Cultures of Segments of Human Fetal Intestine: Applications to Cytologic and Virologic Investigations[1] (34887)

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In 1968 Folkman and French (1) described a method of culturing Peyer's patches in the intestine of germ-free adult rats. Segments of intestine containing Peyer's patches were everted over a glass rod and inserted into a special culture chamber. Under these conditions the cells comprising the Peyer's patches were maintained in a viable state for 14–16 days.

In certain human viral diseases proliferation of the etiologic agent occurs in the intestinal mucosa and in Peyer's patches. It was therefore considered that Folkman's technique of organ culture, if applied to human intestinal tissue, might offer unusually favorable conditions for the growth and isolation of such viruses as well as others, perhaps, that have not yet been propagated in the laboratory. Because of the absence of microorganisms and because of the abundance of lymphoid tissue, as documented by Cornes (2), fetal intestine was selected for this purpose. Organ cultures of human fetal intestine were established and the capacity of a variety of viruses to induce cytopathic changes in cells derived from this tissue was determined. Thus, it was found that, as a result of the proliferation and detachment of intestinal cells, monolayers developed on the walls of the vessel. Because of the ease with which cellular changes could be observed in this outgrowth, the cytopathogenicity of the viruses selected for examination was studied mainly in these monolayers. Viral effects on the cells comprising the original intestinal tissue were examined only indirectly by noting changes in the rate of monolayer formation or its failure to develop and by observing the presence and state of the cells which were shed from the segment into the medium. In a few cases conventional monolayer cultures prepared in test tubes with trypsinized suspensions of cells derived from the original monolayers were also employed.

Materials and methods. Primary organ cultures of human fetal intestine (POC). Fetal intestine obtained under aseptic conditions from therapeutic abortions performed at 12 to 16 weeks gestation was everted and then threaded onto a thin glass rod. The tissue was secured on the rod by a silk tie and was inserted into a modified Leighton tube containing 5 ml of Eagles' basic medium supplemented with 10% fetal calf serum (Fig. 1). The optimal length of the segment was found to be about 2 cm. Shorter segments tended to roll up and detach from the rod. Usually 20 such specimens could be obtained from one fetus. The preparations were incubated at 37°. Culture medium was withdrawn and replaced with fresh material on every fourth day. POC were observed under an inverted and dissecting microscope.

Secondary monolayer outgrowth (SMO). Cells from intestinal segments which grew out along the glass rod from the cut edges of the gut and which spread subsequently on the walls of the Leighton tube to form a monolayer are referred to as secondary monolayer outgrowth (SMO). SMO was enhanced when the rod was adjusted in such a way as to allow the lower surface of the intestinal segment to touch the glass.

Propagation of cells from secondary monolayer outgrowth. Cells comprising SMO were

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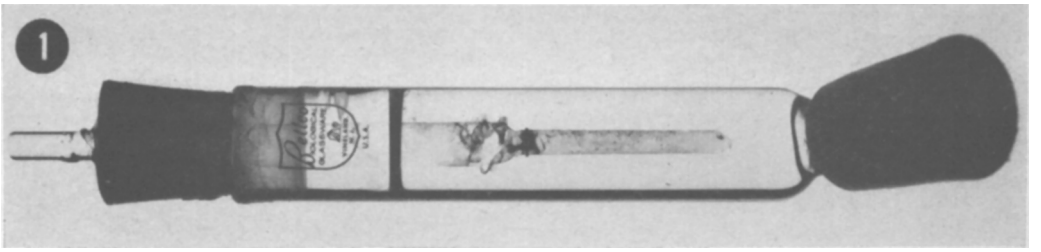

FIG. 1. Illustrates everted intestinal segment on a glass rod of the Leighton tube. The organ is immersed in Eagles' medium (×1).

further propagated according to the usual procedure for the preparation of monolayer tube cultures. After removal of the old medium, cells were washed three times with Puck's saline (3). Subsequently, 2.5 ml of Puck's trypsin (3) was added to each Leighton tube and the tubes were then incubated at 37° for 30 min. Any adherent cells were scraped off the walls with a metal scraper. Cells from each tube were resuspended in 5 ml of fresh Eagles' medium containing 10% fetal calf serum and 1 ml of this suspension was added to each of five 6-in. test tubes. Cultures were then placed in a slanting position and were incubated at 37°. Such preparations will be referred to as test tube monolayer cultures (TMC). Subsequent cell passages were conducted in the same manner, except that 0.5 ml of Puck's trypsin was employed to remove the cells from each tube which were used to prepare two daughter cultures.

Monolayer cultures (SMO and TMC) were stained with hematoxylin and eosin (H & E) following fixation for 24 hr in Bouin's reagent and embedding in collodion (4).

Lymphocytes from Peyer's patches. Numerous cells were found suspended in the media removed from POC. For examination, the cells were separated from the medium by centrifugation at 900 rpm for 5 min. Cover slip spreads were then prepared with the sediment, air-dried, and stained either with hematoxylin and eosin or Jenner's Giemsa. The effect of the addition of phytohemagglutinin on such cells was determined by adding this agent in the concentration of 200 μg/1 ml of medium to POC. Cells from POC with and without phytohemagglutinin were stained and examined at intervals of 3, 8, and 15 days after addition of the reagent which was not replenished when the medium was changed at these intervals. For the study of chromosomes in these cells, preparations were made according to the technique of Moorhead *et al.* (5).

Viruses. Six viral species, isolated originally from patients, were tested. Of these, five were in the form of suspensions which had been held at —60 to 65° in Enders' laboratory for varying periods as stock viruses. Relevant details in respect of these agents are summarized in Table I. To challenge the capacity of rubella virus to induce resistance in cultures of human intestinal cells, 10^3 ID_{50} of stock Sindbis virus was added to each preparation.

Inoculation of viruses. Aliquots (0.25 ml) of undiluted stock suspensions of five of the viruses were added to POC. Primary isolation

TABLE I. Viruses Tested for Cytopathogenicity in Human Intestinal Cells.

Virus	Strain	Source	Remarks
Measles	Edmonston	Blood	31st pass. hum. kid. cells
Herpes simplex	Rhodanus	Lung	5th pass. hum. am. cells
Adenovirus I	Kibrick	—	3rd pass. hum. am. cells in E's lab
Cytomegalo	AD 169-22A-Lang	—	Virus from D. Lang
Rubella	Bell	Throat?	Adapted to Syrian hamster cells
Varicella-zoster	Primary Isol.	Vesicle	Isolated in hum. intest. cells

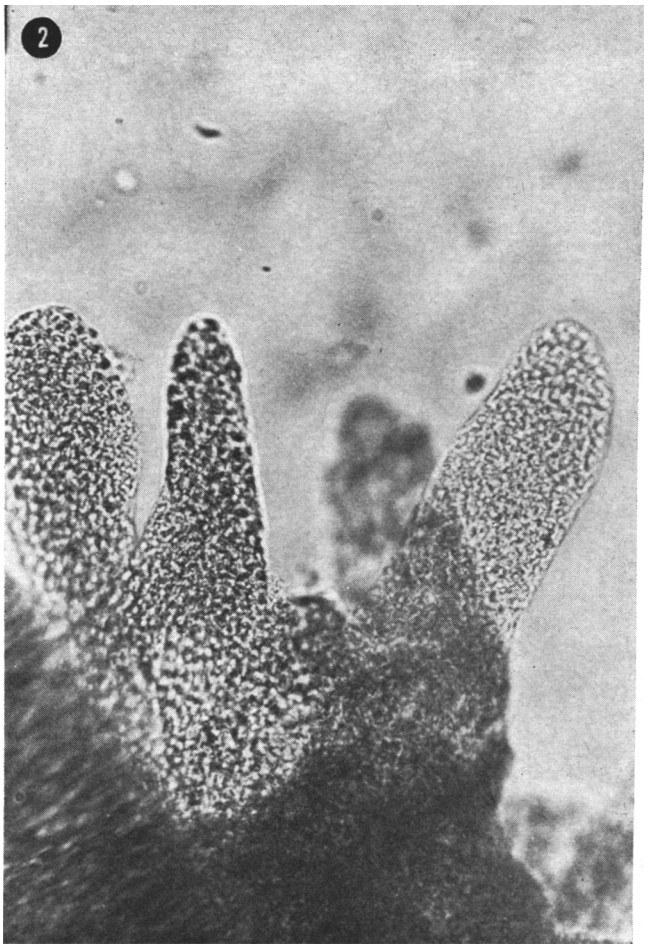

FIG. 2. Unstained intestinal villi as seen under the inverted microscope. Desquamated cells float in the medium (×600).

of varicella-zoster virus was accomplished in TMC. Fluid from a skin vesicle of a 19-year-old boy with Hodgkin's disease and disseminated herpes zoster was removed aseptically with a tuberculin syringe and added directly to the cultures.

Results. Characteristics of primary organ cultures and secondary monolayers. POC. The anatomical structure of the intestine appeared to be well preserved in POC during 14–21 days (Fig. 2). Villi and blood vessels were easily distinguishable under the inverted microscope. The red color of the intravascular blood, however, usually changed to a dark brown hue within 5 to 7 days after inauguration of the culture. Lymphoid tissues under the dissecting microscope appeared as fusiform prominences. The follicular arrangement of the Peyer's patches observed by Folkman in the cultured adult rat intestine was not seen. Progressive flattening and thinning of the intestinal segment which occurred as cultivation was extended was accompanied by shedding of dead cells into the media. Degeneration became complete when the tissue was no longer visible on the glass rod, usually by the end of the third or fourth week but sometimes even later.

SMO. Before the segments completely degenerated a new population of cells became apparent adjacent to the cut edges (Fig. 3). The outgrowth consisted of both fibroblastoid and epithelioid cells (Fig. 4). These cells spread rapidly along the glass rod and more

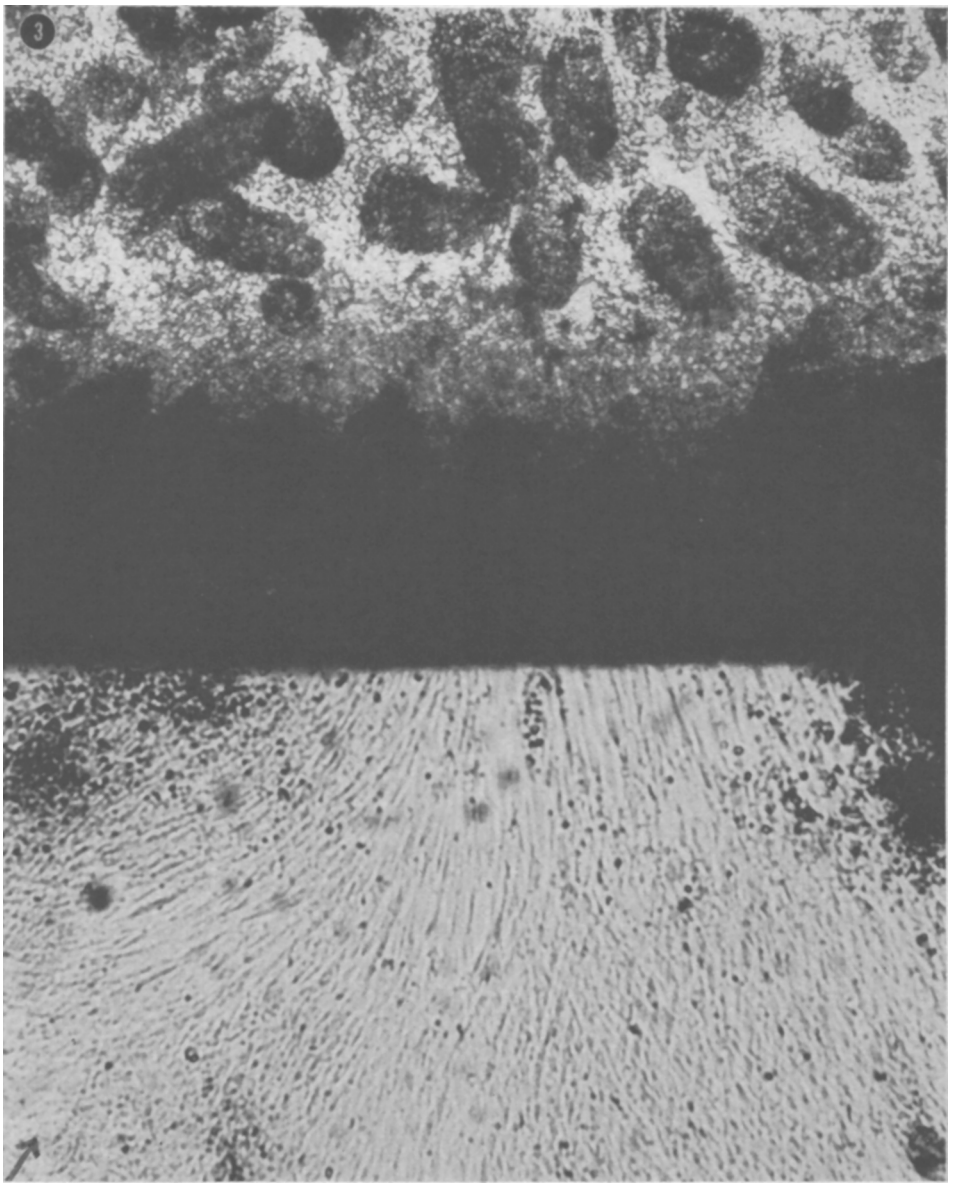

FIG. 3. Everted intestinal segment (top view) 6 weeks in culture. Note good preservation of villi in some areas. New fibroblastoid outgrowth spreading on the walls of the Leighton tube. Arrow indicates edge of a small area of epitheloid cells (×300).

slowly along the walls of the vessel. As a rule smooth monolayers were established at these sites within 7 to 14 days after the beginning of cell migration. The two kinds of morphologically distinguishable cells grew in adjacent patches and both remained in good condition for at least 3 months.

Cells from TMC were easily cultivated through 4 serial passages which were not carried further.

Characteristics of intestinal lymphocytes in culture. In contrast to the continuing proliferation of fibroblastoid and of epithelioid cells replication of lymphocytes did not occur in POC without phytohemagglutinin. Thus, in preparations without phytohemagglutinin when the number and condition of the lymphocyte content in the culture medium was assayed every fourth day during and after the degeneration of the lymphoid tissue in

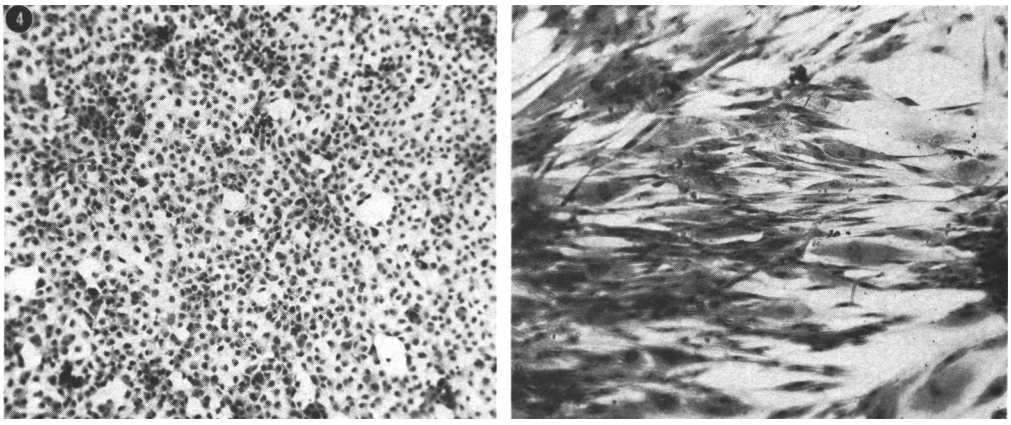

FIG. 4. Secondary monolayer outgrowth comprised of epithelioid (left) and fibroblastoid (right) cells (hematoxylin and eoxin stain × 250).

the segment, no normal appearing lymphocytes were detected at any time. However, when phytohemagglutinin was added to the medium of newly established POC, typical transformation of lymphocytes was observed in the culture fluid after 3 days of incubation (Fig. 5). Although no additional phytohemagglutinin was added, transformed cells were also present even after 8 days but were not found on the 15th day of incubation. Examination of chromosomal preparations of the transformed lymphocytic populations revealed numerous cells in metaphase.

Cytopathic effects of viruses. The effects of the 6 viruses, listed in Table I, on human intestinal cells were determined. In three cases (measles, adenovirus I, rubella) POC were exposed to the agents before as well as after the appearance of SMO and in two cases (herpes simplex and cytomegalo) only after SMO had developed. In the case of varicella-zoster virus, only TMC were exposed.

Measles virus. When this agent was added

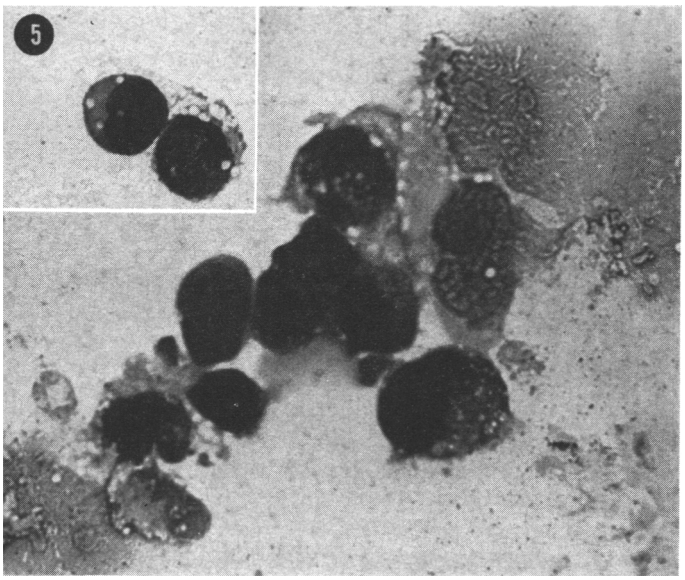

FIG. 5. Transformed lymphocytes emanating from the intestinal segment. Appearance 3 days after addition of phytohemagglutinin to primary organ culture (Jenner's Giemsa stain × 1100).

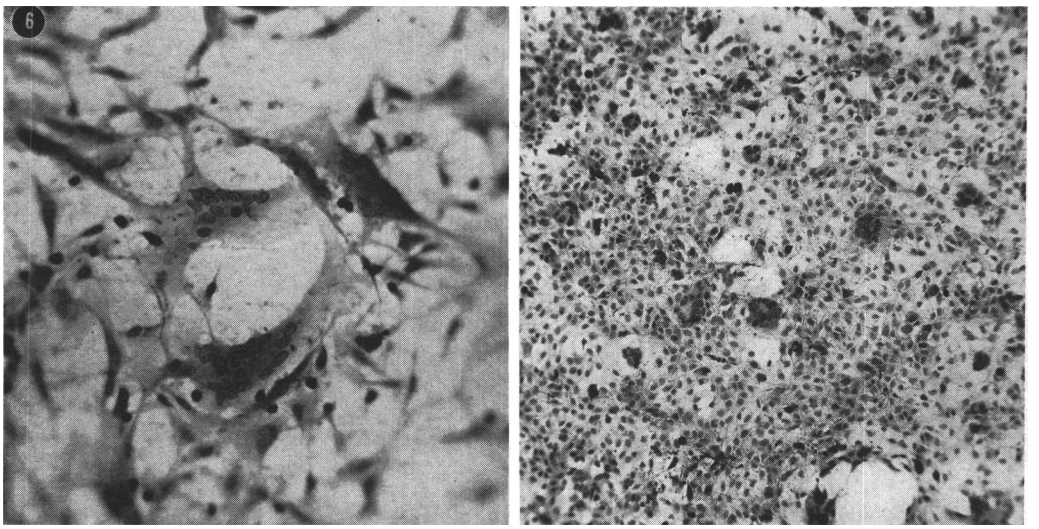

FIG. 6. Cytopathic effect of the measles virus on the intestinal cells. Scanty delayed syncytial outgrowth (1 month since onset of cell migration) following exposure of POC to the virus (left). Early giant cell formation (1 week) following exposure of fully established SMO. Typical eosinophilic intranuclear inclusion bodies are demonstrated by staining with hematoxylin and eosin (×250).

to newly established POC a marked delay in the development of secondary outgrowth was observed. Migration of cells from the segment occurred only after 1 month and the number of migrating cells was small as compared with that in uninoculated control preparations. Among this scanty population, however, syncytial formation characteristic of measles virus infection occurred. When stained, typical eosinophilic intranuclear inclusion bodies were seen within the syncytia as shown in Fig. 6. Such inclusions were also present in the nuclei of extrasyncytial, single fibroblasts. The lymphocytes present in two measles virus-infected POC were examined. In neither was there evidence of lymphoblastic transformation.

When POC in which SMO was well established were exposed to measles virus, syncytia were first seen in the monolayers after 7 days (Fig. 6). Discrete at first, the original syncytia became larger and others appeared. As a result of this spreading infection the entire monolayer was converted by the end of the third week into a continuous syncytium in which the presence of intranuclear inclusion bodies was demonstrated by staining with H and E.

Herpes simplex virus. In SMO this agent rapidly induced extensive cytopathic changes as shown by the rounding of numerous cells 24 hr after inoculation. Single mononuclear cells as well a typical multinuclear giant cells exhibiting characteristic intranuclear eosinophilic inclusion bodies were found in stained preparations.

Cytomegalo virus. Extensive rounding and heaping up of the cells was observed 5 days after this agent was added to well-developed SMO. When stained, eosinophilic intranuclear inclusions were present in many cells. Addition of fluid, removed from such cultures to fresh preparations of SMO, induced comparable cellular changes after 5 days.

Adenovirus I. In POC exposed to this agent, SMO, which developed normally in recently established uninoculated control cultures, failed completely. During the first 5 days of cultivation, cells which had become detached from the segment were found in the fluid. These contained dense lysophilic masses. Later only cellular debris was present in the fluid. Lymphoblastic transformation was not observed in the infected cultures. When added to fully established SMO this virus induced widespread characteristic cyto-

pathic changes by the fifth day.

Varicella-zoster virus. Aliquots of vesicle fluid (see section on technique) were added directly to TMC. After 13 days foci of morphological altered cells became apparent. In stained preparations typical eosinophilic intranuclear inclusions were seen. When crude fluid from such cultures was added to fresh TMC, these changes were reproduced.

Rubella virus. The addition of this agent to recently established POC was associated with a pronounced delay (4 weeks as compared with 7–14 days for the controls) in the development of SMO. In monolayers which eventually formed, the cells presented a lace-like pattern in which the edges of the interstices consisted of rounded cells. This appearance contrasted sharply with the uniform arrangement of unexposed SMO. In stained preparations, however, no intranuclear masses or cytoplamic inclusions were seen in the virus-exposed cells such as described by Neva *et al.* (6) in human amnion cells infected with rubella virus. Fluids from original infected cultures were added to TMC. On the 14th day thereafter areas of cells with increased granulation were seen. The cells in the TMC proved to be resistant to the cytopathic effect of Sindbis virus which rapidly destroyed uninoculated control preparations. The resistance of these cells provided evidence that replication of rubella virus occurred in this system.

Discussion. Folkman's technique of organ culture has been shown to be applicable to segments of human fetal intestine. Under the conditions of the present experiments the principal anatomical features of this tissue have been preserved for periods up to 3 to 4 weeks. During this period epithelioid and fibroblastoid cells emigrating from the segment form monolayers on the vessel wall and lymphocytic cells appear in the fluid phase. Accordingly, the system, which can be readily assembled in any well-equipped laboratory, provides a convenient means for the study of the effect of a variety of agents on the surviving human tissue as well as on some of its separate cell components. Such cultures have the advantage over certain other techniques that in their preparation the use of trypsin or other substances which might induce cellular changes is avoided.

In the present investigation it has been shown that a number of common viruses of man readily induce characteristic cytopathic effects in cells derived from the fetal intestine. Furthermore, it has been demonstrated that certain of these agents affect the cells comprising the intestinal segment itself, since delay or complete suppression of cell migration and the formation of monolayers occurs when newly established organ cultures are infected. It has also been found that the addition of phytohemagglutinin induces transformation in lymphocytes emanating from the segment of intestine. Taken together these observations suggest that the technique may prove useful in the isolation of viruses directly from the infected fetus as well as in the recognition of fetal genetic or physiologic abnormalities. Furthermore, it remains to be seen whether the procedure may be effective for the cultivation of hepatitis viruses or other agents that so far have defied efforts to propagate them *in vitro*. Potentially the system would also seem to be applicable to studies on the immunological response of human fetal lymphocytes to various antigens and to the capacity of these cells to synthesize antibodies.

Summary. Cultures of human fetal intestine have been established and their usefulness for the study of the cellular elements and for the propagation of viruses have been indicated.

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