METAMORPHOSIS OF HUMAN AMNION CELLS INDUCED BY PREPARATIONS OF INTERFERON*

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In the course of assaying interferons in primary cultures of human amnion (HA) cells, cellular morphologic changes were observed which were correlated with the activity of the interferon employed. To our knowledge, such alterations have not been previously described. Accordingly, a preliminary analysis of the phenomenon was undertaken, the results of which are presented in this communication.

Methods and Materials.—Cell culture: Preparation and maintenance of human amnion (HA) and human kidney (HK) cells have been previously described. Maintenance medium consisted of 5% inactivated horse serum, 5% beef embryonic extract, 45% bovine amniotic fluid, and 45% Hanks’ balanced salt solution. Rhesus monkey kidney cells were obtained from Microbiological Associates, Inc. Details of the preparation and maintenance of a continuous cell line (WS)† derived from human amnion cells have also been previously described.

Viruses: Relevant data on viruses employed are as follows:
- Sendai virus (F. Davenport) allantois of embryonated egg, 27 passages
- Sindbis virus (R. Taylor AR-339) HA cells, 10 passages
- Measles virus, Edmonston strain, adapted to HA cells, 35 passages
- Poliovirus II (MEF) chick embryo cell cultures 143 passages, HA cells, 2 passages

Preparation of interferon: Interferon was produced by HA cells infected with Sendai virus. Medium from infected cultures was harvested on day 3, centrifuged at 500 rpm to remove cells and then ultracentrifuged at 110,000 g for 2 hours at 4°C (Spinco L preparative centrifuge #40 rotor). Anti-Sendai rabbit serum§ in excess was added to the supernatant fluid which was stored at 4° or −20°C until use. Preparations of Sendai interferon were shown to be bacteriologically sterile. No infectious virus could be demonstrated after inoculation of undiluted material into the allantoic sac of 9–11-day-old embryonated eggs and incubation at 35–37°C for 72 hours.

Measles (HA cells) and Sindbis (WS cells) interferon preparations¶ were similarly ultracentrifuged and homologous antiserum added prior to use. The medium from cultures was removed 10 days after infection in the case of measles virus and 2 days in the case of Sindbis virus. Both preparations were shown to contain no infective virus.

Assay of interferon: Interferons were assayed in primary tube cultures of HA cells previously incubated in stationary racks at 35–37°C for 4 or more weeks. Twofold dilutions of Sendai or measles interferon (2–3 tubes/dilution) were incubated with cells for 18–24 hours prior to inoculation of 100 TCID₅₀ of Sindbis virus which was employed with these materials as the test or “challenge” agent. Complete destruction of cells in control cultures regularly occurred in 3 days. Interferon titer was expressed as the highest dilution which protected more than 75% of the cell sheet in at least 1 of 2 cultures for at least 3 days after complete destruction of cells had occurred in control cultures. Sindbis interferon was assayed in essentially the same manner. In this instance the challenge virus was Poliovirus Type 2, strain MEF₇.

Properties of interferon: The preparations of interferon employed were found to exhibit the
properties characteristic of this factor, i.e., sensitivity to trypsin, resistance to DNAase and RNAase; thermolability at 60°C for 1 hour; non-dialysability, and stability at a pH of 4. In addition they were not inactivated by antiserum against its producing virus and not sedimented at 110,000 g for 2 hours at 4°C.

Results.—Two to three days after the introduction of interferon (produced by HA cells infected with Sendai virus) into primary cultures of human amnion cells,

Fig. 1.—(a) Uninoculated human amnion cells. Hematoxylin and eosin stain. ×125. (b) Human amnion cells 3 days after introduction of Sendai interferon (200 interferon units) showing fusiform cells. H & E stain. ×125.

Fig. 2.—Human amnion cells 3 days after introduction of Sendai interferon demonstrating fibroblast-like cells; unstained. Mag. ×225.
many of the normally polygonal cells became fusiform and formed whorls of fibroblast-like cells (Figs. 1 and 2). These changes were often so marked that recognition of the original cell type was difficult. After fixation of the monolayer in Bouins' solution and staining with hematoxylin and eosin the cytoplasm of interferon-treated cells appeared retracted and a number of the nuclei more oval than in control cells (Fig. 3).

These changes were largely if not completely reversible. Cells resumed their normal appearance within 24 hours after removal of medium containing interferon, or spontaneously unless additional interferon was added. The extent and duration of these changes were proportional to the initial concentration of interferon, as determined by the viral inhibitory titer.

Though all primary cultures of human amnion cells to which interferon was added have shown this effect, with different lots of cultures some variation was noted in the rapidity, extent, and duration to which metamorphosis became manifest. In some experiments in which interferon was replaced when the medium was changed, the fibroblastic appearance lasted more than 1 month; in others the effect persisted for only 2–3 days. Variations were also observed which appeared to be attributable to the age of the cells. Older cells (1 month or more in culture) underwent metamorphosis more readily than younger cells.

Preparations of interferons produced in HA cells infected with measles and Sendai viruses, as well as WS cells infected with Sindbis virus, were all shown to be capable of inducing this effect in HA cells maintained in stationary racks or in a roller drum. In addition, interferon produced by suspensions of human leucocytes infected with Sendai virus also elicited these changes.

Metamorphosis did not occur if preparations of interferon were preincubated with crystalline trypsin (1 mg/ml, 37°C, 1 hour). Heating at 56°C for 1 hour, dialysis for 24 hours at a pH of 4, ultracentrifugation, and the addition of antiserum to the interferon-inducing virus did not destroy the metamorphogenic effect of interferon preparations. Tissue culture media in which interferon could not be
demonstrated had no effect. Although addition of interferon was regularly followed by spindle cell formation, similar changes were rarely observed in cultures of amnion cells without interferon, when the medium was not renewed, or in some instances when "toxic" medium was inadvertently employed.

The activity of an interferon preparation to inhibit cytopathic effect (CPE) after viral challenge was shown to bear a close relationship to its metamorphogenic capacity. Thus in one experiment (Table 1) it can be seen that the dilution end-

<table>
<thead>
<tr>
<th>Dilution of interferon</th>
<th>1:4</th>
<th>1:16</th>
<th>1:64</th>
<th>1:256</th>
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<tbody>
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<td>Interferon alone</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>O</td>
</tr>
<tr>
<td>Interferon and sindbis virus</td>
<td>●</td>
<td>●</td>
<td>●</td>
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<tr>
<td>Control cells no interferon or virus</td>
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<td>O</td>
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<tr>
<td>Virus alone</td>
<td>+</td>
<td>+</td>
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**TABLE 1**

MORPHOLOGIC ALTERATIONS IN HUMAN AMNION CELLS AFTER INCUBATION WITH SENDAI INTERFERON ALONE AND WITH SENDIBIS VIRUS (100 TCID<sub>50</sub>)

The same endpoint was obtained when the capacity of the interferon to prevent Sindbis CPE was assayed. In other experiments titration endpoints were not always equivalent but it was found that interferon preparations possessing a high viral inhibitory titer also exhibited a high morphogenic titer. In spite of this parallelism, however, cells which underwent spontaneous reversion to normal morphology after exposure to interferon continued to remain resistant for several days to challenge with Sindbis virus.

Interferons prepared in primary cultures of chick embryo (mumps virus)<sup>7</sup> and rhesus monkey kidney cells (Sendai virus)<sup>4</sup> were also incubated with HA cells. The former preparation had a titer of 1:32 in chick embryo cells and <1:2 in HA cells against challenge with Sindbis virus. It did not alter the shape of HA cells. The monkey kidney interferon had a titer of 1:512 as assayed in HA cells against Sindbis virus. Marked morphologic changes in HA cells were not observed but there did appear to be some cytoplasmic retraction and a few foci of fibroblast-like cells as compared to control cultures.

When Sendai interferon was incubated with primary cultures of human kidney or WS cells, morphologic changes were not observed. Human kidney cells are, however, often fusiform in appearance and slight alterations may have passed unnoticed.

It has been noted by Isaacs that the pH of the medium nourishing interferon-treated chick embryo fibroblasts was lower than that of untreated control cultures.<sup>8</sup> Similar differences in pH were observed by us in the media of interferon-treated human amnion cells and controls.

Isaacs and his colleagues have recently summarized the similarities between interferon and various substances capable of uncoupling oxidative phosphorylation.<sup>9</sup> Among these are Janus Green, sodium azide, and 2-4 dinitrophenol. When employed at concentrations suggested by Isaacs, these compounds proved toxic for HA
cells. Lower concentrations compatible with cell survival, i.e., $10^{-7}$ molar, $10^{-3}$ molar, and $10^{-6.3}$ molar respectively, induced no change in the normal appearance of the cells.

Discussion.—The association of the metamorphogenetic effect with the viral inhibitory effect of interferon preparations and the similarity of their various physicochemical properties strongly suggests that interferon itself and not an associated factor is responsible for these cellular changes. Furthermore their extent and duration have been shown to depend on the initial concentration of interferon.

Though in our experiments the titration endpoint of morphologic change appeared to approximate that of the viral inhibitory capacity (Sindbis virus), it is possible that had other challenge viruses been employed a different relationship would have been found. The minimal morphologic changes observed after incubation of rhesus monkey kidney cell interferon with HA cells, despite a high viral inhibitory titer in HA cells (1:512), suggests that these two activities (i.e., metamorphogenetic and viral inhibitory) are dissociable. The virus inhibitory activity of rhesus monkey cell interferon for cells of human origin has been previously reported by Sutton and Tyrrell. It must be emphasized that the results described were obtained with crude preparations of interferon. Experiments in the future with purified materials might show that the metamorphogenic factor is unrelated to interferon.

Ho has described prolonged cell survival associated with morphologic alterations in cultures of human amnion cells infected with the RMC strain of poliovirus. Though these changes bear a superficial similarity to those we have observed, the time of their appearance (30 or more days following inoculation of virus), their irreversibility, Ho's failure to induce similar changes with poliovirus interferon, and the association of the changes with viral infection are all differences that serve to distinguish these effects from those we have described.

Several other points appear worthy of comment. (1) Transformation to spindle cells has been observed in HA cells infected with measles and mumps viruses. The present observations raise the question whether this sort of cytopathic change, i.e., spindle cell transformation, may not in part be mediated by interferon, since it has been demonstrated that infection with these agents is followed by production of interferon in HA cultures. (2) In examination of materials suspected of containing a virus, the presence of interferon might mask viral CPE. Under such conditions the cell-transforming capacity of interferon might still be apparent. Such manifestations would obviously lead to further efforts to demonstrate the virus itself. (3) The suggestion has recently been made that interferon might be therapeutically employed in man. It would, therefore, seem of interest to determine whether cell metamorphosis induced by interferon or an associated factor may occur in vivo and if so to discover whether the change is accompanied by any manifestation of cellular dysfunction.

Summary.—Preparations of several different virus-free interferons induced marked morphologic changes in primary cultures of human amnion within 2–3 days. The usual polygonal configuration of the amnion cells became fibroblast-like and their regular arrangement was transformed into a pattern of interlacing whorls. The extent and duration of these effects depended on the initial concentration of interferon. The changes appeared to be reversible.
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§ Kindly provided by Dr. J. Lewis of the Sterling Winthrop Company.

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THE PATHS OF RAYS OF LIGHT IN GENERAL RELATIVITY OF THE NONSYMMETRIC FIELD \( V_4 \)

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In the first two editions of The Meaning of Relativity, the fundamental tensor \( g_{ij} \) is symmetric. In later editions, \( g_{ij} \) is nonsymmetric, and in the fourth edition (1953) on page 134, it is written in the form

\[
g_{ij} = g_{ij}^s + g_{ij}^s,
\]

where \( g_{ij}^s \) is the symmetric part and \( g_{ij}^s \) the skew-symmetric part of \( g_{ij} \). This equation and the type of notation are used in what follows.

Einstein in the fifth edition (1955) on page 92 characterized the paths of light by the equation

\[
ds^2 = 0,
\]
called minimal curves with the equation

\[
g_{ij} \frac{dx^i}{ds} \frac{dx^j}{ds} = 0,
\]

which in accordance with (1) becomes

\[
g_{ij} \frac{dx^i}{ds} \frac{dx^j}{ds} = 0.
\]