both liver and spleen of virus-infected animals and in liver of mice with CCl_4 poisoning.

1. De Ritis, F., Coltorti, M., Giusti, G., Science, 1956, v124, 32.
2. ———, J. Infect. Dis., 1957, v101, 219.
3. Giusti, G., Galanti, B., Mancini, A., De Ritis, F., Proc. Soc. Exp. Biol. and Med., 1964, v117, 610.
4. Dick, G. W. A., Niven, J. S. F., Gledhill, A. W., Brit. J. Exp. Pathol., 1956, v37, 90.
5. Piazza, M., Giusti, G., Piccinino, F., De Ritis, F., Riv. Ist. Sierot. Italiano, 1962, v37, 551.
6. Piazza, M., Piccinino, F., Matano, F., Nature, 1965, v205, 1034.
7. Mancini, A., Galanti, B., Giusti, G., Arch. Virusforsch., in press.
8. Coltorti, M., Budillon, G., Di Simone, A., Barbieri, A. M., Enzymol. Biol. Clin., 1965, v5, 14.
9. ———, Life Sciences, 1965, v4, 607.
10. Allfrey, V., Mirsky, A. E., J. Gen. Physiol., 1952, v36, 227.
11. McDonald, M. R., in Methods in Enzymology, Colowick, S. P., Kaplan, N. O., eds., Acad. Press, N. Y., 1955, v2, 437.
12. Laskowski, M. Sr., in The Enzymes, Boyer, P. D., Lardy, H., Myrback, K., eds., Acad. Press, N. Y., 1961, v5, 123.
13. Tonhazy, N. E., White, N. G., Umbreit, W. W., Arch. Biochem., 1950, v28, 36.
14. De Ritis, F., Coltorti, M., Giusti, G., Sanita. Publ., 1957, v18, 637.
15. Keir, H. M., Gold, E., Biochim. Biophys. Acta, 1963, v72, 263.
16. Armstrong, S. A., Niven, J. S. F., Nature, 1957, v180, 1337.
17. Svoboda, D. M. D., Nielson, A., Werder, A., Higginson, J., Am. J. Path., 1962, v41, 205.
18. Del Vecchio, B. C., Boll. Soc. It. Biol. Sper., 1964, v40, 1587.
19. Mallucci, L., Virology, 1965, v25, 30.

Received July 22, 1965. P.S.E.B.M., 1965, v120.

Characteristics of the Growth Cycles of Four Simian Enteroviruses (SV2, SV6, SV42, SV49).* (30666)

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During the course of our studies on the characteristics of simian enteroviruses it was found that they could be placed into 4 groups on the basis of their plaque morphology(1). Group A viruses produced hazy plaques with an indistinct border, 8-12 mm in diameter in 7 days; group B produced large, sharp plaques with islets of viable cells within their periphery, 10-20 mm in diameter; group C produced small, clear plaques with irregular borders, 5-8 mm in diameter; and group D produced large polio-like plaques, 20-25 mm in diameter. In order of their time of appearance, group D plaques were generally first, followed by B, A and lastly C.

While it was apparent that the plaque morphology was largely dependent on the type of cytopathology (CPE) observed in cell cultures under liquid media(1), we decided to study several aspects of the growth cycle of representatives of each plaque group to try to determine what role they might play in the size and rate of appearance of plaques. For this purpose rates of adsorption, penetration and multiplication in primary rhesus monkey kidney cell cultures (PMK) by SV6 (group A), SV42 (group B), SV2 (group C) and SV49 (group D) were determined.

Materials and methods. Viruses. SV2 was obtained from the American Type Culture Collection, Viral and Rickettsial Registry, and SV6 from Dr. R. N. Hull. SV42 and SV49 were isolated in this laboratory from rectal swabs of a cynomolgus monkey and a rhesus monkey, respectively(1). All 4 viruses were plaque purified in LLCMK2 cells and passed several times in PMK cells prior to their use in this study.

Tissue culture. One- and three-ounce bottle monolayer cultures of PMK cells were prepared as previously described(2).

Rate of adsorption of viruses to PMK cells. PMK monolayers in 3-ounce bottles

* This investigation was supported in whole by USPHS Research Grant AI-02535, from Inst. of Allergy and Infect. Dis.

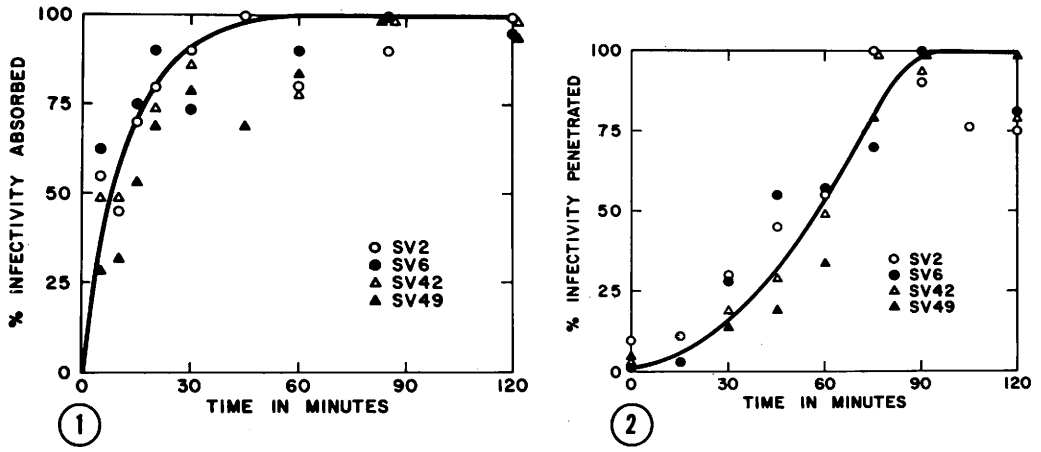

FIG. 1. Rate of adsorption of SV2, SV6, SV42 and SV49 to PMK cells.
FIG. 2. Rate of penetration of SV2, SV6, SV42 and SV49 into PMK cells.

drained of medium, were inoculated with 25-50 plaque forming units (PFU) of virus in 0.1 ml aliquots. The virus was allowed to adsorb at room temperature for varying lengths of time before washing off unadsorbed virus with PBS (pH 7.2-7.4). The monolayers were then overlaid with 10 ml of a modification of Hsiung and Melnick's agar medium (1) and, after solidification of the medium, incubated at 37°C. Plaques were counted daily for 1 week.

Rate of penetration of the viruses into PMK cells. Penetration of the viruses was determined by the rate of loss of sensitivity to light of virus grown in the presence of neutral red(3). Virus was grown in PMK cells with an overlay medium composed of Earle's saline containing 2% calf serum and 4 μg/ml neutral red. This virus was used to inoculate 3-ounce bottles containing PMK monolayers with 25-50 PFU. After adsorption at room temperature for 30 minutes, the bottles were incubated at 37°C for various periods of time when they were irradiated for 5 minutes at a distance of 8 cm from 3-15 W fluorescent lamps. They were then overlaid with 10 ml agar medium and incubated at 37°C for plaque count determination.

Rate of multiplication of viruses on PMK cells. Methods for determining the rate of multiplication have been described(2). Multiplicities of infection were between 5 and 10. Incubation temperature was 35°C.

Results. Rates of virus adsorption. Fig. 1 demonstrates that all 4 viruses adsorb to PMK cells at about the same rate, *i.e.*, 80-90% of the virus is adsorbed in ½ hour at room temperature. Approximately the same rate was observed at 37° for all viruses.

Rates of virus penetration. Fig. 2 demonstrates that rates of penetration of all 4 viruses are essentially the same, *i.e.*, penetration of the viruses as measured by resistance to photoinactivation is complete between 75 and 90 minutes.

Rates of virus multiplication. Fig. 3 illustrates growth curves obtained for the 4 viruses in a representative experiment. While these curves are representative of those obtained in repetitive experiments some slight variations have been noted. An eclipse period of 3 to 4 hours before infective intracellular virus appeared was noted with all 4 viruses. This intracellular viral synthesis was complete at the end of 6 to 7 hours. Cytopathology was noted at this time Extracellular virus infectivity generally began to appear in the medium between 7 and 8 hours or about the time intracellular virus was approaching a maximum. Extracellular virus reached a maximum between 16 and 24 hours. The only significant difference noted for the 4 viruses was that SV42 and SV48 extracellular virus generally exceeded the intracellular virus in titer between the 12th and 24th hour. SV6 and SV2 infectivity, on the other hand, remained mostly intracellular throughout the period of observation.

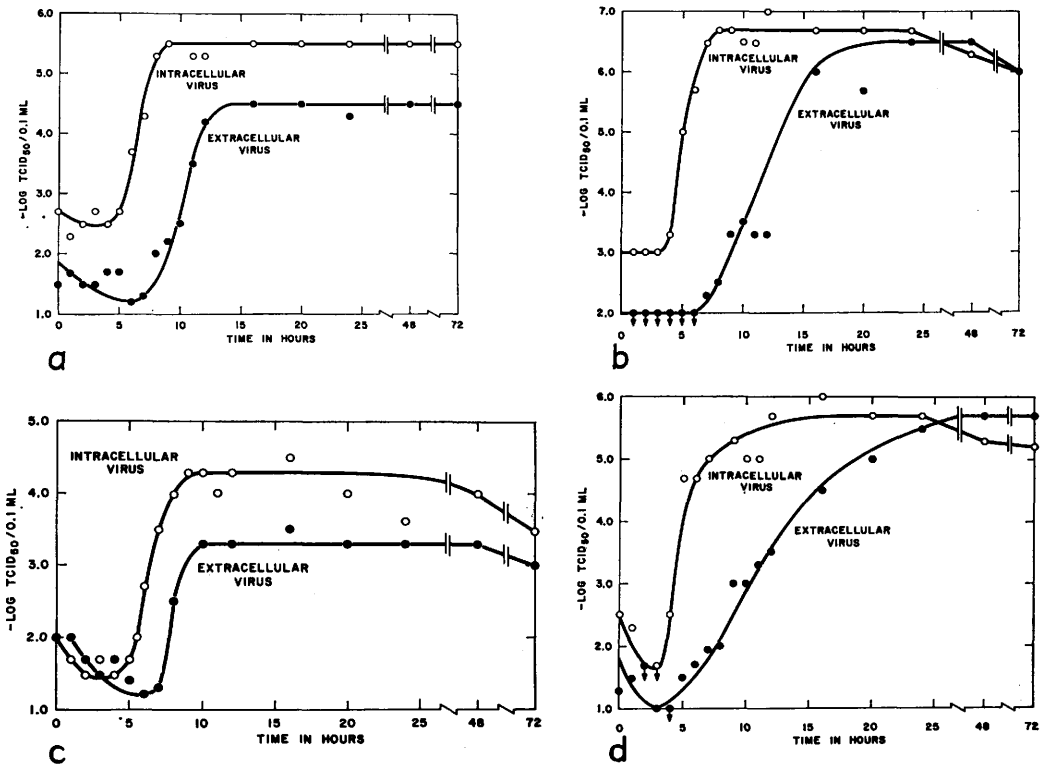

FIG. 3. Rates of extracellular and intracellular virus production in PMK cells. a. SV6, b. SV42, c. SV2 and d. SV49.

Virus yields were generally highest for SV42 (approximately 1000 PFU/cell) followed by SV49 and SV6 (100-500 PFU/cell). SV2 was lowest of all in yield per cell at 30-50 PFU/cell.

Effect of protamine on efficiency of plaquing. Because we were interested in determining the factors responsible for the plaque differences noted for the viruses under study, we measured the plaquing efficiency of Hsiung and Melnick's medium(4) with and without protamine. By this means the effect of agar inhibitor on plaque formation can be determined(5). Table I shows that only SV6 plaque formation was enhanced by the addition of protamine sulfate (salmine) to the overlay medium. In addition to numbers, SV6 plaque size was also increased by protamine.

Discussion. We have previously shown that the simian enteroviruses vary in the type CPE they produce in PMK cell cultures under liquid media and that this may be related to the appearance of plaques formed by these viruses, *i.e.*, group A viruses, including SV6, produce hazy plaques containing many viable cells and a CPE characterized by a few refractile cells on an almost confluent sheet of cells of normal appearance; group B (SV42) viruses produce large, sharp plaques which are clear except for a few patches of viable cells and a CPE which destroys all of the cell sheet except for small patches of cells; SV2 (group C) produces small, clear plaques and a CPE which slowly destroys all of the cells, and SV49 (group D) produces large,

TABLE I. Effect of Protamine on Plaquing Efficiency.

Virus	Overlay medium	
	Hsiung-Melnick	Hsiung-Melnick + protamine*
SV2	2×10^5	2×10^5
SV6	2×10^4	4×10^5
SV42	8×10^6	8×10^6
SV49	2×10^6	2×10^6

* 0.4 mg/ml protamine sulfate (salmine).

clear plaques and a CPE which rapidly destroys all of the cells(1).

It was not apparent from these observations, however, why plaques formed by SV2 and SV6 were smaller and slower in appearing than those of SV42 and SV49. The studies presented here show that all 4 viruses adsorb to, penetrate and multiply in PMK cells at approximately the same rate. The one difference noted was that SV2 and SV6 tended to remain intracellular while SV42 and SV49 were readily released into the medium. Therefore spread of infection from cell to cell in a plaquing system would be facilitated for the latter 2 viruses and it might be expected they would produce larger plaques.

A second difference noted was that SV6, SV42 and SV49 produced a considerably higher yield of virus per cell than did SV2.

Finally, it was found that SV6 was inhibited by the agar inhibitor in the overlay medium. Even with protamine in the medium, however, plaque size did not approach that of SV42 and SV49.

One further characteristic of these viruses might be noted. SV2 and SV42 are more heat labile than SV6 and SV49(1,6). This may explain why SV49 consistently produces larger plaques than SV42 even though the virus yield is higher for SV42.

In conclusion, it can be said that while gross morphology of the plaques formed by SV2, SV6, SV42 and SV49 is related to the type of CPE observed in cell cultures under liquid media, the plaque size is determined by a number of factors including virus yield per cell, ability of the virus to be released from the cell once synthesized, influence of factors such as protamine in the overlay medium and possibly thermal stability of the virus.

Summary. A study was made of the rate of adsorption, penetration and multiplication in PMK cell cultures by 4 simian enteroviruses which form different kinds of plaques. The effect of protamine on plaque formation by these viruses was also noted. The results obtained were used to explain differences observed in the size of plaques formed.

The authors wish to acknowledge the technical assistance of Miss Mary Ann Sides.

1. Heberling, R. L., Cheever, F. S., Am. J. Epidem., 1965, v81, 106.

2. ———, Proc. Soc. Exp. Biol. and Med., 1965, v118, 151.

3. Wilson, J. H., Cooper, P. D., Virology, 1963, v21, 135.

4. Hsiung, G. D., Melnick, J. L., J. Immunol., 1957, v78, 128.

5. Liebhaber, H., Takemoto, K. K., Virology, 1961, v14, 502.

6. Heberling, R. L., Cheever, F. S., Ann. N. Y. Acad. Sci., 1960, v85, 942.

Received July 22, 1965. P.S.E.B.M., 1965, v120.

Effect of Graded Levels of Insulin on Feed Consumption in Normal Female Rats.* (30667)

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The variation in the voluntary feed consumption of normal mature female rats of Sprague-Dawley-Rolfsmeyer strain has been observed(1). The mean feed intake of 265 g rats was 5.3 g/100 g body weight (bw) with a range from 2.9 to 7.5 g/100 g bw/day. While the neural regulation of feed intake has been studied extensively(2) the possible role of the endocrine glands and their hormones has been neglected. It is interesting to speculate as to the cause of the difference in feed consumption of individual animals. It has been suggested that the difference may be due, in part, to variation in the secretion rate of various hormones.

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2958. Approved by Director.

† Aided in part by a grant from USPHS.