THE REAGGREGATION AND METABOLISM OF DISSOCIATED ADULT GUINEA PIG CELLS IN FILTER-WELL CULTURES

J. A. DICKSON and I. LESLIE. From the Biochemistry Department, Queen's University, Belfast, Northern Ireland

When embryonic tissues are dissociated by treatment with tryptic enzymes, the individual cells are able to reaggregate to form the essential architecture of the tissue of origin (1–3). The variables involved in this aggregation phenomenon have been minimized by Moscona (4) by subjecting the cell suspensions to controlled rotatory motion in flasks. Under such conditions, embryonic kidney cells become organized into nephrogenic epithelium, renal corpuscles, and tubules (4, 5). This type of reaggregation is most pronounced with tissue from the youngest embryos, and the ability of the cells to produce organized colonies declines with increasing embryonic age.

The present paper reports results obtained with a technique which was developed with the aim of maintaining dissociated cells in a viable but nonproliferating state, in conditions which favoured cell-surface interactions. This was achieved by allowing cell suspensions of known number to settle on Millipore membranes which formed the base of filter-wells set within Trowell organ culture units. The paper describes the reaggregation and associated metabolism of adult guinea pig kidney cells maintained for 6 to 10 days in filter-well cultures.

MATERIALS AND METHODS

Prefasted young adult guinea pigs weighing 500 to 600 gms were used. Following bilateral nephrectomy, the two kidneys were cut into fifteen to twenty small pieces which were washed free of blood in four changes of Ca^{++}- and Mg^{++}-free saline (6). The tissue was then disaggregated with 1 per cent trypsin (Difco Laboratories, Inc., Detroit, 1:250) in this saline to give a unicellular suspension. After two washes in Waymouth MB 752/1 medium containing 4 per cent human AB serum and supplemented with 1 unit insulin (Boot's once-crystallized insulin), the cells were enumerated on a haemocytometer and examined for their viability by the nigrosin dye-uptake test (7). Volumes containing 10×10^6 to 100×10^6 cells were transferred to the filter wells, and the cells were allowed to settle on the membrane. The cultures were incubated at 37°C and the medium was renewed at intervals of two days.

The Millipore filters and their attached cultures were treated as a unit and transferred directly from the culture vessel to saturated picric acid–ethanol at −70°C, then fixed overnight at −35°C. The specimens were then allowed to reach room temperature before being passed through changes of ethanol and xylene (1 hour each). A 4 hour impregnation with paraffin wax at 57°C was followed by embedding in wax. All sections were cut at 10 μ and stained with haematoxylin and eosin.

Cell Metabolism

The use of modified Trowell organ culture units makes it possible to determine glucose metabolism and cell respiration. As emphasized elsewhere (8), particular care must be exercised in the preparation of glassware and filter-wells, and the level of the medium in the Petri dishes is critical. The Millipore membrane should be horizontal on the surface of the medium, and the layer of cells should lie in the gas phase (20 per cent of O_2, 75 per cent N_2, and 5 per cent CO_2). The medium initially contained 0.25 to 0.5 μc of uniformly labelled ^{14}C-glucose per ml. Radioactivity of the CO_2 produced by the cells was determined by releasing bound CO_2 in the medium with acid, and transferring the total CO_2 to an evacuated ionisation chamber for evaluation of ^{14}C with a Dynacon 6000 Electrometer (Nuclear Chicago Corporation, Des Plaines, Illinois). Glucose and lactic acid in initial and used medium were also determined as described previously (8).

RESULTS

From an evenly dispersed plaque at four hours (Fig. 1 *a*) the cells migrate to form organized histological patterns. An annular colony is formed at 4 days (Fig. 1 *b*); on further migration this becomes a central nodule (Fig. 1, *c* and *d*). Initially the cell layer shows no structural features (Fig. 1 *e*). As the cells migrate, they become arranged into a distinctive histological pattern which is increasingly organized as the cell colony moves centrally (Fig. 1, *f* to *h*). The final, central nodule exhibits a well-defined architecture with distinctive histological features (Fig. 1 *h*). Differential staining (Van Gieson) of sections similar to Fig. 1 *h* shows many of the aggregates to consist of epithelial cells surrounding central areas of connective tissue.

Downloaded from http://rupress.org/jcb/article-pdf/19/2/449/1562272/449.pdf by guest on 01 December 2023

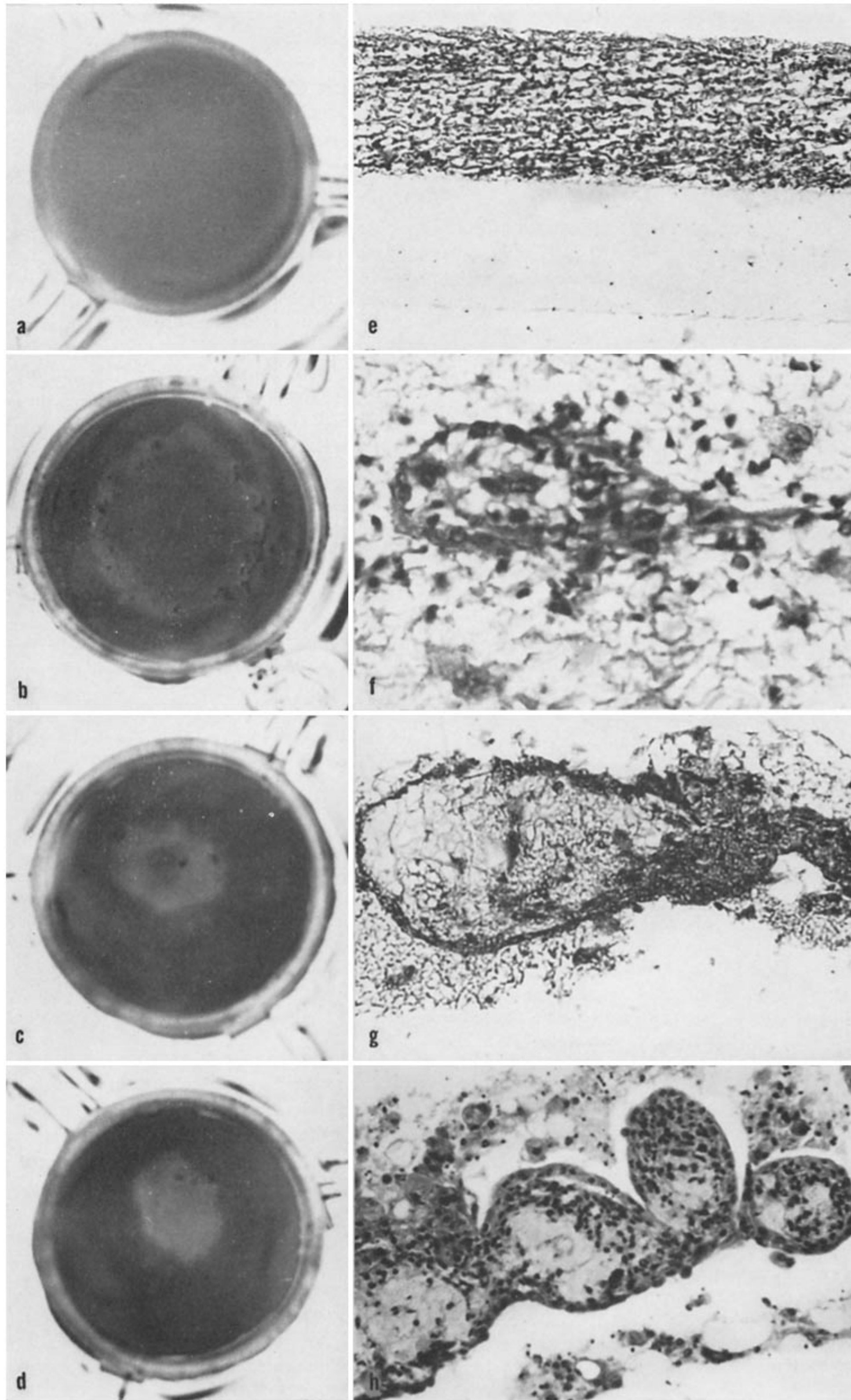

FIGURE 1

Downloaded from http://rupress.org/jcb/article-pdf/19/2/449/1562272/449.pdf by guest on 01 December 2023

TABLE I

Metabolism of Migrating and Reassociating Adult Guinea Pig Kidney Cells in Filter-Wells

Time	No. of Cultures	Glucose uptake	LA production / G uptake	Percentage $^{14}C\text{-}G(U) \rightarrow CO_2$
days		μM/ml	μM	
2	6	7.3 to 8.7	0.65 to 0.75	2.4 to 2.7
4	3	9.3 to 10.0	1.12 to 1.20	—
6	3	8.9 to 9.2	1.20 to 1.28	1.6 to 1.8

The filter-wells each contained *37 million cells.*
Glucose at an initial concentration of 27 μM/ml, and activity of ^{14}C-glucose was 0.27 μc/ml.
Viability of initial suspension > 90 per cent.

This centripetal migration of the cells has been found to occur with initial suspensions containing twenty to forty million cells per well, and the central nodule can be formed in as little as 6 days. Even with cell numbers within this range, migration to form a central nodule as shown in Fig. 1, *a* to *d* does not always occur, the cells forming instead discrete aggregates of varying size on the Millipore filter.

Table I shows the changes in glucose metabolism which occurred when kidney cells were migrating and reassociating over a period of 6 days. There was a slight increase in glycolysis, as measured by the molar ratio of lactic acid produced to glucose utilised, and this was accompanied by a decrease in the percentage of ^{14}C-glucose converted to carbon dioxide. Histological examination of the cultures revealed very few mitotic figures. This was consistent with the observed decrease of DNA content of replicate filter-well cultures to 25 per cent of the initial value over a period of 10 days. There was, however, a considerable increase in the ratios of RNA and protein to DNA in the kidney cell cultures over the same period.

A feature of the present results is that reaggregation of adult guinea pig cells has occurred in the presence of Waymouth MB 752/1 medium supplemented with 4 per cent human AB serum and 1 unit insulin/ml.

The authors are glad to acknowledge the help of Dr. D. H. Strangeways in the interpretation of histological material. This research was supported by grants from the Medical Research Council and the British Empire Cancer Campaign. Dr. Dickson received a Medical Research Council Research Scholarship.

Received for publication, April 30, 1963.

REFERENCES

1. Moscona, A., *Exp. Cell Research*, 1952, **3**, 535.
2. Moscona, A., *Proc. Nat. Acad. Sc.*, 1957, **43**, 184.
3. Moscona, A., and Moscona, H., *J. Anat., London*, 1952, **86**, 287.
4. Moscona, A., *Exp. Cell Research*, 1961, **22**, 455.
5. Moscona, A., *in* Growth in Living Systems, (M. X. Zarrow, editor), New York, Basic Books Publishing Co., Inc., 1961, 197.
6. Rinaldini, L. M., *Exp. Cell Research*, 1959, **16**, 477.
7. Kaltenbach, J. P., Kaltenbach, M. H., and Lyons, W. B., *Exp. Cell Research*, 1958, **15**, 112.
8. Dickson, J. A., and Leslie, I., *Exp. Cell Research*, 1963, **31**, 214.

Figure 1 Centripetal migration and reaggregation of adult guinea pig kidney cells. Filter-wells photographed with Ilfocolour negative film. Filter-wells (*a* to *d*), × 2. Sections (*e* to *h*) stained with haematoxylin and eosin.

Fig. 1, *a* and *e*: Uniformly distributed plaque of cells on Millipore filter (*a*) and random arrangement of the cells at 4 hours in section (*e*). × 100.

Fig. 1, *b* and *f*: Initial migration of the cells at 4 days to form a well defined annulus (*b*) which on section shows elements of organization (*f*). × 400.

Fig. 1, *c* and *g*: Continued centripetal movement of the annulus at 6 days (*c*). The histological pattern shows further organization (*g*). × 150.

Fig. 1, *d* and *h*: Formation of central nodule at 10 days (*d*). On section the nodule shows a highly organized structure in which epithelial cells enclose well defined areas of connective tissue (*h*). × 150.

Downloaded from http://rupress.org/jcb/article-pdf/19/2/449/1562272/449.pdf by guest on 01 December 2023