In vitro Effects of Guinea Pig Serum on the Jensen, JA-1 and JA-2 Sarcomas. (30082)

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The *in vivo* regression of certain mouse and rat tumors following injection of normal guinea pig serum (GPS) has been described (1-9). Some tumors of similar morphology, however, were insensitive. Recent evidence has indicated that L-asparaginase of GPS was responsible for the tumor regression effect(7-9).

Recently, Broome(8) found that if lymphoma 6C3HED cells showing *in vivo* sensitivity to GPS were cultivated in medium devoid of L-asparagine, the cells lost their *in vivo* sensitivity to the effects of the serum. Previously, McCoy *et al*(10) isolated 2 nutritional variants (JA-1 and JA-2) from cell cultures of the Jensen sarcoma exposed to media devoid of asparagine. Although the parent Jensen required an exogenous source of asparagine(11) for growth *in vitro*, the JA-1 and JA-2 cells did not. Further, this change was stable and heritable over hundreds of transplant and subculture generations.

In view of these factors, it seemed that the Jensen, JA-1, and JA-2 sarcomas could be used to investigate the action of GPS on cells as well as to develop a tool for further studies into the development of the JA tumors from the parent Jensen. The present report describes such a study.

Materials and methods. The stock tumors were carried as intramuscular transplants in female Holtzman rats. Six to 10 days after transplantation the animals were sacrificed, the tumors removed, and cell suspensions prepared for culturing by techniques previously described(12,13). The cells were established in T-15 flasks containing McCoy's basal Medium 5a(14) for 48 hours prior to introduction of the experimental media. A cell count at 48 hours was considered the "cell take." The medium was changed every 24 hours during the test period. At termination of an experiment, the medium was changed to remove any floating dead cells, and the remaining cells were released from the flask floor by chilling at 4°C. These cells were counted in a Model B Coulter Counter. The GPS was purchased from Pentex, Inc., Kankakee, Ill. Guinea pig asparaginase was purified and assayed according to the procedure of Meister(15) using the Chaney-Marbach (16) method to detect the ammonia released during the assay. A 3-fold increase of enzyme activity was achieved with removal of 82% of the serum protein. The preparation was dialyzed against distilled water at 4° prior to experimental use.

Results and discussion. The effect of 0, 0.01, 0.1, and 1.0% supplement of GPS to the basal medium on the growth of the Jensen sarcoma is shown in Fig. 1. It can be seen that GPS inhibited the growth of the Jensen cells at all levels tested and the degree of inhibition was essentially the same irrespective of the concentration of GPS. The actual loss of cells did not become pronounced, however, until they had been exposed to GPS for 2 or 3 days.

Since the JA-1 and JA-2 tumors did not require exogenous asparagine for growth, these cells and the parent Jensen were grown in the presence of 0.01% GPS. The results are shown in Fig. 2. While GPS has a marked inhibitory effect on the Jensen sarcoma, it appeared to be innocuous to the JA-1 and JA-2 cells. If the primary action of GPS was the destruction of asparagine and since the JA-1 and the JA-2 tumors have been shown to synthesize asparagine(17), then the inhibitory site of action of GPS appeared to be extracellular.

Replicate Jensen cell cultures were established for 48 hours and then exposed to GPS or partially purified asparaginase. The medium was changed daily and triplicate cultures harvested and counted every 24 hours for the first 10 days and every 48 hours thereafter (Fig. 3). During the first 4 days the cell population decreased and then became

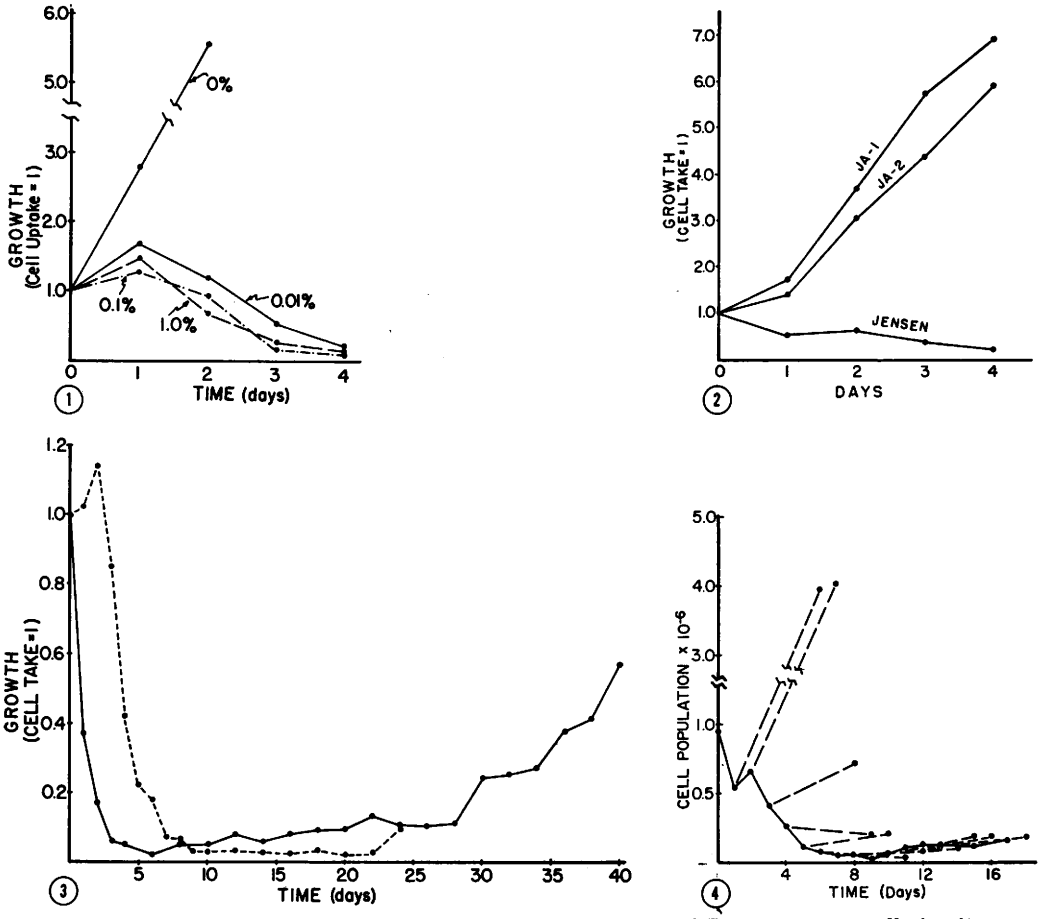


FIG. 1. Concentration effect of guinea pig serum on growth of Jensen sarcoma cells *in vitro*.

FIG. 2. Effect of 0.01% guinea pig serum on growth of Jensen, JA-1, and JA-2 tumor cells *in vitro*.

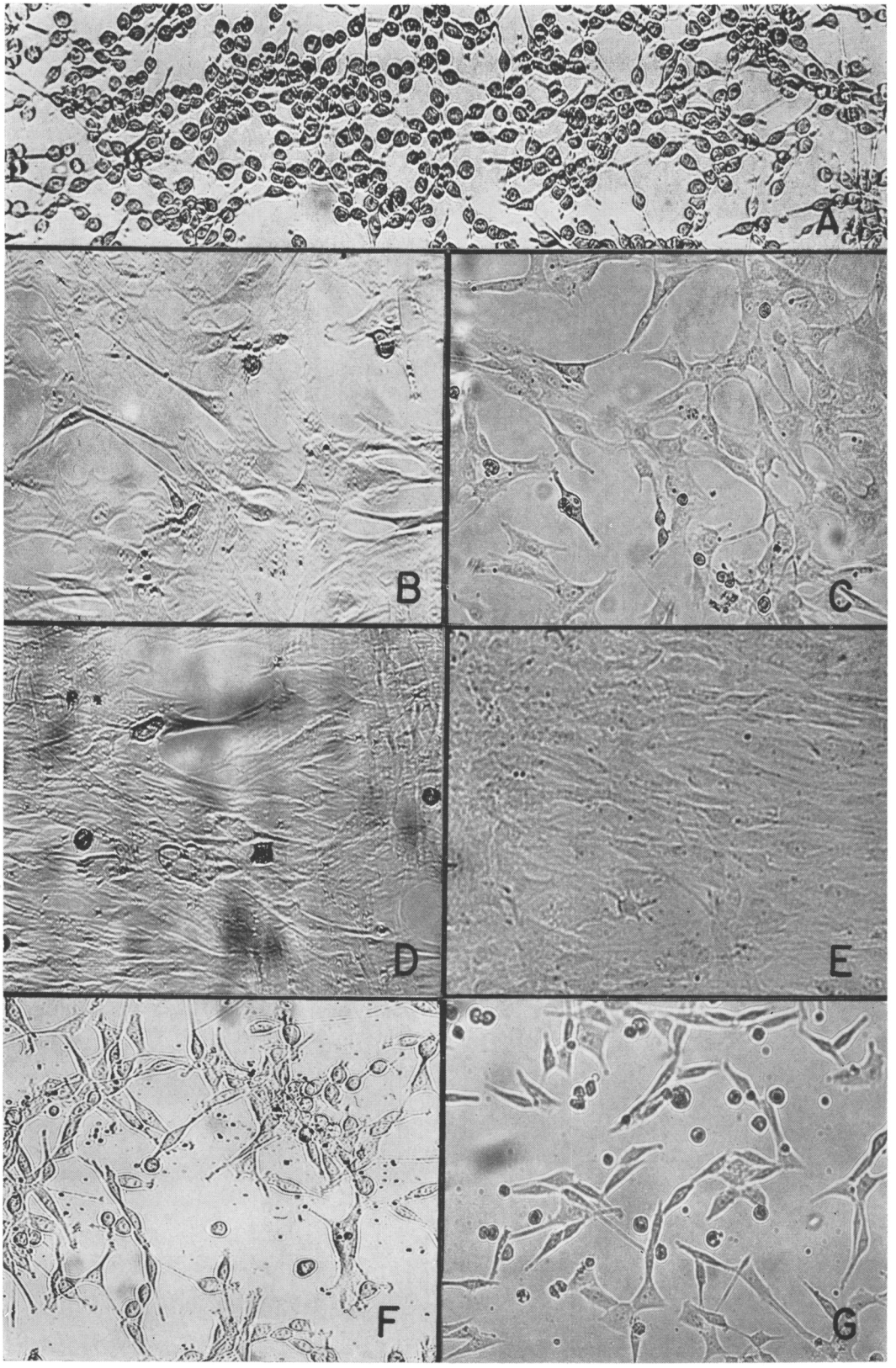
FIG. 3. Development of J_{gps} tumor by exposure of Jensen sarcoma to 0.01% guinea pig serum (solid line) or partially purified asparaginase (broken line). The asparaginase supplement to the media had an equivalent deamidase activity as 0.01% guinea pig serum.

FIG. 4. Recovery of Jensen sarcoma cells in complete media following exposure to 0.01% guinea pig serum at different time intervals. Solid circles denote population in presence of guinea pig serum; open circles denote population after 5 days on complete media.

more or less stationary. Eventually the cell population recovered and growth proceeded. The recovery of replicate cultures during the latter phase, however, was variable. For example, individual flasks harvested at any one time had as much as a 3-fold difference in cell populations. Cells exposed to the GPS for 8, 12, and 22 days were inoculated into animals and produced tumors. These tumors (J_{gps}) when excised and cultured showed no asparagine requirement after 44 subculture and 20 transplant generations and were insensitive to *in vitro* GPS effects.

Because there was a variation in the time at which the cultures started the rapid proliferation phase, an experiment was designed to study the randomness of this phenomenon. Replicate cultures of the Jensen tumor were

FIG. 5. Morphology of Jensen sarcoma cells during incubation in the absence of asparagine or the presence of 0.01% guinea pig serum. (A) Jensen sarcoma cells *in vitro*; (B) 5 days minus asparagine; (C) 5 days guinea pig serum; (D) 16 days minus asparagine; (E) 15 days guinea pig serum; (F) 33 days minus asparagine (JA-1); (G) 40 days guinea pig serum (J_{gps}).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exposed to the partially purified asparaginase and allowed to incubate until confluent populations were noted. The time for such growth to appear varied from 21 to 54 days with the majority of the flasks requiring 23 days incubation. Cells isolated at this time showed no asparagine requirement and gave rise to transplantable tumors following injection into the animal.

Since the response of the Jensen cells to GPS and partially purified asparaginase was essentially the same and JA-1, JA-2, and J_{gps} tumors were not affected by GPS or asparaginase, it appeared that the primary effect of GPS was the destruction of exogenous asparagine. It was of interest, therefore, to grow Jensen cells in the presence of GPS followed by removal of the GPS at different time intervals. Thereafter the cells were retained in fresh Medium 5a for 5 days (Fig. 4). Cultures exposed to GPS for 24 and 48 hours recovered very rapidly, but recovery was not noted when the cells remained in the presence of GPS for longer periods. Apparently the cells' metabolism was irreversibly altered after exposure to GPS for 72 hours. Similar results were obtained when the cells were exposed to the partially purified asparaginase.

A further comparison of the effect of medium devoid of asparagine or containing GPS was made on the morphology of the cells during growth. Fig. 5(a) shows the typical morphology of the Jensen cells grown in tissue culture. During the first several days in medium devoid of asparagine [Fig. 5(b)] or in the presence of GPS [Fig. 5(c)], the cells showed degeneration of the general population with some cells appearing as flat, nonrefractile bodies. These cells then formed colonies [minus asparagine, Fig. 5(f); GPS, Fig. 5(g)]. These cells showed a fusiform morphology which they maintained throughout the subsequent subculture and transplant generations.

The results obtained in these studies indicated that the active site of GPS was in the extracellular fluid surrounding the tumor cells and supports the supposition that the active component of GPS was asparaginase by virtue of the fact that (a) JA tumors that did not require exogenous asparagine were insensitive to GPS; (b) Jensen cells exposed to GPS for several days would produce tumors in the host, but these tumors no longer required asparagine for growth *in vitro* and were insensitive to the addition of GPS in the medium; and (c) Jensen cells grown in medium devoid of asparagine show growth and morphology similar to cells grown in the presence of GPS.

Summary. The *in vitro* growth response of the Jensen sarcoma and its nutritional variants (JA-1 and JA-2) to media containing normal guinea pig serum and partially purified asparaginase and media devoid of asparagine was studied. A comparison of the morphology of the cells grown in the presence of guinea pig serum and media devoid of asparagine showed a remarkable similarity. Further, the growth characteristics of cultures exposed to guinea pig serum and partially purified asparaginase were essentially the same. These results suggested the active component of guinea pig serum was asparaginase which catalyzed the destruction of extracellular asparagine.

The technical assistance of Mr. Gerald Orr in part of these studies is gratefully acknowledged.

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Received January 25, 1965. P.S.E.B.M., 1965, v119.

Inhibitory Effect of Mononucleotides on Virus Plaque Formation.* (30083)

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Ribonucleic acids obtained from foreign cells will often induce formation of interferon in cells treated with such preparations(2). It was the original intent of this study to determine the minimal molecular weight of ribonucleic acid preparations required to induce interferon. However, it was found that when yeast ribonucleic acid preparations were completely hydrolyzed, the resultant hydrolysates were more inhibitory to virus plaque formation than the original RNA preparations. This observation led to the present study. Recently, it has been shown that mononucleotides would induce small amounts of interferon in chick cell systems(3) and would also induce resistance to influenza virus in embryonated eggs or mice(4).

Materials and methods. Viruses. Vaccinia and chikungunya viruses were employed in the assays. Vaccinia virus was either the V-1 strain employed by Lindenmann and Gifford (5) or a similar strain(6), and was grown on the chorioallantoic membranes of 10- to 12-day-old chick embryos. Chikungunya virus was grown in the brains of newborn mice. Viruses were stored at —60°C in sealed glass capillaries or ampules.

Tissue cultures. Chick embryo tissue cultures were prepared essentially as described (5). Gey's, instead of Hanks' balanced salt solution was employed in both the growth and maintenance media. Proteose peptone, 0.1%, was routinely added to growth and maintenance media(6). For studies with mononucleotides, the yeast extract was omitted from the maintenance medium since it was thought to contain unknown amounts of these compounds. Omission of the yeast extract had no measurable effect on the plating efficiency of vaccinia virus nor on plaque size.

Virus assays. Vaccinia virus was assayed as previously reported(5) except for the slight change in medium composition and for a reduction in final volume from 2.5 to 2.0 ml. Chikungunya virus was assayed by plaque technique described by Ruiz-Gomez and Isaacs(7).

Compounds. Yeast nucleic acid was purchased from L. Light and Co., Ltd., Colnbrook, England. Mononucleotides were obtained from the same source or from the California Corporation for Biochemical Research, Los Angeles.

Results. Effect of yeast nucleic acid and alkaline hydrolyzed yeast nucleic acid on virus plaque formation. Yeast nucleic acid was hydrolyzed with 0.3 M potassium hydroxide for 18 hours at 37°C(8). It was then neutralized with 0.3 M hydrochloric acid and tested on chick cells to determine the effect on the plating efficiency of vaccinia and chikungunya viruses. Preparations were added with virus in the case of vaccinia virus assays and usually 1 hour before virus when chikungunya virus was employed. Chikungunya virus was allowed to adsorb for 1 hour before the agar overlay was added and the RNA or RNA hydrolysates were permitted to remain in the overlay. Yeast RNA preparations were usually dialyzed against balanced salt solutions before use, especially after the effect of mononucleotides became known. Results of one such experiment are

* Supported in part by USPHS Fellowship ESP-17932 while the author was at Nat. Inst. for Medical Research, London, England, and continued with support of U.S.P.H.S. Research Grant AI 04361.