CYTOPATHIC CHANGES IN CELLS OF CHICK EMBRYO TISSUE CULTURE AFFECTED BY NEWCASTLE'S DISEASE VIRUS

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One of the particular problems of cellular determination [4] is the capacity of tissue cells to alter their sensitivity to the action of viruses during culture in vitro. Embryonic cells, the determination of which changes with development and, possibly, is therefore not the same in different organs, are especially suitable for such studies. In addition, cells of embryonic origin are easily cultured, are subject to the action of viruses, and are widely accessible for study. In this connection, a considerable part of the research devoted to experimental study of the cytopathic effects of viruses, has been carried out on embryonic tissue cultures. However, rather frequently the cultivation of viruses on the same embryonic culture under nearly the same conditions has led to different results. In this situation the age of the embryo from which the culture was prepared has rarely been considered.

This paper presents the results of morphological study of cells in kidney, liver and lung tissue culture of chick embryos prepared at relatively different stages of development (nine and 16 days of incubation) and subjected to the action of Newcastle's disease virus.

METHODS

Cells were taken by the usual method from the kidney, liver and lung tissues of nine- and 16-day chick embryos. Medium No. 199 containing 10% bovine serum was used for cultivation of the liver and lung tissue. Kidney tissue was cultivated on medium containing 0.5% lactalbumin hydrolysate in Hanks' solution, with the same amount of serum. Penicillin, 100 units/ml was added to the nutrient medium. A cell inoculum, 2×10^6 (liver and lung) and 1.5×10^6 (kidney), was placed in Carrel dishes eight cm in diameter in which a mica or glass disc had been put. Monolayers were obtained in 3-4 days.

A vaccine strain of Newcastle's disease virus was used that was virulent for embryos. The virus titer in lung tissue culture of 16-day chick embryo was $10^{5.5}$–10^6 TCD/0.1 ml (tissue cytopathic dose).

In control tissue cultures non-infected allantoic fluid from 10-11 day chick embryo was added to a heated culture of the virus. A viral dose not exceeding $10^{5.5}$ TCD_{50}/0.1 ml was used for infection. After thirty minutes of exposure the virus was washed off and nutrient medium added. The cultures were fixed in Bouin's fluid and by Shabadash's method and were stained with hematoxylin and eosin for cytological studies.

RESULTS

In connection with the significant differences in morphological characteristics between liver, lung and kidney tissue in the organism and in culture we feel it is necessary first to describe the normal tissue cultures.

Tissue culture of liver (Fig. 1, a) consists of two types of cells: 1) resembling the cells of liver parenchyma, epithelial, comparatively small, polygonal forms, with round nuclei, growing in clearly delimited islands and 2) surrounding them, considerably larger cells, fibroblast-like, with oval large nuclei.

Tissue culture of lung in different experiments varied, depending on the predominance of fibroblast-like or

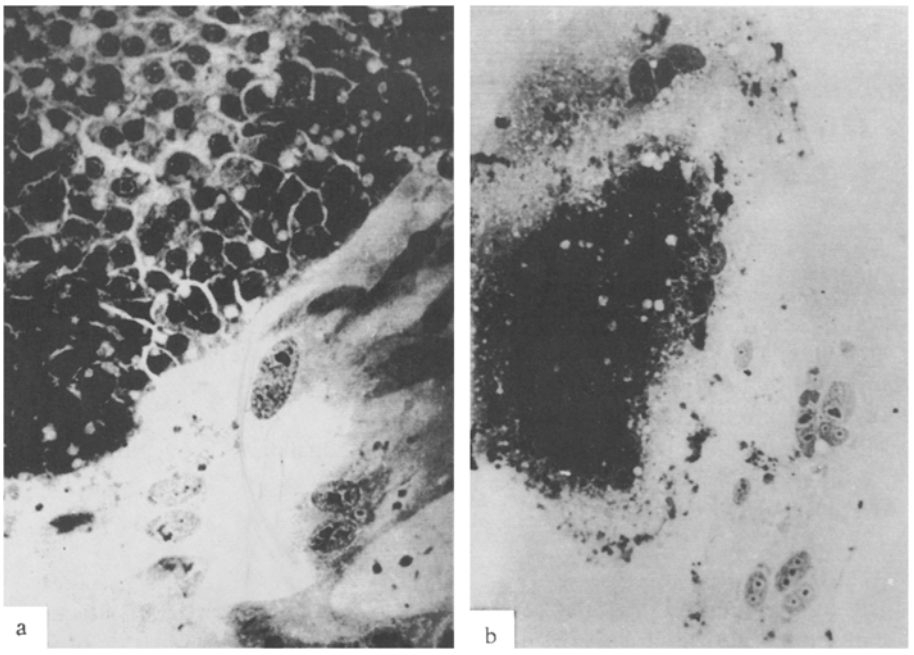

Fig. 1. Culture of liver tissue from nine-day chick embryo, fourth day of cultivation. a) Normal culture, magnification 500 × ; b) six hours after infection with virus, magnification 300 × . Bouin's fixation. Hematoxylin-eosin.

epithelial-like cells. The latter with rather large, oval or round nuclei lie in groups and do not always have clearly outlined borders. Attention should be paid to the very delicate structure of the cytoplasm. The principal experiments were performed on cultures containing epithelial-like cells (Fig. 2, a).

Tissue culture of the kidney (Fig. 3, a) consists of fibroblast-like cells, creeping out from islands of growth. In our opinion, the latter represent aggregated cells in the suspension which have settled and adhered to the mica discs. In certain experiments a considerable portion of the culture consisted of epithelial-like cells (Fig. 3, b). We found no difference in the morphology of cultures grown from embryos of nine- and 16-days' incubation. Small eosinophilic, round inclusions, probably of a metabolic nature, were found in cells of all cultures used.

The dynamics of the development of cytopathic changes will be examined on tissue from 16-day embryos. The first, minor changes, connected with the action of the virus, appeared after 18 h post infection. Portions of the cytoplasm lying near the nucleus became eosinophilic and the nucleolus was observed to hypertrophy. Subsequently, at 24 h post infection, polymorphic, cytoplasmic eosinophilic inclusions appeared in the perinuclear zone, at first smaller and multiple, rounded (which is especially characteristic of lung tissue), then fewer, larger and varied in shape. At this time appeared the first small multinuclear cells, bearing up to ten nuclei. They appeared in all parts of the cellular layer (Fig. 2, b). At 48 h post infection with the virus the cytopathic effect involved a large portion of the cells, in many of which cytoplasmic vacuolization was pronounced, as had been observed also in cells which preserved a normal morphology. The number of cells containing inclusions increased (Fig. 3, b). At the end of the third day individual cells might be seen in the preparations, sometimes small islands of them and gigantic symplasts consisting of several hundred nuclei and often occupying the entire microscopic field. The nuclei in the symplast often stained poorly and looked "empty;" they were very variable in size. In certain symplasts fragmentation of the nuclei was observed; mitoses were not observed. In other multinuclear cells large cytoplasmic inclusions were visible. By 96 h post infection almost all the cells usually had fallen from the glass, whereas in control cultures were seen multilayered areas of morphologically unchanged cells.

Symplast formation was not noticed in cultures infected by small doses of the virus—100-1000 TCD_{50}/0.1 ml, whereas cytoplasmic inclusions were also found there.

In cultures made from nine-day embryos, the nature of the disease and the dynamics of cytopathic effect development were similar to those described, but were significantly advanced in time. The first cytoplasmic inclu-

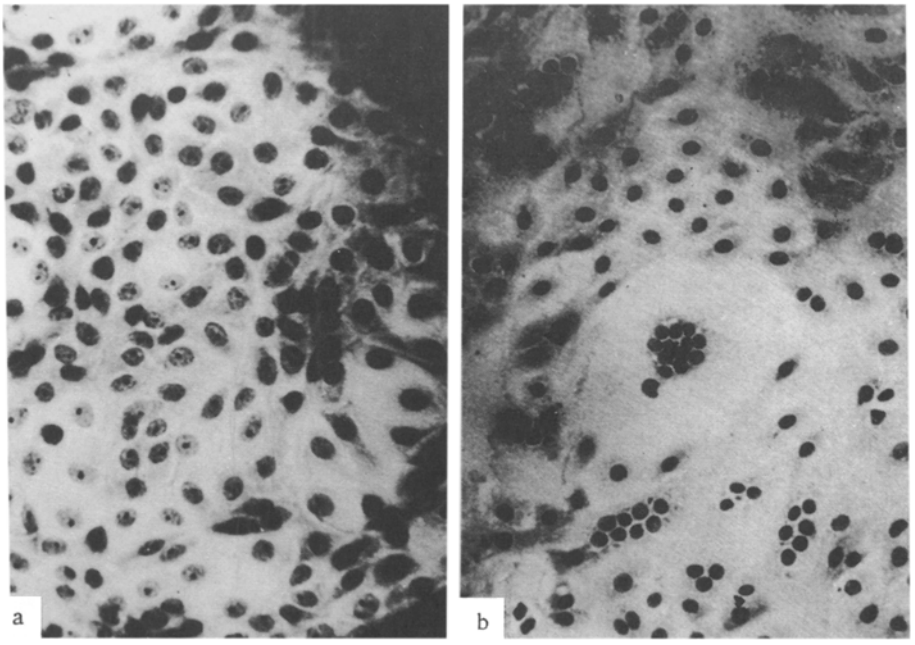

Fig. 2. Culture of lung tissue from 16-day chick embryo, fourth day of cultivation. a) Normal culture; b) 24 h after infection with virus, magnification 500 ×. Bouin's fixation. Hematoxylin-eosin.

sions were already observed four hours after inoculation with virus, and at six hours, giant symplasts were seen in all the tissues, particularly well developed in kidney tissue (Fig. 1, b).

In liver and kidney tissue from the nine-day embryo there was an increase during the first six hours post infection in the mitotic activity, which continued in the kidney tissue for 18 h, at which time as later on, mitoses were not detected in experimental cultures. On the contrary, in the control a decrease in the mitotic activity occurred only after seven to eight days of cultivation.

The dynamics of virus multiplication were studied on tissue culture of the lung from 16-day embryos. As a result of the study it was noted that the virus in low titer (100 TCD_{50}/ml) is present in the culture fluid already at 18 h post-infection and its concentration reaches a maximum (about $10^6 TCD_{50}$/ml) at 72 h.

Study of the cytopathic effect of Newcastle's disease virus on three embryonic tissues of different ages suggests the characteristic feature of the cellular reaction: the formation of eosinophilic cytoplasmic inclusions and of symplasts.

The cytopathic changes produced by the virus in tissue culture of embryonic tissue nine- and 16-days old are similar but they appear much earlier (by six hours) in the tissue from the nine-day embryo.

The capacity for Newcastle's disease virus to produce in cells of primary and established cultures eosinophilic, cytoplasmic inclusions and multinuclear cells, as previously described by other authors [1, 6-10, 12, 13] suggests that this virus acts with little specificity.

The symplast formation is another example of its poor specificity of action. It is characteristic of a whole variety of viruses: Herpes, measles, parotitis, (mumps), vaccinia, etc. The mechanism of symplast formation is not always the same [6, 11]. It may be by cytophagia, cytolosis or nuclear division without division of the cytoplasm.

In our experiments, together with the pictures of cell confluence we also noted nuclear fragmentation, suggesting that symplasts form in this instance by two paths: merger of cells after destruction of the cellular membrane and nuclear division without subsequent cytokinesis.

The phenomenon of increasing mitotic activity in cells one day post-infection has been observed before [2, 3, 5], and has been related by the authors to a defensive stimulation of cellular metabolism and to growth of the virus.

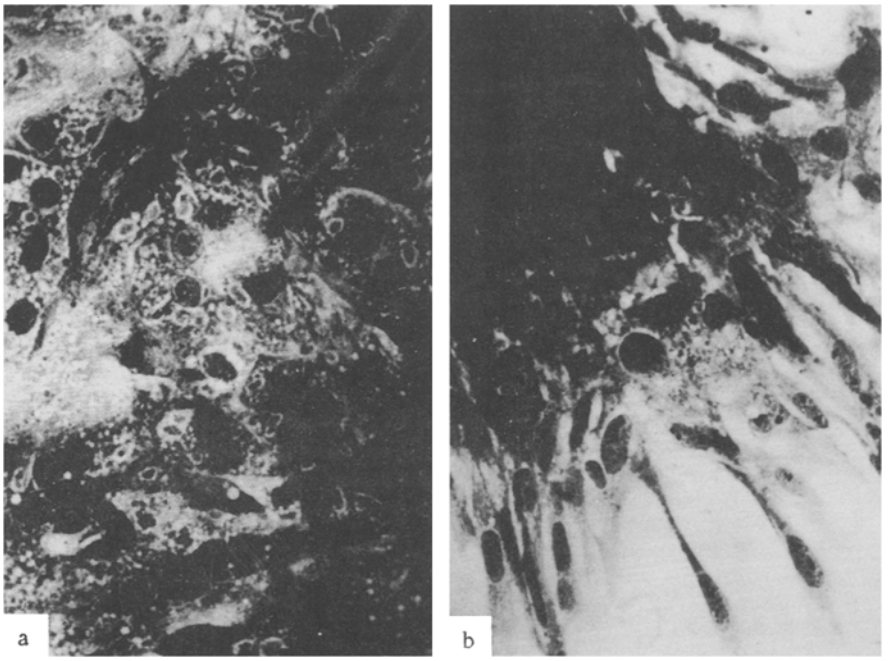

Fig. 3. Culture of kidney tissue from 16-day chick embryo, fourth day of cultivation. a) Normal culture; b) 48 h after infection with virus. Magnification 500×. Bouin's fixation. Hematoxylin-eosin.

The data obtained in this work, attesting to the single type of cellular reaction of embryonic cultures independent of tissue origin or age, indicates to a great degree the unification of cellular reaction to the effects of virus under in vitro conditions. However, for definite conclusions about the marked changes in the nature of the cell response to a given virus, further investigation of cytopathic changes in cells from different embryonic organs at different stages of development in vivo and in vitro will be required.

LITERATURE CITED

1. T. A. Bektemirov and I. M. Sokkar, Vopr. virusol., No. 3, (1963), p. 330.
2. S. Ya. Zalkind and L. G. Stepanova, Byull. exsper. biol., No. 12, (1960), p. 76.
3. S. Ya. Zalkind, K. S. Kulikova, N. V. Borisoglebskaya, et. al., Vopr. virusol., No. 5, (1962), p. 586.
4. N. N. Sokolov, M. I. Parfanovich, and L. B. Mekler, Acta virol. 7, (1963), p. 209.
5. F. V. Sushkov, In book: Morphology of Viral Cytopathic Effect [in Russian], Moscow (1963), p. 106.
6. J. André and G. Audebaud, Ann. Inst. Pasteur, 98, (1960), p. 829.
7. R. A. Bankowski and J. Hyde, Am. J. vet. Res., 18, (1957), p. 743.
8. C. D. Brandt, Virology, 5, (1958), p. 177.
9. R. M. Chanock, Proc. Soc. exp. Biol. (N. Y.), 89, (1955), p. 379.
10. C. Chany, et al., Ann. Inst. Pasteur, 95, (1958), p. 721.
11. E. J. Mason and N. Kaufman, Am. J. Path. 31, (1955), p. 883.
12. D. A. J. Tyrrell, J. Immunol., 74, (1955), p. 293.