Influence of Glutamine on Multiplication and Cytopathic Effect of Respiratory Syncytial Virus.* (31674)

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The cytopathic effect produced by respiratory syncytial (RS) virus in cell cultures is distinctly different from that of the other animal viruses. It is characterized by the formation of multinuclear giant cells, the syncytia. Thus, the name of RS virus, in part, is a description of its cytopathic effect in a culture system(1).

In a study of the growth characteristics of RS virus in cell cultures, Jordan noted that the composition of the medium may influence the formation of syncytia by the RS virus, but the specific component in the medium was not identified(2). Tyrrell reported that different Hep-2 cell lines may have different degree of susceptibility to RS virus(3). In our laboratory variable results have been obtained from time to time with respect to the cytopathic changes produced by RS virus in Hep-2 cells. The present report describes an observation of the influence of glutamine, an essential amino acid of Eagle's medium(4), on the multiplication and cytopathology of RS virus in Hep-2 cell cultures.

Cell cultures. Several sources of Hep-2 cell cultures were used. 1) A Hep-2 cell line carried in this laboratory for several years, originally obtained from Dr. A. B. Sabin, 2) a cell line obtained from Dr. F. L. Black who has carried the cell line for some time in the study of measles virus, and 3) a cell line recently purchased from Flow Laboratories (lot no. 08996).

Culture medium. Growth medium consisted of Eagle's minimal essential medium in Hanks' balanced salt solution(4) with 10% fetal calf serum and 2.9% glutamine. Maintenance medium (MM) consisted of Eagle's basal medium in Earle's balanced salt solution(5) with 3% fetal calf serum and 2.9% glutamine. Both media were purchased from Grand Island Biological Co. and were made without either calf serum or glutamine, both of which were added in appropriate concentrations just prior to usage.

Virus strain. The Simons strain of RS virus was obtained through the courtesy of Drs. H. V. Coats and R. H. Chanock. The virus stock was made from infected Hep-2 cells maintained in MM containing fetal calf serum and glutamine.

Virus assay. Infectivity titrations were made in tube cultures, 4 tubes per dilution. Virus infectivity was determined by the presence of multinucleated syncytial cells as observed under microscopic examination. The degree of CPE was scored as + or ++ depending upon the proportion of cells which became multinucleated. Advanced CPE, *i.e.*, ++++, indicated that multinucleated giant cells were involved in the complete cell sheet with some cellular degeneration. The end point was calculated by the method of Reed and Muench(6).

Stained preparations were also used. Hep-2 cells grown on coverslips were infected with RS virus. At different time intervals coverslips with infected cells were removed from culture tubes, fixed in Zenker's acetic solution and stained with hematoxylin-eosin(7).

Determination of virus growth. Semi-confluent Hep-2 tube cultures were washed twice with Hanks' balanced salt solution (BSS) and each was inoculated with 0.2 ml of RS virus suspension containing 4 log/TCD_{50}. After a period of 2 hours at 35°C to allow adsorption of the virus, unadsorbed virus was removed and the infected cultures were washed 3 times

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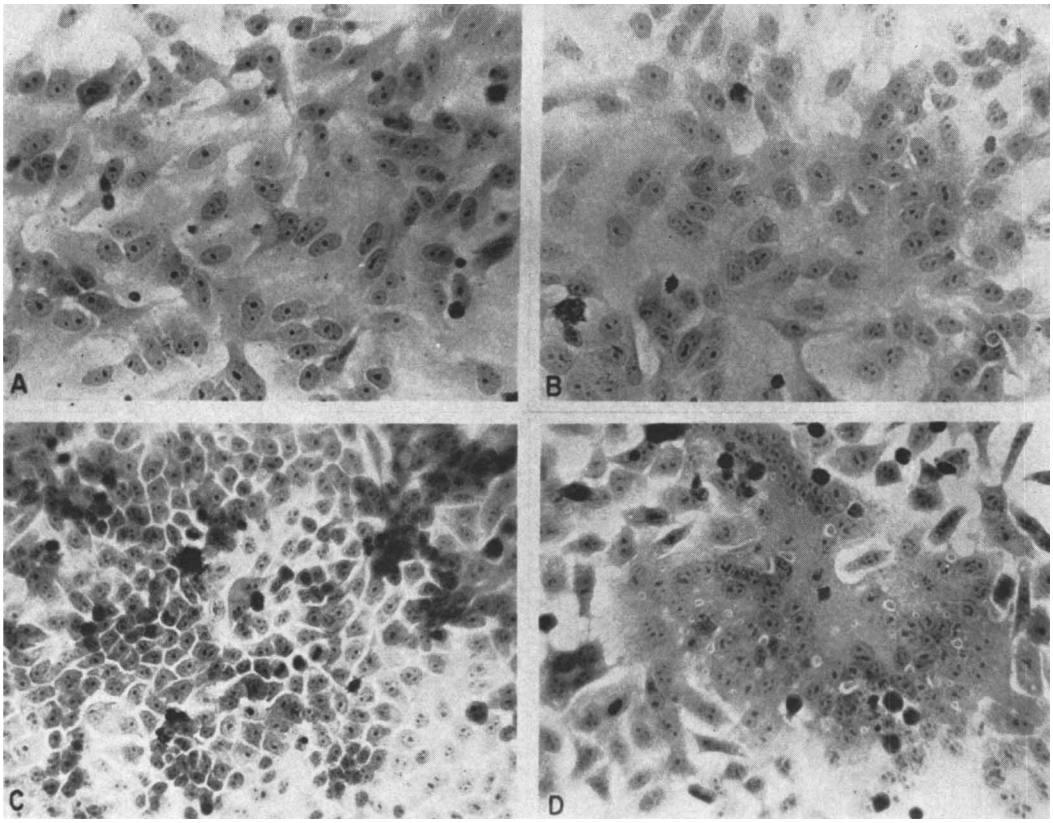
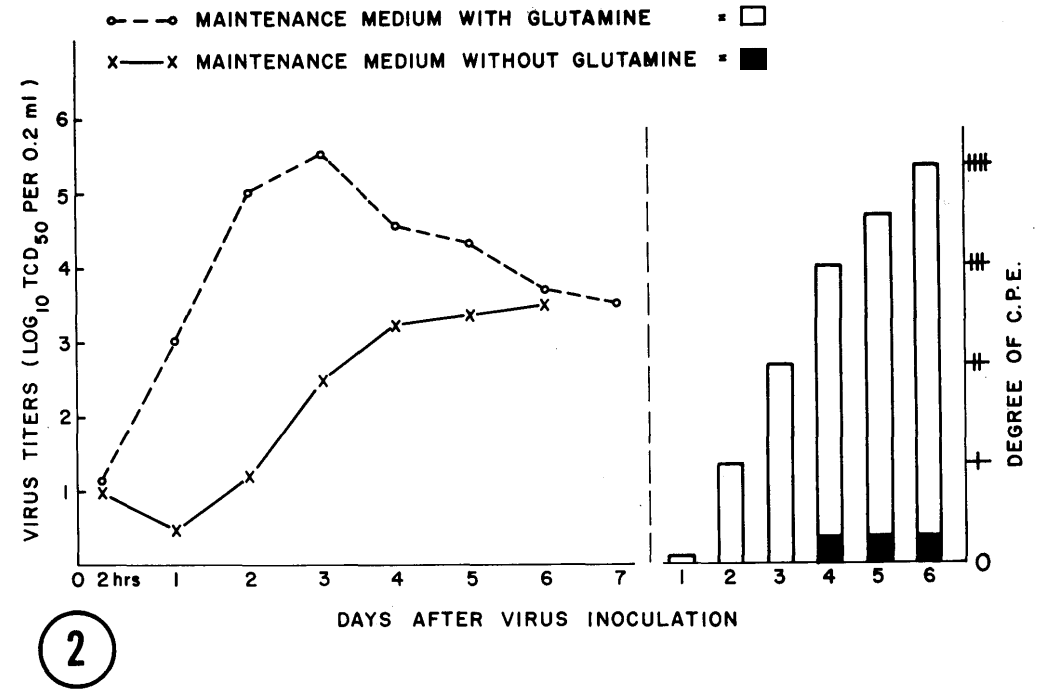


FIG. 1. Effect of glutamine on growth of Hep-2 cells and syncytial formation of RS virus. Preparations were fixed in Zenker's acetic acid solution and stained with hematoxylin-eosin. 200 ×. A. Hep-2 cells maintained in MM without glutamine for 4 days. Note thin cell sheet and absence of mitotic figures. B. Same as A but infected with RS virus for 4 days. Note absence of multinuclear giant cells. C. Same as A but maintained in MM containing glutamine, 2.9%. Note compact cell sheet and many mitotic figures. D. Same as C but infected with RS virus for 4 days. Note multinucleated giant cells.

with Hanks' BSS. Half of the infected culture tubes received complete MM, containing calf serum, with glutamine, while the other half received MM containing calf serum but without the addition of glutamine. Daily microscopic examinations were made and the degree of CPE was recorded. Two samples from each group of infected cultures were taken daily, frozen in a CO_2 alcohol bath, then stored at —70°C. Subsequently all samples were thawed and individually titrated in the same lot of Hep-2 cultures maintained in complete MM with glutamine and incubated at 35°C.

Results. Effects of glutamine on cellular changes and virus growth. As shown in Fig. 1-A, Hep-2 cells maintained in the MM without glutamine for 4 days showed no evidence of cellular division but maintained uniformly thin monolayers. Similarly, Hep-2 cells infected with RS virus showed no apparent changes when maintained in such a glutamine deficient medium (Fig. 1-B). On the other hand, when the same lot of Hep-2 cells was maintained in MM containing the standard concentration of glutamine, compact cell sheets with significant numbers of mitotic figures were noted (Fig. 1-C). Typical syncytial cells were observed in the infected cultures which were maintained in MM with glutamine (Fig. 1-D). The growth rate of RS virus in Hep-2 cells maintained in the two-culture media was then determined. The results are shown in Fig. 2. In addition to cellular changes, RS titers in cultures containing glutamine reached their maximum



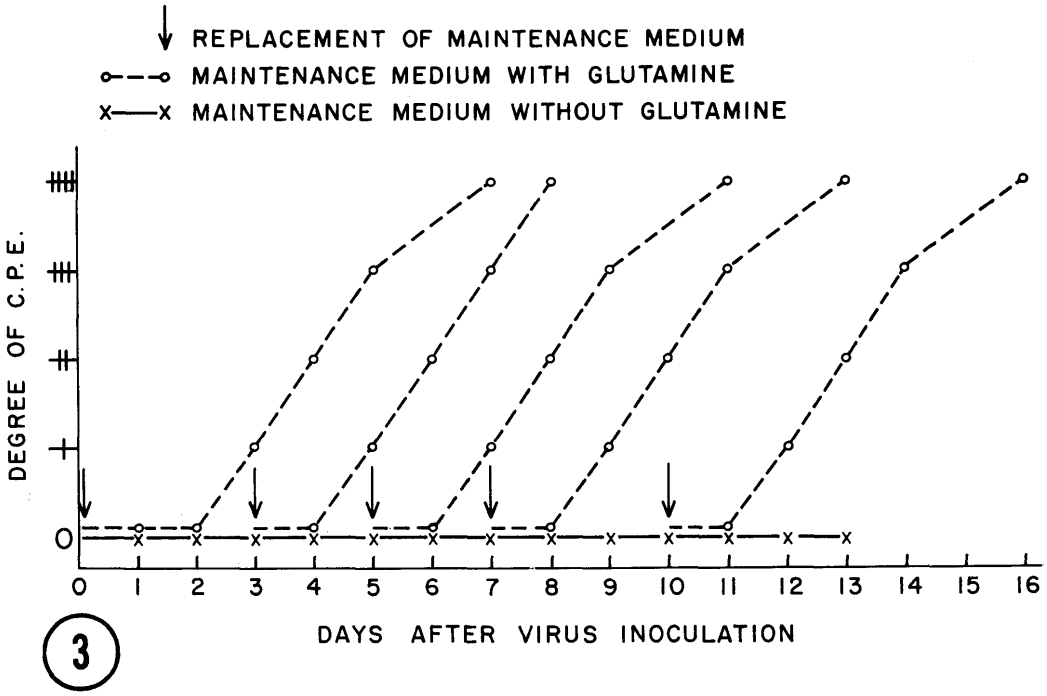


FIG. 2. Growth curves and cytopathic effect (CPE) of RS virus in maintenance medium with glutamine and without glutamine.

FIG. 3. Induction of RS virus CPE by replacement of MM without glutamine with MM containing glutamine. Replacements of fresh MM containing glutamine were made at points of arrows.

3 days after inoculation, and decreased slowly thereafter. Since these infected cultures were not replaced with fresh medium daily, inactivation of RS virus may have occurred at 35°C. Cytopathic changes were not observed in the infected cultures maintained in MM without glutamine but virus growth was confirmed. In the latter case the maximum titers were considerably lower than in the former, and were attained 2-3 days later. Apparently the yield of virus is directly related to the development of CPE.

Induction of RS virus CPE by addition of glutamine. In view of the fact that infected cultures maintained in glutamine free MM supported RS virus multiplication but showed no apparent CPE, it was of interest to determine whether the addition of glutamine into such infected culture would promote the appearance of CPE. The results of one representative experiment are illustrated in Fig. 3. Three days after infection, cellular changes were observed in the inoculated cultures maintained in the MM containing glutamine, but no obvious changes were detected in the same lot of infected cultures maintained in glutamine free MM. However, when the fluid of the latter cultures was replaced with MM containing glutamine, typical CPE appeared in 2 days. CPE was obtained not only in the cultures which had been maintained in the deficient medium for 2 to 3 days, but also for those in the glutamine free medium 5, 7, and 10 days (Fig. 3). In all instances, typical syncytia appeared consistently 2 days after the replacement of MM containing glutamine, regardless of the length of time that the infected cultures had been maintained in the deficient medium. Virus yields were not determined in these experiments.

Optimal amounts of glutamine required for production of CPE. A preliminary study of the optimum amount of glutamine required for RS virus to produce CPE was performed by determining RS virus infectivity in a series of Hep-2 cell cultures. Each series of cultures was maintained in a medium containing different concentrations of glutamine. As shown in Table I, 18 μg of glutamine per ml of medium appeared to be the minimum amount of this amino acid necessary for RS virus to produce syncytial cells. When the standard concentration of glutamine used in Eagle's medium, *i.e.*, 290 μg/ml, was diluted to 1:2 or 1:4 no obvious reduction in virus titers was noted, although delayed CPE was observed in the cultures maintained in the MM containing 72.5 μg/ml.

TABLE I. Infectivity Titration of RS Virus in Media Containing Different Concentrations of Glutamine.

Dilutions of glutamine stock solution	Conc of glutamine in medium, μg/ml	CPE first appeared (days*)	Virus titer log TCD_{50}/ml†
Undiluted stock	290.0	2	5.6
1:4	72.5	4	5.6
1:16	18.0	7	3.5
1:64	4.5	None	1.0
No glutamine	0	None	1.0

* Number of days post-inoculation of undiluted virus suspension containing log 4.6 TCD_{50}.

† Readings of titration were taken 10 days after inoculation, cultures were replaced intermittently with MM containing the respective concentrations of glutamine.

Discussion. At present, recognition of the presence of RS virus depends entirely upon the presence of characteristic cytopathic changes in cell cultures, the formation of syncytia. The present study indicates that these cellular changes are influenced by glutamine, one component of the MM. It would appear that the presence of RS virus in a clinical specimen could be overlooked if Hep-2 cultures were maintained in a glutamine deficient medium. Similar situations regarding nutritional requirements for virus multiplication have been observed with other viruses (8,9). In the case of influenza or measles virus, however, glutamine had an inhibitory effect on their multiplication(10,11).

It has been observed by Jordan that a factor or factors which were not present in Scherer's medium but were present in Eagle's medium promoted the syncytial formation in Hep-2 cell cultures by RS virus(2). In his experiments, however, the omission of glutamine from the Eagle's medium did not show any significant results. Since the present study has demonstrated that the amount of glutamine required for the appearance of syncytia was so small, 18 μg/ml, it probably would be

necessary to wash the test cultures thoroughly if a "glutamine free" experiment is anticipated. This is especially true if the cultures were grown or maintained in a medium containing glutamine prior to inoculation. Our findings, however, do not necessarily rule out the possibility that another factor or factors may be involved in the development of RS virus syncytia, especially when different cell lines are used.

Because of the instability of glutamine in the balanced salt solution, even at refrigerator temperature, glutamine was not generally included in the ready made commercially available Eagle's medium, or in the essential amino acids concentrates. However, precautions should be taken that the culture medium used contains appropriate amount of glutamine, since the deteriation of this amino acid may have occurred during storage.

Summary. Glutamine was found to be an essential component in Eagle's medium for the maximal multiplication of RS virus and for the development of syncytia cytopathic effect in Hep-2 cell cultures. The minimal amount of glutamine needed for syncytium formation was only 18 μg/ml. It is suggested that it be ascertained that the culture medium used contains glutamine when Hep-2 cell cultures are used for the cultivation and recognition of RS virus.

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Migration of Cells to the Thymus Demonstrated by Parabiosis.* (31675)

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Lymphocytes are known to recirculate between the lymph nodes, spleen and blood(1) but the thymus is generally considered not to be involved in this recirculation. Cell injection studies using labelled thymus and lymph node cells have shown cells to lodge only rarely in the thymus(2,3). However, the lymphoid population of thymus grafts is repopulated completely by host cells(4,5) and parabiotic studies suggest the occurrence of continuous repopulation of the lymphoid cortex of the thymus by blood-borne cells(6). Further, spleen and bone marrow cells are capable of repopulating the thymus after whole body irradiation(3).

In the present studies one partner of parabiotic pairs of mice was labeled with tritiated thymidine and the unlabeled partner monitored for the presence of labeled migrant cells, to determine the frequency of cell migration to the thymus and the location of migrant cells in the thymus.

Materials and methods. Pairs of inbred C57Bl or AKR strain mice, maintained in this Institute, were united in parabiosis using silk sutures between opposing scapulae and glutei and the skin wounds united with skin clips. One member of each pair was always a 2 months old animal and the other partner was

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