forces acting on the satellite are represented in the program completely and accurately, these elements will remain constant. Hence, any variations which appear are the result of other forces such as atmospheric drag, inaccurate or neglected gravitational terms, and the radiation pressure considered in the present note.

It appears surprising at first that the effect of solar radiation on the satellite orbit should be comparable to that produced by the gravitational attraction of the sun, since the gravitational force of the sun is larger than the force of solar radiation by a factor of 10⁵. However, the major part of the solar gravitational effect disappears because the earth undergoes approximately the same acceleration as the satellite; that is, the net effect of the geocentric solar gravitational force around a complete orbit is nearly zero. In the case of radiation pressure, the resultant acceleration of the earth is many orders of magnitude less than that of the satellite.

We have also applied this theory to the 100-foot balloon satellite which is planned for launching by the National Aeronautics and Space Administration as a communications experiment. This satellite represents a high ratio of area to mass, and is therefore one in which the effect of solar radiation pressure will be substantial. We find that for representative values of the orbit elements of the balloon satellite, solar radiation can in fact produce orbit perturbations on the order of hundreds of kilometers in a few months.

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References and Notes

- 1. P. Musen, paper on the influence of solar radiation pressure on the motion of an artificial satellite, J. Geophys. Research, in press.
- Calculations of the effect of solar radiation pressure have also been carried out by H. M. Jones and I. I. Shapiro of Lincoln Laboratory, Massachusetts Institute of Technology (private communication)
- 3. E. Upton, A. Bailie, P. Musen, Science 130, 1710 (1959).
- J. A. O'Keefe, A. Eckels, R. K. Squires, Astron. J. 64, 245 (1959).
- 5. Y. Kozai, "On the effects of the sun and moon upon the motion of a close earth satellite," *Smithsonian Astrophys. Observatory Spec. Rept. No.* 22 (1959).
- *Kept. No. 22* (1937).
 6. P. Musen, "Contributions to the theory of actallita arbite" COSPAR Meeting, Nice, satellite orbits," COSP France, 11-15 Jan. 1960. COSPAR Meeting,
- 2 March 1960

Virus Production by Subcultured Monkey-Kidney Cells

Abstract. Cultured monkey-kidney cells, like the rabbit cells previously studied, have a relatively high folic acid requirement for growth. Subcultures can be readily prepared if the medium is supplemented with a high level of the vitamin. The sensitivity of the resultant cells to poliovirus and the yields of virus obtainable with them are the same as with primary cultures.

Examination of primary cultures (1) from various rabbit organs revealed a common, relatively high folic acid requirement for growth, a property not shared with the permanently cultivable mammalian cells tested. Thus, with the primary cells, 10^{-3} to 2×10^{-3} µmole of folic acid per milliliter was needed to provide a maximum growth rate and yield, while a concentration about 1/100, or less, of the vitamin satisfied the cell lines (2). With high levels of folic acid, further cultivation of the primary rabbit cells became readily achievable for at least an additional six or eight doublings.

These observations suggested a possible application of some value to the virologist. While monkey-kidney cells cultured directly from the animal provide luxuriant growth, subcultures not initiated with extremely large inocula grow poorly, and they rarely, if ever, provide cells useful for virus studies. It was of interest, therefore, to determine whether the growth of subcultured monkey-kidney cells is enhanced by high levels of folic acid and whether the resultant cells can well support virus multiplication.

To test this, primary cultures of trypsin-dispersed monkey-kidney cells were prepared in a medium similar to that of Healy et al. (3), containing 15 percent of beef serum. Subcultures were prepared with populations which allowed about a sixfold increase in cell numbers, and growth and virus studies were made with the various cultures.

The growth response of the monkeykidney cells to a high level of folic acid was similar to that of the rabbit cultures. With no further supplementation of the medium $(1.4 \times 10^{-5} \mu \text{mole of})$ folate per milliliter), growth of the subcultured primary cells occurred initially with a doubling time of about 36 hours but ceased before a confluent sheet was made. Replacement of the medium with fresh medium did not stimulate further growth. On the other

Table 1. Poliovirus production in subcultures of monkey-kidney cells. Plating efficiency was estimated as plaque-forming units per mil-liliter of a single pool of Parker type 1 virus, by a procedure similar to that of Dulbecco and Vogt (4). Virus yield per cell was measured by inoculating cultures containing 5 million to 6 million cells with the Parker virus at a multiplicity of about 1. After 48 hours, the supernatant fluids were obtained by centrifugation, and the yields of plaque-forming units were determined (5).

Monkey-kidney culture	Plating efficiency	Virus yield per cell
Primary	4.2×107	450
First transfer	9.4×10^{7}	470
Second transfer	2.2×10^{7}	240

hand, in the same medium supplemented with folic acid (2 \times 10⁻⁸ μ mole/ ml), the doubling time during the exponential phase of growth was only 22 to 23 hours, confluent monolayers were formed, and these growth characteristics were maintained during the two additional transfers studied. Furthermore, the ability of the subcultures to support poliovirus multiplication was not detectably diminished (Table 1). Similar results were obtained with cells subcultured in a lactalbumin hydrolyzate-5 percent beef-serum medium enriched with a high level of the vitamin.

As had been anticipated from studies with rabbit cells (2), it was found that in a medium containing glycine, the folic acid requirement of the monkeykidney cells is completely replaceable by a mixture of thymidine and a purine (6).

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References and Notes

- 1. The term *primary culture* is used to describe the product of the initial cultivation of cells
- the product of the initial cultivation of cells obtained from the animal.
 2. I. Lieberman and P. Ove, unpublished.
 3. G. M. Healy, D. C. Fisher, R. C. Parker, *Proc. Soc. Exptl. Biol. Med.* 89, 71 (1955). The modified medium contained the amino acids (including glycine and the other "nonessential" ones), inorganic salts, glucose, reducing agents, vitamins, and antibiotics of the medium of Healy *et al.* For the growth of primary cultures, the medium was not supplementary folic acid. The ability of the primary cultures to multiply without supplementary folic acid is thought to result from the liberation of growth factors from the cell debris. the cell debris.
- 4. R. Dulbecco and M. Vogt, J. Exptl. Med. 99,
- K. Dubecco and M. Vogt, J. Expt. Med. 99, 167 (1954).
 J. S. Youngner, J. Immunol. 76, 50 (1956).
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- 3 November 1959