during exsanguination of the donor animal.

Summary. Behavior of liver to changes in portal concentration of FFA was studied. Rapid infusion of serum containing large amounts of FFA into portal veins of dogs caused increased hepatic uptakes of this metabolite. Uptake of FFA was a function of portal concentration. Liver cells behave in a passive manner to fluctuations in serum FFA concentration, and local hepatic mechanisms are responsible for changing degree of FFA uptake.

1. Gordon, R. S., Jr., *J. Clin. Invest.*, 1957, v36, 810.

2. Spitzer, J. J., in *Blood Lipids and the Clearing Factor,* Brussels: Royal Flemish Acad., 1956, 243.

3. Roheim, P. S., Spitzer, J. J., *Am. J. Physiol.*, 1958, v195, 288.

4. Olson, R. E., Piatnek, D. A., *Ann. N. Y. Acad. Sci.*, 1959, v72, 466.

5. Balourdas, T. A., Spitzer, J. J., Croll, M. N., Scott, J. C., *Circulation,* 1959, v20, 981.

6. Bing, R. J., *Harvey Lectures,* Series L., 1954-55, 27.

7. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.

8. Gordon, R. S., Cherkes, A., *ibid.*, 1956, v35, 206.

9. Bierman, E. L., Dole, V. P., Roberts, T. N., *Diabetes,* 1957, v6, 475.

10. Roheim, P. S., Spitzer, J. J., *The Physiologist,* 1958, v1, 66.

11. Havel, R. J., Goldfien, A., *J. Lipid Res.*, 1956, v1, 102.

12. Bradley, S. E., Ingelfinger, F. J., Bradley, G. P., Curry, J. J., *J. Clin. Invest.*, 1945, v24, 890.

13. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.

14. Dole, V. P., *Chemistry of Lipids as Related to Atherosclerosis.* C. C. Thomas, Springfield, Ill. 1958, 189.

15. Bierman, E. L., Schwartz, I. L., Dole, V. P., *Am. J. Physiol.*, 1957, v191, 359.

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Growth of SE Polyoma Virus in Primary and Established Cell Cultures.* (25713)

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The SE polyoma virus multiplies *in vitro* with cytopathic effect (CPE) in primary tissue cultures from mouse embryo(1) and monkey kidney(2) and in an established cell line (P388 D_1) derived from a murine lymphoma(3), but fails to grow in rat embryo tissue cultures(4). This study reports results of experiments to determine if polyoma virus multiplies in other primary and established cell cultures to determine further the host range spectrum of this virus *in vitro*.

Materials and methods. The SE polyoma virus (strain 210-22) was obtained from Dr. Bernice Eddy and propagated in mouse embryo tissue cultures. Presence of virus was determined by infectivity titrations as described previously(1). Cultures were observed 3 weeks for CPE and fed at weekly intervals. $TCID_{50}$ end points were calculated according to method of Reed and Muench (5). Four different tissue culture systems were used: mouse embryo cells, L cells, rabbit embryo cells and HeLa cells. Tissue culture tubes of mouse embryo cells were prepared with trypsin-dispersed cells from primary cultures of 14- to 18-day-old mouse embryos by modification of trypsin digest method of Youngner(6). Eagle's medium(7) modified by using Hanks(8) BSS and 10% bovine fetal serum was used as growth medium. Medium consisting of 8 parts Eagle's medium with double concentration of amino acids and vitamins, one part tryptose phosphate broth and 2% horse serum was used as maintenance medium. L cells were maintained in Hanks BSS with lactalbumin hydrolysate 0.5%, yeast extract 0.01% (HLYE) and 2% horse serum. In one experiment, an additional set of cultures of L cells was maintained in syn-

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TABLE I. Growth of SE Polyoma Virus.

Tissue	Inoculum	Virus content 18 hr Fluids	18 hr 3rd washing	1 wk Fluids	1 wk 3rd washing	2 wk Fluids	2 wk 3rd washing	3 wk Fluids
Mouse embryo	$10^{4.5}$*	—	—	$10^{7.0}$	$10^{5.1}$	$10^{7.3}$		
L cells HLYE†	"	—	—	$10^{5.2}$	$10^{3.1}$	$10^{5.6}$	$10^{2.6}$	$10^{5.8}$
" " C‡	"	—	—	$10^{4.8}$	$10^{3.3}$	$10^{4.6}$	"	$10^{4.8}$
HeLa	"	—	—	$10^{3.8}$	$10^{2.1}$	$10^{3.4}$	$10^{1.6}$	$10^{2.3}$
Mouse embryo	$10^{5.3}$	$10^{4.5}$	$10^{4.5}$	$10^{7.5}$	$10^{6.9}$	$10^{8.0}$	$10^{6.5}$	$10^{7.0}$
L cells HLYE†	"	"	$10^{2.3}$	$10^{4.2}$	$10^{1.6}$	$10^{3.5}$	$<10^{1}$	$10^{3.7}$
Rabbit embryo	"	$10^{5.3}$	$10^{2.2}$	$10^{2.8}$	$<10^{1}$	$10^{1.2}$	"	$<10^{1}$
HeLa	"	$10^{4.2}$	$10^{2.3}$	$10^{2.5}$	10^{1}	"	"	"

* $TCID_{50}$. † Hanks' BSS lactalbumin yeast extract. ‡ Synthetic medium.
—, not done.

thetic medium(9) containing only amino acids and water-soluble vitamins. HeLa cells were maintained in HLYE with 2% horse serum. Rabbit embryo cells derived from primary culture of trypsinized whole rabbit embryo were maintained in the same media as mouse embryo cells. To determine capacity of different cells to support propagation of polyoma virus, 6 culture tubes of each cell system were inoculated with approximately 10^5 $TCID_{50}$ of the virus. Four tubes for each cell system served as controls. At daily intervals tissue cultures were observed microscopically for CPE. At weekly intervals fluids were removed, pooled and stored at −70°C for titration; each culture was washed 3 times with 2 ml of Hanks BSS and pooled fluids from third washing were taken for titration. In a second experiment, procedure was modified as follows. After infection of cultures, an 18-hour period of incubation was allowed for adsorption. Fluids were removed, pooled and stored for titration. Each tube was washed 3 times with 2 ml of BSS, and the fluid of third washing was pooled and stored for titration. Tubes were refed with proper medium and incubated at 37°C as before.

Results of experiments to determine ability of different cell systems to support virus growth are summarized in Table I.

In Exp. 2 it was evident that during the 18-hour period allowed for attachment, about 90% of the virus was adsorbed in all cell systems except rabbit embryo tissue, in which no decrease in virus titer of fluid was detectable. Active multiplication of the virus was evident in both experiments when mouse embryo tissue was used, the virus reaching titers as high as 10^8 $TCID_{50}$ ml. Multiplication of virus did not take place in HeLa and rabbit embryo cells. The moderate rise of virus in fluids bathing HeLa cells after washing with BSS in the first experiment appeared due probably to slow release into supernatant fluids of virus associated with the cells. In the first experiment with L cells, active multiplication of virus took place, since amount of virus in samples taken at any week was higher than that of inoculum. In the second experiment such a clear-cut rise of virus titer did not occur. However, the virus released into fluids at the end of each week was much greater than that seen with HeLa and rabbit cells.

Microscopic observation of both control and infected tubes of the cell systems revealed typical CPE(1) only in mouse embryo cell cultures. Rabbit embryo, HeLa and L cells showed no differences between infected and control cultures.

In the first experiment, CPE in mouse embryo cultures was complete at end of second week and the tubes were discarded. In the second experiment, cultures could be followed for the 3-week period.

Discussion. Results of experiments in which different cell systems were inoculated with polyoma virus illustrate a clear difference in their ability to support virus growth. Mouse embryo cells, as shown by Eddy *et al.* (1), are suitable for propagation of the agent. Rabbit embryo and HeLa cells do not support multiplication of the virus. L cells seem to support growth of polyoma virus but at a

slower rate than mouse embryo cells. Failure to reach a comparable rate of multiplication may be a characteristic of this cell line or could depend on nutritional conditions in our experiments, since fluids were changed only weekly and a low concentration of horse serum was used. Furthermore, media used for L cells contained less bicarbonate than that employed with mouse embryo cells, and it was recently reported(10) that virus yield is dependent on bicarbonate concentration of the medium.

Through analysis of host-virus relationships with polyoma virus so far studied, it appears that capability to induce tumors in animals is not necessarily correlated with ability to multiply *in vitro* in cells from the same animals nor with ability to produce CPE in the same cells. Moreover, a correlation does not exist between ability to multiply and CPE. For instance, polyoma virus induces tumors in mice(11) and multiplies in mouse cells *in vitro* with CPE(1). On the other hand, in rats(12) and rabbits(13) tumors are produced, but neither virus growth nor CPE takes place *in vitro*. In hamsters, tumors are induced(14), multiplication of virus takes place *in vitro,* but no CPE is detectable(15). Then, when multiplication *in vitro* occurs, it may result in killing of infected cells (CPE) or in production of virus without cell degeneration, as shown in our experiments with L cells and by Vogt and Dulbecco in hamster tissue cultures(15). Therefore, 3 different kinds of relationships between polyoma virus and cells exist: malignant transformation, multiplication with CPE, and virus production with survival of infected cells. Whether the type of response depends on predetermined and genetic characteristics of cells or on modifiable conditions is now under investigation.

Summary. Propagation of SE polyoma virus in 4 different cell systems *in vitro* has been studied. Mouse embryo cells support multiplication of this virus with CPE. Growth occurs to a lesser extent in L cells without CPE, while it does not take place in HeLa and rabbit cells.

1. Eddy, B. E., Stewart, S. E., Berkeley, W., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 848.

2. Stewart, S. E., Eddy, B. E., Gochenour, A. M., Borgese, N. G., Grubbs, G. E., *Virology*, 1957, v3, 380.

3. Rabson, A. S., Legallais, F. Y., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 229.

4. Kilham, L., Olivier, L. J., *Virology,* 1959, v7, 428.

5. Reed, L. J., Muench, H., *Am. J. Hyg.,* 1938, v27, 493.

6. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.

7. Eagle, H., *J. Exp. Med.,* 1955, v102, 595.

8. Hanks, J. H., Wallace, R. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 196.

9. Bader, J. P., Morgan, H. R., *J. Exp. Med.,* 1958, v108, 617.

10. Eddy, B. E., Stewart, S. E., *J. Publ. Health,* 1959, v49, 1486.

11. Stewart, S. E., Eddy, B. E., Borgese, N., *J. Nat. Cancer Inst.,* 1958, v20, 1223.

12. Eddy, B. E., Stewart, S. E., Stanton, M. F., Marcotte, J. M., *ibid.,* 1959, v22, 161.

13. Eddy, B. E., Stewart, S. E., Kirschstein, R. L., Young, R. D., *Nature,* 1959, v183, 766.

14. Eddy, B. E., Stewart, S. E., Young, R., Mider, G. B., *J. Nat. Cancer Inst.,* 1958, v20, 747.

15. Vogt, M., Dulbecco, R., in press.

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Biochemical Polymorphisms in Animals: Haptoglobins and Transferrins. (25714)

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Ford(1) has defined polymorphism as "the occurrence together in the same habitat of 2 or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation." Biochemical polymorphic traits of men