Glycerol Effect on the Loss of Solid Constituents from Hepatocytes Isolated in a Potassium Chelating Fluid[1]

The cell suspensions can be used for quantitative cytological studies and for quantitative analysis of the cell constituents to yield information which cannot be easily or reliably obtained by studies on tissue slices. For such studies, it is important that the tissue cells are obtained in suspension in biochemically, physically and structurally undamaged condition. Especially, it is necessary that no loss of cell constituents occurs during and/or after the tissue cell dispersion.

In the case of the hepatocytes from small animals, the above requirements may be satisfied by the perfusion of the liver in situ with a Ca^{++} chelating fluid, followed by a mild mechanical dispersion (JACOB and BHARGAVA[2]). Such dispersion has been proposed to be carried out directly in anhydrous glycerol (TONGIANI and PUCCINELLI[3]). In fact, it can be assumed that either organ perfusion (BHARGAVA and BHARGAVA[4]) or the tissue dispersion in anhydrous glycerol (TONGIANI[5]) do not induce any noticeable loss of solid substances from the hepatocytes. This method, difficult to use for large animals, cannot be used for humans.

For the latter situation, a suitable procedure might be the method described by RAPPAPORT and HOWZE[6], which involves: a) tissue fragments incubated in a fluid containing Na Tetraphenylboron (TPB), a potassium complexing agent; b) cell dispersion by pipetting the tissue fragments up and down in a series of pipettes with decreasing bore size.

In previous studies (TONGIANI[5]; TONGIANI and PUCCINELLI[7]) the hepatocytes, isolated with the JACOB and BHARGAVA's method[2] and suspended in an aqueous medium (for example BARNES, ESNOUF and STOCKEN's fluid[8]), have been observed to undergo a loss of about 40% of their solid content (probably soluble protein, RNA and glycogen).

On the other hand, we found that the cells isolated in the aqueous medium containing TPB also lose part of their protoplasmic constituents.

Therefore, an attempt was made to improve the procedure by modifying the incubation medium by means of the addition of glycerol in order to limit the amount lost. The effect of different glycerol concentrations was also studied.

The experiments were carried out on rat and hamster hepatocytes, following this procedure: a liver lobe was tied, removed and cut in small blocks. Some fragments were incubated in a potassium chelating medium (PCM), containing: Na TPB $0.001\,M$, sucrose $0.05\,M$, NaCl $0.14\,M$, Na phosphate buffer $0.005\,M$, pH 8.5. Other fragments were incubated in PCM supplemented with glycerol (G) at final concentrations of $0.1368\,M$, $0.2736\,M$ and $0.4105\,M$. Satisfactory yields of isolated cells were not obtained with higher glycerol concentrations. After a 2 h incubation at the cracked ice temperature, the liver cells were dispersed. The cell dispersate was washed at $1000 \times g$ for 5 min in a centrifuge at 0 °C and then resuspended in anhydrous glycerol.

As soon as the tied lobe was removed, the remaining liver was perfused with a Ca^{++} chelating fluid and small tissue fragments were dispersed in anhydrous glycerol (TONGIANI[5]). The cells obtained in this way were used as controls. The total weight of the solid substances constituting the isolated cells was determined by means of a microinterferometric integrating apparatus (TONGIANI[9]).

The results, reported in Figures 1 and 2, point out that:

1. Hepatocytes isolated in PCM and in PCM + G are grouped with gaussian distribution into cell classes (TONGIANI[10]; TONGIANI and PUCCINELLI[7]). Their modal weights form an arithmetical progression as 1:2:3:4 and as 2:3:4:5:6:7:8 as regards the rat and hamster, respectively.

2. These classes correspond for number, reciprocal ratios and cell percentage to those of hepatocytes isolated by dispersion in anhydrous glycerol after liver perfusion.

3. The classes, on the other hand, differ as concerns the value of the period (= difference between the modal weight of contiguous classes). The class period of hepatocytes directly dispersed in anhydrous glycerol is 132 pg

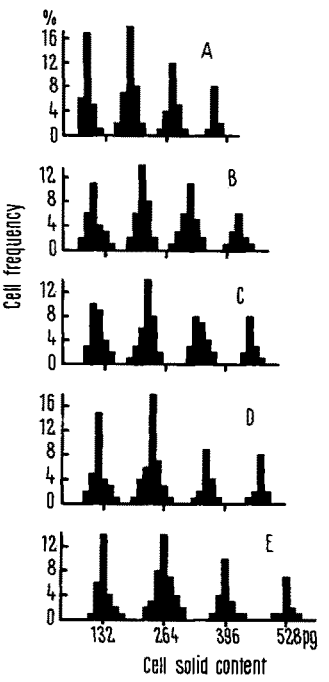

Fig. 1. Hepatocytes of 21-day-old Wistar rat. In ordinate: cell frequency in per cent; in abscissa: cell solid content in pg. The cells were isolated from a same liver either after liver tissue incubation in PCM (A), in PCM + G $0.1368\,M$ (B), in PCM + G $0.2736\,M$ (C), in PCM + G $0.4105\,M$ (D) or directly in anhydrous glycerol after liver perfusion with Ca^{++} chelating fluid (E).

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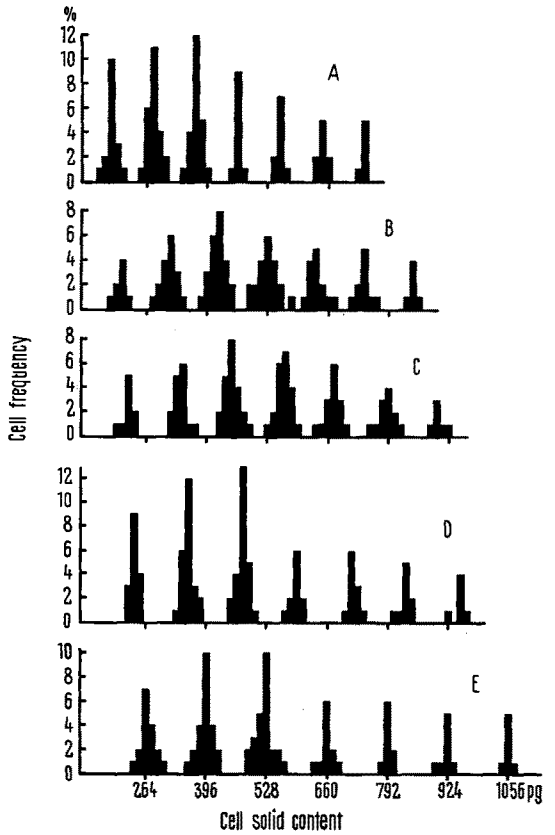

Fig. 2. Hepatocytes of 120-day-old golden hamster. As in Figure 1.

(1 pg = 1 g × 10^{-12}). On the contrary, it is 118.8 pg, 112.2 pg, 105.6 pg and 92.4 pg when the liver tissue was incubated in PCM supplemented with 0.4105 *M*, 0.2736 *M*, 0.1368 *M* and no glycerol, respectively.

4. Consequently, it is apparent that the hepatocytes of the same class weigh differently according to the isolation method (dispersion in PCM, PCM + G, anhydrous glycerol).

5. Thus weight differences are as higher as the glycerol concentration in the incubation medium is lower: under our experimental conditions, −30% when no glycerol was added, −20.4%, −14.8% and −10% when 0.1368 *M*, 0.2736 *M* and 0.4105 *M* glycerol was supplemented, respectively.

6. On the basis of the Student's *t*-test, the weight differences observed are highly significant ($P < 0.001$).

We can therefore conclude that the addition of glycerol to the PCM reduces the loss of protoplasmic substances in the incubation fluid. This effect is the same for both, rat and hamster hepatocytes.

Riassunto. Epatociti isolati mediante dispersione meccanica dopo incubazione del tessuto epatico in un mezzo acquoso contenente tetrafenilborato di Na, un agente chelante il K, perdono circa il 30% del loro contenuto in sostanze solide. L'aggiunta al mezzo di incubazione di glicerolo riduce tale perdita.

MARA ROSSINI and R. TONGIANI

Istituto di Patologia Generale dell'Università, Via Roma 55, I-56100 Pisa (Italy), 30 November 1970.

An Unusual Basement Membrane Underlying Intestinal Epithelium of the Platypus (*Ornithorhynchus anatinus*)

The functional role or roles of the basement membrane which separates epithelial and connective tissue components of the gastrointestinal mucous membrane is far from clear. In mammalian gut 2 relatively distinct components can be distinguished, a narrow layer of collagen fibres surmounted by a thin basal lamina which is demonstrable only at high resolution, and these apparently represent contributions from non-epithelial and epithelial tissues respectively.

In the platypus (*Ornithorhynchus anatinus*), the basement membrane subjacent to the surface epithelium of the intestines is especially prominent[1]. Histochemical and ultrastructural studies reported here show that it presents a number of unusual features.

Specimens of stomach, duodenum, mid-jejunum, terminal ileum, colon and rectum were removed under anaesthesia from 1 adult female and 3 adult male animals, all of which appeared free of disease. For light microscopy tissues were fixed in cold 10% neutral formalin or Bouin's solution, or were freeze-dried. Following routine processing and embedding in paraffin, sections were stained by each of the following: haematoxylin and eosin; PAS, with and without prior digestion with diastase; Alcian blue (pH 1.0 and 2.5) either alone or combined with PAS; toluidine blue (pH 3.5, 7.0 and 9.0); aldehyde fuchsin, with or without prior oxidation with potassium monpersulphate; Verhoeff's elastin stain; Van Gieson stain; and Masson's original trichrome. In addition, unstained sections were examined microscopically under polarized light, and sections of freeze-dried tissues were examined for the presence of amine fluorophores using the formaldehyde-induced fluorescence histochemical technique[2]. For ultrastructural study small blocks of mucosa were fixed in 4% phosphate-buffered glutaraldehyde and subsequently treated in osmium tetroxide[3]. Thin sections were stained with uranyl acetate and lead citrate[4] and examined with a Siemens Elmiskop 1 A electron microscope.

The basement membrane situated beneath the surface epithelium was found to be uniformly thickened throughout the intestines. It was continuous with a much less prominent basement membrane enveloping the intestinal glands. In respect of structure and histochemical reactions the glandular basement membrane was indistinguishable from that found in the stomach of the platypus and in the gastrointestinal tract generally of other mammals. The basement membrane associated with the

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