N. WILLMER; Academic Press, London 1965), vol. 1, p. 70.
[2] L. L. BRANDBORG, C. E. RUBIN and W. E. QUINTON, Gastroenterology *37*, 1 (1959).
[3] P. G. JOHANSEN, C. M. ANDERSON and B. HADORN, Lancet *1*, 455 (1968).
[4] Fungizone, 25 μg/ml, and Streptomycin sulphate, 500 μg/ml, plus Penicillin, 600 units/ml or Neomycin sulphate, 500 μg/ml.
[5] O. A. TROWELL, Expl. Cell Res. *16*, 118 (1959).

Biopsies from 40 patients with cystic fibrosis and from normal controls have been compared in this study, using histochemical methods and $^{35}SO_4^{=}$ incorporation (autoradiography) for examining mucus production; however, no consistent differences were demonstrated. An exhaustive histochemical examination of rectal mucus likewise has failed to reveal any chemical abnormality in cystic fibrosis mucus[6].

The potential of this method for investigating a variety of other problems appears to be considerable, and at present it is being used for studying the toxic effects of gluten digests on duodenal and rectal biopsies from patients with treated coeliac disease[7].

Zusammenfassung. Bei der zystischen Pankreasfibrose ist eine abnorme Schleimabsonderung vorhanden. Bei der einfachen Rektummucosabiopsie sind keine sicheren Befunde zu erheben. Wichtige Veränderungen wurden jedoch an der Duodenalmucosa mit Hilfe einer Organzüchtungsmethode festgestellt.

PATRICIA G. JOHANSEN

Gastroenterological Research Unit,
Royal Children's Hospital Research Foundation,
Parkville 3052, Melbourne (Australia), 7 July 1969.

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Crystallization and Properties of L-Asparaginase from *Escherichia coli*

EC II L-asparaginase from *E. coli*[1] is crystallizable from fractionated cell extracts[2]. Favourable conditions for crystallization are protein concentrations of 3–5%, low ionic strength of buffer solution, pH ranging from 5.0–6.5 and cold room temperature. Ethanol is slowly added until a slight turbidity becomes apparent, which is immediately removed by centrifugation. Crystallization then starts and is completed after several hours. The yield of crystalline L-asparaginase is about 80% of the starting material. Cations such as Mg, Ca, Sr, Ba, Zn and Co are not necessary for crystallization, which even starts in the presence of EDTA. Rhombs originate at pH 6.5, whereas at pH 5.0 slender rods are formed (Figures 1 and 2). This phenomenon is to be explained by a favoured growth of one dimension at pH 6.5. When crystallization is performed, starting with a rather impure material, the contaminating proteins which migrate in PAA-gel electrophoresis faster towards the anode than L-asparaginase, remain in the supernatant (Figure 3). The specific activity of 300 IU/mg is reached by repeated crystallization (Figure 4, sample 1).

Purified L-asparaginase gradually loses up to 90% of enzyme activity on exposure to 37 °C for several days. However, the inactivated product is as easily crystallized as the enzyme, from which it has originated. The loss of activity is represented in PAA-gel electrophoresis by a splitting into 3–4 components of minute higher mobility than the active enzyme which exhibits a single component only (Figure 4). In the ultracentrifuge the Schlieren patterns of two 37 °C treated samples are entirely homogenous. Sedimentation constants $S^{\circ}_{20,w}$ are identical with $S^{\circ}_{20,w}$ of the active enzyme (Table).

The molecular weight of L-asparaginase, inactivated at 37 °C, was determined from equilibrium sedimentation

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[2] P. K. HO and E. B. MILIKIN Fedn. Proc. *28*, 728, Abstr. 2625 (1969).

[3] T. SVEDBERG and K. O. PEDERSEN, *The Ultracentrifuge* (Oxford University Press, New York 1940), p. 262.

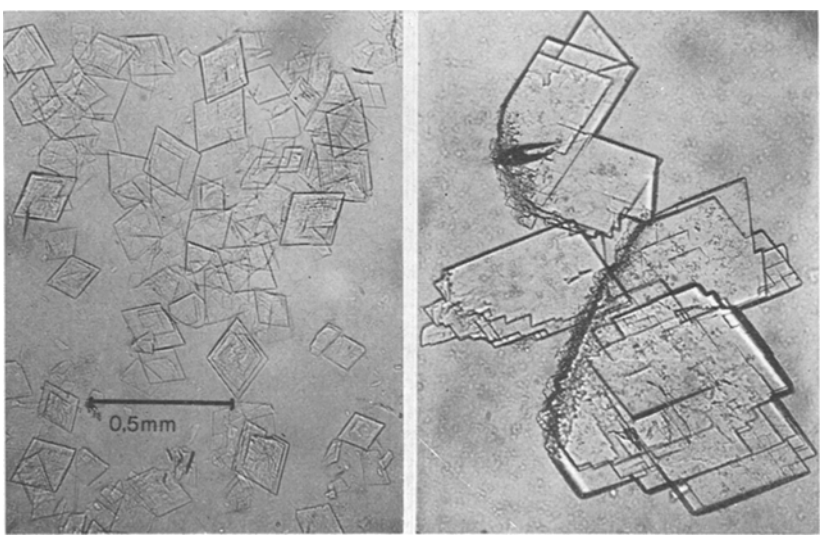

Fig. 1. Rhombs of crystallized L-asparaginase from *E. coli*. Conditions are: 4.8% protein in 0.025 *M* sodium phosphate, pH 6.5; addition of approx. 20% v/v ethanol at 4 °C. Left: fast crystallization within 5 min. Right: slow crystallization with 24 h. The scale applies to both exposures.