LETTERS TO THE EDITORS

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Animal Tissue Cells in Protein-Free Media

FEW attempts have been made to maintain (or grow) animal tissue cells in protein-free media, although it has been shown[1] that the low molecular weight substances of blood plasma are of the utmost importance for the life of tissue cells, which rapidly perish in dialysed blood plasma. It has further been possible[1,2], by the addition of a number of amino-acids together with glutamine, glutathione and hexose diphosphate to a medium containing only dialysed plasma proteins and dialysed embryonic extract, to obtain survival and growth of the cells. The few attempts made to devise completely synthetic media have met with only partial success because of lack of a suitable technique, and because very little is really known about the fundamental nutritional requirements of animal tissue cells during survival and growth.

M. R. Lewis and W. H. Lewis[3] obtained migration and growth of cells in hanging-drop cultures in media containing inorganic salts, dextrose or maltose, amino-acids and polypeptides. As they also obtained migration and growth in a medium containing inorganic salts only, there could, however, be no doubt that their results were complicated by insufficiently defined residual growth phenomena originating from the use of rather large tissue explants and small volumes of media. P. R. White[4] has recently, by means of a modified roller tube technique, obtained migration and growth of long duration in a synthetic medium containing a large number of substances supposed to be of importance for the maintenance and growth of animal tissue cells *in vitro*. The manner in which White prepared the tissue explants and placed them in the cultivation tubes does not seem to ensure, however, that the reaction of the cells was due solely to the constituents of the synthetic medium (cf. also ref. 2).

In order to be able to study the effects of some protein-free media, I have made use of hanging-drop cultures made according to the technique of Lewis and Lewis[3]. By the use of very small explants (of embryonic heart tissue ; 8–9 day embryos) it apparently became possible to avoid contamination of the media by substances given off from the explants. The following results were obtained :

(1) In Tyrode solution no migration of cells was obtained, undoubtedly because a solution containing only inorganic salts and dextrose cannot maintain the life of animal tissue cells.

(2) Tyrode solution containing the following nine amino-acids was made up : *d*-lysine dihydrochloride (0·006 per cent) ; *d*-arginine (0·003 per cent) ; *l*-tryptophane (0·002 per cent) ; *d,l*-methionine (0·001 per cent) ; *l*-histidine monohydrochloride (0·001 per cent) ; glutamic acid (0·006 per cent) ; *l*-aspartic acid (0·002 per cent) ; *l*-proline (0·002 per cent) ; *l*-cystine (0·0006 per cent), (cf. ref. 1). Zones of migrating cells were formed around the explants. The number of migrated cells showed great variation (from a few hundred to about 1,500 cells). The mitotic frequency was very low (about 1 per 4,000 cells). The cells survived two or three days, looking apparently normal. The addition of glutamine (0·05 per cent), glutathione (0·01 per cent) and hexose diphosphate (0·03 per cent) did not change the result.

(3) Dialysate obtained by dialysis of blood plasma (adult cock) against an equal volume of Ringer's solution (0·1 per cent dextrose). Large zones of migrating cells were observed (the number of migrating cells varying between a few hundred and more than 2,000). The mitotic frequency was very low (1 per 2,500). Dialysates of pig and ox serum have so far given no cellular migration.

A number of experiments have also been made, placing the tissue explants at the bottom of Carrel flasks (volume of medium 0·5–1 c.c., one explant in each flask). The *p*H was adjusted to 7·6–7·8 by regulation of the amount of carbon dioxide present. The large volume of the medium, and frequent renewals, made it possible to keep its composition constant—this is scarcely possible in hanging-drop cultures for any long period of time.

In this way, survival of zones of migrated cells in the amino-acid mixture (in Tyrode solution) or in plasma dialysate up to ten to twelve days was achieved ; no migration of cells could be obtained in Tyrode solution alone. It is, moreover, possible to isolate zones of migrated cells placed at the bottom of Carrel flasks, the original explants being removed after one or two days.

Using the procedures described here makes it possible to study the effects of protein-free media on tissue cells *in vitro*.

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[1] Fischer, A., *Acta physiol. Scand.*, **2**, 143 (1941).
[2] Fischer, A., Astrup, T., Ehrensvärd, G., and Oehlenschlager, *Proc Soc. Exp. Biol. Med.* (in the press).
[3] Lewis, M. R., and Lewis, W. H., *Anat. Rec.*, **5**, 277 (1911).
[4] White, P. R., *Growth*, **10**, 231 (1946).

Failure of Sulphanilamide to Inhibit Calcification of Bone

BENESCH and co-workers[1] have reported almost complete absence of calcification in the bones of rat fœtuses at term, following administration of large doses of sulphanilamide and sulphapyridine to the mothers during the last seven to ten days of the gestation period. Their report was based upon hæmatoxylin-staining of decalcified bones, with the reservation that this method might not be reliable, and upon X-ray examination of the bones of the fœtuses from sulphonamide-treated rats, no mention being made of control observations upon normal fœtuses. The results of observations upon mice were stated to be equivocal.

We have administered sulphanilamide to pregnant rats under conditions similar to those reported by Benesch *et al.*, and have observed the new-born rats from both treated and untreated mothers by positive staining of bone salt in undecalcified bones and by X-rays. Our observations do not confirm those of Benesch *et al.*

Four pregnant rats received 720 mgm. of sulphanilamide/kgm./day during the latter 5, 7, 11 and 16 days, respectively, of pregnancy. Three new-born rats of each litter were examined by X-rays and autopsied within fifteen hours after birth. X-ray films were made with 10 m. amp. current and 1 sec.