THE QUESTION OF FREE BACILLARY GROWTH IN THE PLASMA SURROUNDING LEPROMATOUS EXPLANTS DURING TISSUE CULTIVATION

by

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An accumulated turbidity and the presence of many bacilli in the plasma surrounding lepromatous tissue culture explants has been emphasized by Timofejewsky (1) and by Suwo and Kin (2) as indicating the multiplication of free bacilli in the plasma itself. Since this inference seems unwarranted by the observations made during the preceding studies with tissue cultures, it is desired to explain why different conclusions were reached.

The opacity of the plasma surrounding the explants is not explained by the numbers of bacilli present. In preceding communications (3, 4), reference has been made to difficulties due to the deposition of calcium phosphates in the explants and adjacent plasma when 10 or 15 per cent of chicken embryo juice was added to human serum media. A detailed analysis of this problem is to be presented elsewhere (5). Briefly, the fresh embryo juice is saturated with respect to calcium and phosphates, and its high phosphatase activity continues to liberate inorganic phosphates from organic phosphorus, thus producing supersaturation. The embryo juice alone, or mixtures with serum and the diluting fluid, when stored or incubated in plain glass tubes for a few days deposit calcium phosphates which are accompanied by complex materials absorbed from the medium. In the presence of plasma, autolyzing tissue explants, and especially of actively metabolizing cultures, similar deposits accumulate most strikingly in and around the centers of the tissue cultures.

Carrel's earlier notes on tissue cultivation (6) warn against the opacity which characterizes rabbit plasma in tissue cultivation. Rabbit serum, due to its higher calcium content, is more prone than human serum to cause calcification of plasma in the presence of embryo juice (5). Timofejewsky described his best cultures as having been prepared in rabbit and human plasma and having been renewed with undiluted human embryo juice. Suwo and Kin employed chicken plasma and renewed the liquid phase with chicken embryo juice. From what has been said, it appears correct to infer that the cloudy plasma described by Timofejewsky was due to the

phenomenon under discussion, while figure 3 in the article of Suwo and Kin clearly shows that a dense precipitate occupied nearly the entire explant of a culture only five days old. Though calcification never occurred so promptly in the media used in the present work, its incipient stages were precisely as shown in the figure from Suwo and Kin. Due to solubility in the acid decolorizing solutions, these precipitates always disappear during staining of the cultures.

The use of total preparations in the present studies permitted a review of the entire series of stained cultures accumulated in several experiments. Observation of the relative concentrations of free bacilli in the plasma showed a fairly orderly increase in the numbers of such bacilli as incubation progressed. Their conspicuousness was related (a) to their concentration in the original explants and to the fate of the primary cell growth and (b) to the duration of the incubation period. A brief summary, and a plausible explanation, of these observations may be made as follows:

First, the largest numbers of bacilli were found in the plasma surrounding explants rich in bacilli, a characteristic which has been shown to be associated with high cell content and a potentiality for early cell outgrowth and the greatest transport of bacilli in fibroblasts. The importance of conveyance by cells was easily recognized, since early cultures which had shown a partial corona of active outgrowth and subsequent damage to the cells revealed numerous bacilli in the plasma in the region of outgrowth and very few in the areas not penetrated by cells. Timofejewsky and Suwo and Kin describe their cultures as deteriorating in the third week of cultivation, and both articles state that this was the interval when the bacilli increased most remarkably in the plasma. It appears reasonable that these bacilli had been carried into the plasma by migrating cells, and that their great numbers appeared more impressive as soon as the cells no longer occupied so important a place in the picture.

Secondly, the numbers of free bacilli in the plasma immediately surrounding the explants increased during the incubation period; this was due in part to softening of the explants and of the plasma. Thus, in older cultures which had been partially surrounded by cells, or even in those which remained negative for cell growth, the bacilli were distributed quite evenly around the explants. The influence of these purely mechanical factors is perhaps augmented by the manipulations required to remove the cultures from the culture vessels, and might play a more important role when large volumes of plasma must be rolled on a needle and squeezed through the narrow necks of the Carrel flasks used by the earlier workers.

As noted before, the advancing border of a tuberculoid lesion suggests that the bacilli in these tissues, though not numerous, are active and viable. The results from such lesions must also be considered. Since deterioration of cells, in this case, also results in deposition of bacilli in the plasma, and active tissue resistance may be presumed to disappear, it should leave a situation which is ideal for rapid bacillary growth. The numbers of bacilli, nevertheless, correspond only with the concentrations seen while studying the cultures with active cells.

It is concluded that the turbidity of the plasma described by the earlier workers was due to calcification rather than the numbers of bacilli present. The bacilli in the plasma surrounding explants from lepromatous tissues are derived by cell transport or other mechanical means, and they do not arise by free multiplication in the plasma itself.

REFERENCES

(1) TIMOFEJEWSKY, A. D. Explantationsversuche von leprösem Gewebe. Arch. f. exper. Zellforsch. 9 (1930) 182-202.

(2) SUWO, M. and KIN, S. Ueber Kulturen von leprösem Gewebe *in vitro*. I. Mitteilung. Gewebskulturen von Hautknötchen bei Menschenlepra. Internat. J. Leprosy. 7 (1939) 57-66.

(3) HANKS, J. H. Fate of leprosy bacilli in fibroblasts cultivated from macular and tuberculoid lesions. Internat. J. Leprosy. 15 (1947) 31-47.

(4) HANKS, J. H. The fate of leprosy bacilli in fibroblasts cultivated from lepromatous lesions. Internat. J. Leprosy. 15 (1947) 48-64.

(5) HANKS, J. H. The calcification of tissue cultures in mixtures of mammalian sera and embryo juice. In preparation.

(6) CARREL, A. Filterable Viruses, p. 101. Edited by T. M. Rivers. Williams & Williams, Baltimore, Maryland, U.S.A.