THE INFLUENCE OF IRON-DEXTRAN COMPLEX ON THE STRAIN L FIBROBLAST

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Iron-dextran complex has been found to be carcinogenic in animals. Richmond (9, 10), Haddow (3), and Haddow and Horning (4) have reported that subcutaneous injections of massive doses of this complex in the rat and mouse have given rise to sarcomas originating from iron-laden connective and reticuloendothelial tissues.

It was thought that it would be interesting to grow the mouse subcutaneous fibroblast in culture in the presence of massive doses of the iron-dextran complex. In such cultures the morphology, growth, and metabolism, particularly the respiration, could be studied. The experimental evidence to be reported here are the changes which have occurred in these cultures during the first 3 months in which they were grown in medium containing iron-dextran complex.

METHODS

Cultures: Stock cultures of the mouse subcutaneous fibroblast, strain L, clone 929, were grown in Roux flasks under standard culture conditions (6). The nutrient medium used was a modified Eagle's medium (6) plus 2 per cent calf serum and 5 per cent Bacto-peptone. 0.1 milliliter of iron dextran complex, Imferon, (equivalent of 500 μg Fe) per milliliter was added to the nutrient fluid. The fluid was changed twice weekly. The cell population was maintained at approximately 10^4 to 10^5 cells per milliliter.

For detailed studies, the cells were grown in test tube cultures. Cells from Roux flasks were trypsinized ($1/250$ Difco), washed, and resuspended in nutrient fluid containing the same amount of iron-dextran as in the stock cultures. A tricarboxylic acid supplement (1) was added to the medium in order to insure constant maximal respiration during the culture period. Two milliliters of this cell suspension, containing approximately 100,000 cells per milliliter, were introduced into regular test tubes.

Cell Counts: For growth studies, cell counts of duplicate test tube cultures were done daily. Each culture was drained and 2 milliliters of 0.25 per cent trypsin ($1/250$ Difco) were added. After 5 minutes the test tube was shaken to disperse the cells. A sample was examined microscopically to be sure that the cells had been dispersed. The cell concentration was then determined using a Ljungborg celloscope.

Preparations for Cytological Study: In order to obtain cells for cytological study, coverslips were introduced into both test tube cultures and Roux flasks at the time the cultures were set up (6). Cells adhered to the upper surface of the coverslips. The coverslips were removed from the cultures at various intervals, washed in balanced salt solution, fixed in formalin for 5 minutes, and stained for the Prussian-blue reaction (2) or with Ehrlich's hematoxylin. The coverslips were then mounted for cytological study.

Respiration: Cellular respiration was measured using a modified Cartesian diver technique which has been described previously in detail (7). Two milliliters of cell suspension were introduced into the Cartesian diver for respiration determinations.

OBSERVATIONS

Morphology: Soon after the cells were grown in medium containing iron-dextran complex, the cytoplasm contained discrete yellow pigment (Figs. 1a, b). The multinucleated cells had appreciably more cytoplasmic inclusions than the mononuclear cells.

This cytoplasmic pigment gave a positive Prussian-blue reaction (Fig. 1c). The pigment granules in the mononuclear cells were single and discrete (Figs. 1a, b) whereas the cytoplasm of the multinucleated cells was packed with large masses of material giving a positive Prussian-blue reaction (Fig. 1c).

The proportion of the population which were multinucleated cells did not alter nor was there any increase in abnormal mitotic figures.

Growth: Cell growth was definitely inhibited by massive doses of iron-dextran complex in the nutrient medium. As determined by cell counts, growth was progressively curtailed (Fig. 2 and

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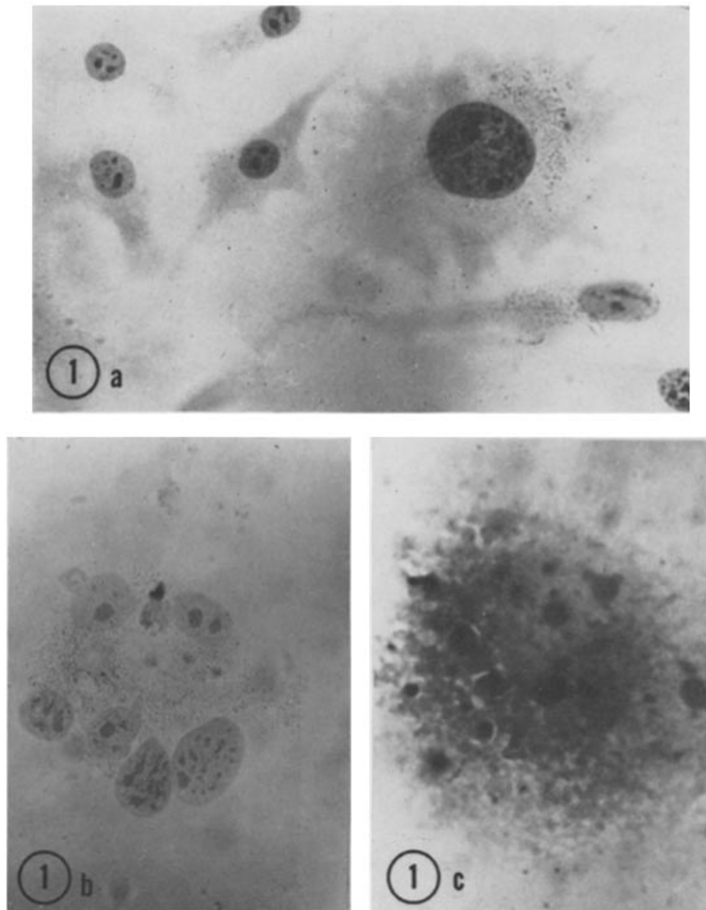

FIGURE 1

Cultures of mouse subcutaneous fibroblast, strain L, clone 929, grown on coverslips in test tube cultures.

Cells grown in medium containing 500 μg iron-dextran complex, Imferon, for 6 weeks.

1a. Mononuclear cells stained with Ehrlich's hematoxylin. Pigment in cytoplasm had deep yellow color. × 250.

1b. Multinucleated cell stained with Ehrlich's hematoxylin. Yellow pigment in perinuclear position. × 600.

1c. Multinucleated cell stained for Prussian blue reaction (2). Cytoplasm contained many discrete granules and masses taking a deep blue stain indicating loosely bound iron. × 600.

Table I); however, the curtailment of growth during the first week in the presence of iron-dextran was the greatest, 38 per cent. Up until 3 months, the degree of curtailment of growth increased slowly.

Respiration: Respiration of cells grown under control conditions was normally around 5×10^{-6} μl per hour per cell. Cells grown for 6 weeks in iron-dextran–containing medium had a respiration of approximately 3.23×10^{-6} μl per hour per cell. After 3 months, the respiration of these cells was approximately 3.89×10^{-6} μl per hour per cell.

DISCUSSION

The strain L cells were grown in medium containing massive doses of iron-dextran complex (equivalent of 500 μg per milliliter). This concentration was chosen as representative of the

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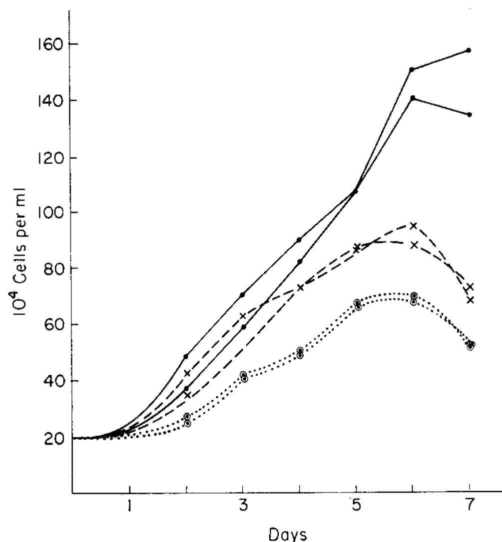

FIGURE 2

Influence of iron-dextran complex, Imferon, (500 μg per milliliter) on growth of the strain L cell.

Cells grown in test tube cultures for 7 days. —•— control, --×-- iron-dextran complex added to medium at beginning of this culture period, ...⊙... cells grown in medium containing complex for 3 months.

concentration of this complex at the site of subcutaneous injections in *in vivo* research (4, 9, 10). However, such concentrations represented an overloading of the organism, in this case the isolated tissue culture cells, with exogenous iron.

When the strain L cells were grown in this iron-dextran–containing medium, the cytoplasm of these cells contained a pigment which gave a positive Prussian-blue reaction indicating loosely bound iron (Fig. 1c). It was proposed that the iron-dextran complex, having a number average molecular weight of approximately 2500 (4), entered the cell by pinocytosis. The actual process of uptake has not been followed. Therefore, it has not been strictly shown that iron-dextran–containing droplets have actually been engulfed by pinocytosis. However, Danes and Andresen (unpublished) have demonstrated that iron-dextran complex enters the ameba by such a process. In the case of the ameba, iron-dextran did not stimulate pinocytosis and was taken up only as a natural outcome of the engulfing of fluid which contained iron-dextran complex. In the concentration used, iron-dextran did not appear to be adsorbed on the cell membrane and thereby stimulate pinocytosis.

The strain L cell, clone 929, used in this study contained both mono- and multinucleated cells (approximately 2 per cent of the population). The multinucleated cells consistently contained more cytoplasmic pigment than the mononuclear cells. It was proposed that the multinucleated cells might have had a greater pinocytotic activity, taking up more nutrient fluid containing the iron complex. This difference in activity of the two cell types originally derived from the same cell (11) was interesting in view of the metabolic differences of these two cells reported by Phillips and Terryberry (8).

The uptake of such a high molecular weight substance as iron-dextran complex (4) was surprising in view of the report by Holter and Holtzer (5) that fluorescein-labeled plasma proteins were not taken up by strain L cells. These experiments were short time (4 to 6 hours) under alkaline conditions whereas the present experiments were carried on over monthly periods under neutral or acid hydrogen ion concentrations.

After being grown in iron-dextran–containing medium for 3 months, the pigment in the cytoplasm of mononuclear cells appeared as small, discrete droplets, the size one would expect of a cytoplasmic vacuole resulting from pinocytosis (Figs. 1a, b). The multinucleated cells however contained small granules as well as large masses of pigment, the result of fusion or aggregation of small vacuoles (Fig. 1c). Whether the iron-dextran complex remained in the pinocytotic vacuoles and therefore was not incorporated into the cellular constituents, or the membranes of the vacuoles became permeable to the iron or iron complex and allowed diffusion into the cytoplasm was not learned.

Both growth and respiration of the cells were

TABLE I

Strain L Cells Grown Continuously in Medium Containing 500 μg Iron-Dextran Complex, Imferon, per Milliliter Medium for 3 Months

Weeks grown in complex medium	% inhibition growth	Δ V per cell
		$\times 10^{-6}$ *μl per hr.*
0–1	38	3.90
6	47	3.23
12	52	3.89

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curtailed by the presence of iron-dextran complex (Fig. 2 and Table I). Danes and Paul (1) reported that growth of the strain L cell was not influenced by such a degree of curtailment of respiration for short time periods. Perhaps this reduction in growth was due to prolonged minimal suppression of respiration. This reduction in growth especially indicated that the iron was not isolated in vacuoles.

Haddow and Horning (4) have suggested that such an excess of iron in the cell would interfere with the cytochrome system and thus respiration. In view of this suggestion, it was interesting to note that respiration was only slightly reduced after the cells were grown in the presence of massive doses (500 μg per milliliter) of iron-dextran complex for 6 weeks. It will be of the upmost importance to follow the respiratory activity of these cells over a longer time period.

SUMMARY

The strain L cell has been grown in medium containing massive doses (500 μg per milliliter medium) of iron-dextran complex, Imferon. Morphology, growth, and respiration of these cells have been studied for a 3 month period.

The cytoplasm of strain L cells grown in iron-dextran medium contained pigment which gave a positive Prussian-blue reaction. The multinucleated cells consistently contained more pigment than the mononuclear cells. Growth was markedly reduced whereas respiration was only slightly curtailed.

The mechanism of entry of the iron-dextran complex into the cell was discussed.

This research was done as a result of discussions with Professor Haddow and Dr. G. C. Easty at the Chester Beatty Institute while Dr. B. Shannon Danes was a visitor in the laboratory of Professor Sir Roy Cameron, F.R.S.

We express our appreciation to Dr. John Paul, H.E.R.T. Tissue Culture Laboratory, Biochemistry Department, The University, Glasgow, Scotland, for supplying the cultures and nutrients for this study and for his comments and suggestions.

Dr. Danes wishes to thank Sir Roy Cameron, Professor Haddow, and Dr. Easty for reading the manuscript and making critical comments.

This research was supported by an American Cancer Society Postdoctoral Fellowship held by Dr. B. Shannon Danes.

Received for publication, March 1, 1961.

BIBLIOGRAPHY

1. Danes, B. S., and Paul, J., *Exp. Cell Research,* 1961, manuscript submitted.
2. Gomori, G., Microscopic Histochemistry, Principles and Practice, University of Chicago Press, 1952, 38.
3. Haddow, A., *in* Ciba Foundation Symposium on Carcinogenesis, London, Churchill, 1959, 300.
4. Haddow, A. and Horning, E. S., *J. Nat. Cancer Inst.,* 1960, **24,** 109.
5. Holter, H., and Holtzer, H., *Exp. Cell Research,* 1959, **18,** 423.
6. Paul, J., Cell and Tissue Culture, Edinburgh, Livingstone Ltd., 1959.
7. Paul, J., and Danes, B. S., *Analytical Biochemistry,* 1961, in press.
8. Phillips, H. J., and Terryberry, J. E., *Exp. Cell Research,* 1958, **14,** 454.
9. Richmond, H. G., *Scottish Med. J.,* 1957, **2,** 169.
10. Richmond, H. G., *Brit. Med. J.,* 1959, **1,** 947.
11. Sanford, K. K., Earle, W. R. and Lilkey, G. D., *J. Nat. Cancer Inst.,* 1948, **9,** 229.

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