Reports

Propagation of Poliovirus, Measles, and Vaccinia in Guinea Pig Spleen Cell Strains

Abstract. A new mammalian cell strain was established from guinea pig spleen tissues and has shown interesting virus sensitivity. Several viruses have been successfully propagated in the cells. For one of these, poliovirus, it represents one of the two successful attempts at propagating the virus in nonprimate or nonhuman cultures.

During the course of a study on the phagocytosis of *Mycobacterium tuberculosis* in tissue culture, an attempt was made to establish a cell line derived from guinea pig spleen. Reports on the successful primary cultivation of spleen tissue have been numerous; however, to our knowledge no report of a stable cell line has been recorded. Several reticular spleen cell lines (*1*) with demonstrated phagocytic activity have been established in these laboratories.

Several investigators have attempted to propagate poliovirus in nonprimate and nonhuman cells by utilizing both primary and cell line type cultures. With but one notable exception—Sheffield and Churcher (*2*), who successfully adapted all three strains of polioviruses (*3*) to the "transformed" embryo rabbit kidney cell line (ERK-1) of Westwood *et al.* (*4*)—no additional reports have been published.

In establishing these cell lines the spleen of guinea pigs was minced and smeared on the surface of two medicine bottles. These were incubated at 37°C for 30 minutes, following which medium 199 containing 10 percent horse serum and 100 units of penicillin and 100 μg of streptomycin per milliliter was added. After 5 days' incubation, at which time patches of mixed cell types were observed, the medium was changed. After 15 days the mixed cell masses had degenerated except for several patches of reticular-type cells which covered approximately one-third of the inoculated area. At this time the concentration of horse serum in the replacement medium was increased to 40 percent. After 29 days the culture was treated with trypsin, and the resulting cell suspension was used to seed two bottles. These cultures grew well, and additional serial transfers were made. During the early stages of growth the cells appeared reticular-like. The confluent cell sheets appeared to be similar to human amnion epithelial cell line cultures. Following the 38th passage the horse serum concentration was reduced to 5 percent. The GPS-1 (*1*) culture has been carried through 56 passages.

A second cell line from guinea pig spleen (*1*), GPS-2, and a cell line from embryonic guinea pig liver have been established in a similar manner. With these cultures 10 percent horse serum was utilized from the outset. After 25 and 10 transfers, respectively, no alteration in appearance has been noted, and there is no detectable difference in the morphology of GPS-2 from that shown by GPS-1.

Submerged cell cultures of GPS-1 have been prepared and maintained on medium 199 containing 5 percent horse serum, according to the method of Cherry and Hull (*5*) as modified by Simpson and Johnson (*6*). This modification eliminates the gassing step during the initiation and maintenance of cultures. Estimates of cell populations were made by a modification (*6*) of the method of Waymouth (*7*).

Fresh cultures of the GPS-1 cells were prepared by two methods: (i) from submerged cultures by direct "planting" into medicine bottles or roller tubes, and (ii) by trypsinizing medicine bottle cultures.

Roller tube cultures were inoculated with undiluted suspensions of the three types of polioviruses (*3*) and observed at 24-hour intervals. A degree of cytopathogenic effect typical of that seen in human amnion and in trypsinized monkey kidney cultures was observed with all three virus types in less than 24 hours. The cytopathogenic effect was complete in less than 48 hours. Serial passages of the three types of viruses were made into additional GPS cultures, and the typical cytopathogenic effect was observed for six serial passages. These results are considered to be a demonstration that polioviruses can be propagated in this cell line. The same criterion was used in determining the sensitivity of the GPS-1 to other viruses. All three strains of poliovirus propagated in GPS-1 were identified by quantitative neutralization tests in trypsinized monkey kidney cultures with standard poliovirus antisera.

Measles virus was also propagated in the GPS-1 cells in the manner described above. The cytopathogenic effect is much the same as that produced in human amnion cells. The effect can be described as masses of multinucleated cells with inclusions and syncitia.

Preliminary experiments indicate that measles virus reaches a somewhat higher titer in GPS-1 cells than in human amnion cells. In addition, measles virus produced in human amnion cells seems to attain a higher titer in GPS-1 cultures than in homologous human amnion cultures.

Vaccinia virus of calf lymph origin was also grown in GPS-1 cultures. The infected cells became enlarged, rounded, and opaque, and they appeared to contain inclusions. The distinctiveness of the cytopathogenic effect in GPS-1 cells is in direct contrast to that observed in primary rabbit kidney cultures. Preliminary experiments indicate that calf lymph vaccinia virus attains higher titers in GPS-1 than in trypsinized rabbit kidney cultures.

Comparative titrations of poliovirus (*3*) produced in trypsinized monkey kidney (TMK) cultures were carried out in both trypsinized monkey kidney and

Table 1. $TCID_{50}$ titers of polioviruses grown in trypsinized monkey kidney cultures assayed in trypsinized monkey kidney and GPS-1 cells.

Negative $\log_{10}$ $TCID_{50}$ titer					
Type I		Type II		Type III	
TMK	GPS-1	TMK	GPS-1	TMK	GPS-1
		Titration #1			
7.5	7.0	7.0	6.4	6.7	6.3
		Titration #2			
7.3	6.8	7.3	6.7	6.8	6.6
		Titration #3			
7.3	7.0	7.1	6.8	7.0	6.6

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

GPS-1 cultures. Table 1 shows the results of these titrations. The results obtained demonstrate that pools of poliovirus grown in trypsinized monkey kidney cultures attained $TCID_{50}$ titers, as calculated by the method of Reed and Muench (*8*), of 0.5 log less in GPS-1 cultures than they do in trypsinized monkey kidney cultures.

A pool of type I poliovirus, Mahoney strain, was prepared from GPS-1 cultures. The cytopathogenic effect for this culture was complete in less than 24 hours. This pool was titrated three times in both trypsinized monkey kidney and GPS-1 cells. On each occasion the titer was higher in the former than in the latter. The titers obtained in trypsinized monkey kidney cell assays were of the order of those obtained when poliovirus produced in trypsinized monkey kidney is titrated in the same cells. This indicates that infective virus particle concentrations are the same when pools are grown in either culture.

The cell strain derived from embryonic guinea pig liver was found to be susceptible to infection by poliovirus. This cell line, as well as GPS-2, is being investigated for virus sensitivity. Preliminary results show that polioviruses, measles, and vaccinia grow and produce cytopathogenic effects in GPS-2 which are identical with those in GPS-1.

Heavy suspensions of GPS-1 cells in complete medium have been inoculated with and without cortisone into adult and suckling guinea pigs by several routes to determine whether or not these cells have undergone transformation to a malignant state. No gross evidence of malignancy has been observed.

Since the first report in 1957 (*2*) of the susceptibility of the nonprimate cell line ERK-1 to poliovirus, there has been considerable controversy. Some critics of the work doubt that the cell is actually of rabbit origin. It is considered a primate cell line contaminant. Some support for this stand can be drawn from the work of Melnick and Habel (*9*), who found by complement-fixation techniques that the ERK-1 cell line possessed an antigen in common with HeLa and other primate cell lines. These latter findings, however, can also be used to support the theory that many cell lines possess a common antigen which was not present in primary cells.

Though primary cell cultures may be refractory to infection by poliovirus, it is possible for cell lines when transformed to become susceptible to infection by this virus. Since primary guinea pig tissues have been found to be refractory to infection and cell lines susceptible, it would seem that some change or transformation has occurred.

In our laboratories a guinea pig embryo liver and two guinea pig spleen cell lines, all susceptible to poliovirus, have been established by the techniques described. It seems very unlikely that all three cell lines are in reality primate cell line contaminants.

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References and Notes

1. Guinea pig spleen cell lines (Stanfield #1 and #2), referred to as GPS-1 and GPS-2.
2. F. W. Sheffield and G. Churcher, *Brit. J. Exptl. Pathol.* 38, 155 (1957).
3. Poliovirus types I, II, and III used in this study were Mahoney, MEF-1, and Saukett, respectively. Stock pools of virus were prepared in trypsinized monkey kidney cultures.
4. J. C. N. Westwood, I. A. MacPherson, D. H. J. Titmuss, *Brit. J. Extpl. Pathol.* 38, 138 (1957).
5. W. Cherry and R. N. Hull, *Anat. Record* 124, 483 (1956).
6. I. S. Johnson, *Ann. N.Y. Acad. Sci.*, in press.
7. C. Waymouth, *J. Natl. Cancer Inst.* 17, 305 (1956).
8. L. J. Reed and H. Muench, *Am. J. Hyg.* 27, 493 (1938).
9. J. L. Melnick and K. Habel, *Federation Proc.* 17, 526 (1958).

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Role of Magnesium in Acetyl Coenzyme A Formation by Acetothiokinase

In a recent discussion (*1*) of the role of magnesium in enzyme-catalyzed reactions involving adenosine triphosphate, Ingraham and Green postulated that magnesium acts by chelating with the substrates and the enzyme in such a way that the net effect is to alter the "entropy of activation" and thereby promote the reaction. Without considering the implications of this hypothesis in the general problem of metal ion catalysis in enzymic reactions, I should like to point out that the specific hypothesis concerning the role of magnesium in acetyl coenzyme A (CoA) (*2*) formation advanced by Ingraham and Green (*1*) is in contradiction to published experimental findings.

In analyzing the sequence of events leading to the formation of acetyl CoA from ATP, acetate, and CoA (see *1*, Fig. 2), Ingraham and Green proposed that a complex is first formed between CoA, Mg^{++}, and the enzyme and that this is followed by reactions with ATP and acetate. This results in the liberation of free inorganic pyrophosphate and in the formation of acetyl AMP, linked through magnesium to the enzyme. It was proposed that in the final step of the reaction there is a transfer of the acetyl group from the magnesium-bound acetyl AMP to the sulfhydryl group of CoA.

While it is clear that Mg^{++} is required for the over-all reaction, the series of reactions proposed by Ingraham and Green (*1*) leads to certain verifiable predictions. These are (i) that CoA is required for the isotope-exchange reaction between P^{32}-labeled inorganic pyrophosphate and ATP and (ii) that Mg^{++} is required for the formation of acetyl CoA from acetyl AMP and CoA. It has already been demonstrated that the formation of acetyl CoA from ATP, acetate, and CoA (reaction 3, below) actually occurs in at least two discrete steps (reactions 1 and 2).

$$\text{ATP} + \text{acetate} \overset{Mg^{++}}{\rightleftarrows} \text{acetyl AMP} + \text{PP} \quad (1)$$

$$\text{Acetyl AMP} + \text{CoA} \rightleftarrows \text{acetyl CoA} + \text{AMP} \quad (2)$$

$$\text{ATP} + \text{acetate} + \text{CoA} \overset{Mg^{++}}{\rightleftarrows} \text{acetyl CoA} + \text{AMP} + \text{PP} \quad (3)$$

Reaction 1, which is easily measured by the acetate-dependent exchange of PP^{32} and ATP or by the conversion of acetyl AMP to ATP, requires the presence of Mg^{++}. Not only is CoA not required for this reaction but if it is added the rate of exchange is decreased (*3*). Reaction 2, which is measured by the conversion of acetyl AMP to acetyl CoA, occurs in the absence of Mg^{++}. Neither the rate nor the extent of the reaction is affected by the addition of Mg^{++}.

That this is not due to the presence of Mg^{++} in the enzyme or reactants can be inferred from the requirement for added Mg^{++} when the over-all reaction (reaction 3) is studied (*3*). The most direct interpretation of these data is that Mg^{++} is involved only in the initial step of the reaction—namely, the reaction sequence involving ATP.

It is conceivable that these conclusions can be interpreted by proposing that exogenously supplied acetyl AMP is utilized by the enzyme at some unrelated transacylating site which does not require Mg^{++} (*4*). I feel that this is probably unlikely, since the presence of acetyl AMP excludes ATP from reacting with the enzyme. This is shown by the fact that if acetyl AMP is added to the complete system (ATP, acetate, CoA, Mg^{++}, and the enzyme), acetyl CoA synthesis continues but pyrophosphate formation is decreased to approximately one-tenth the rate found in the absence of acetyl AMP. When the added acetyl AMP is exhausted, the rate of PP formation is restored to the control level (*3*). This provides evidence that acetyl AMP competes for the site occupied by ATP. Were the acetyl AMP to be used at a second site on the enzyme, one would not expect an inhibition of the reaction in which ATP is utilized.