cumulation of spermine-like activity in culture filtrates of *B. anthracis*. However, the possibility has not been excluded that the active substance was initially present in the culture as an inactive carbamyl compound, which was hydrolyzed during removal of bicarbonate prior to testing. Reaction of putrescine with carbamyl phosphate has been demonstrated in the presence of yeast and mold enzymes(19). It is perhaps significant that arcain, the diguanidyl derivative of putrescine, was inactive in the present system; dicarbamyl derivatives of the active compounds have not yet been tested.

Summary. Spermine was active at a concentration of 3 μ moles per liter in allowing growth of a lysozyme-susceptible strain of *B. anthracis* in the presence of 1-4000 lysozyme. Spermidine, 1-3 propanediamine, putrescine, and cadaverine were respectively 1/2, 1/20, 1/40 and 1/200 as active as spermine. The activity of all of the substances was overcome by addition of 0.005*M* sodium bicarbonate to the medium.

1. Gladstone, G. P., Johnston, H. H., *Brit. J. Exp. Path.*, 1955, v36, 363.
2. Johnston, H. H., D. Phil. Thesis, Univ. of Oxford, England, 1958.
3. Thorne, C. B., In *Bacterial Anatomy*, Cambridge Univ. Press, England, 1956, p68.
4. Puziss, M., Wright, G. G., *J. Bact.*, 1959, v78, 137.
5. Dudley, H. W., Rosenheim, M. C., Rosenheim, O., *Biochem. J.*, 1924, v18, 1263.
6. Weaver, R. H., Herbst, E. J., *J. Biol. Chem.*, 1958, v231, 637.
7. Tabor, C. W., Rosenthal, W. M., *J. Pharmacol.*, 1956, v116, 139.
8. Rosenthal, S. M., Tabor, C. W., *ibid.*, 1956, v116, 131.
9. Herbst, E. J., Glinos, E. B., Amundsen, L. H., *J. Biol. Chem.*, 1955, v214, 175.
10. Martin, W. H., Pelczar, M. J., Hansen, P. A., *Science*, 1952, v116, 483.
11. Sneath, P. H. A., *Nature*, London, 1955, v175, 818.
12. Traub, A., Mager, J., Grossowicz, N., *J. Bact.*, 1955, v70, 60.
13. Kihara, H., Snell, E. E., *Proc. Nat. Acad. Sci.*, 1957, v43, 867.
14. Herbst, E. J., Weaver, R. H., Keister, D. L., *Arch. Biochem. Biophys.*, 1958, v75, 171.
15. Tabor, H., Tabor, C. W., Rosenthal, S. M., *Fed., Proc.*, 1958, v17, 320.
16. Kazin, S., Bachrach, U., Gery I., *Nature*, London, 1958, v181, 700.
17. Weaver, R. H., Herbst, E. J., *J. Biol. Chem.*, 1958, v231, 647.
18. Brumfitt, W., *Brit. J. Exp. Path.*, 1959, v40, 441.
19. Friedman, S., *Fed. Proc.*, 1957, v16, 183.

Received September 5, 1961 P.S.E.B.M., 1961, v108

Multiplication of Echo 9 Virus in Suspensions of Human Leucocytes.* (27053)

ROBERT B. BERG† (Introduced by John P. Fox)
Division of Epidemiology, Public Health Research Institute of the City of New York, Inc.

Isolation of ECHO virus type 9 from the serum of 10 of 18 children(1) indicates that viremia occurs commonly in human infection with this agent. In attempting to investigate a possible role of leucocytes during ECHO 9 viremia, experiments were performed to determine whether human leucocytes *in vitro* were capable of supporting multiplication of the virus. A previous publication(2) reported the multiplication *in vitro* of measles virus in human and simian leucocytes.

Materials and methods. Human blood cell suspensions were prepared by a modification of a previously described method(2). Venous blood was withdrawn from a healthy donor whose serum neutralized 100 TCD_{50} of ECHO 9 virus in 1:16 final dilution. Heparinized blood was centrifuged at 1500 RPM for 10 minutes. Buffy coat was washed 3× and finally resuspended in medium 199(3) containing 20% calf serum, penicillin and streptomycin. Final medium was adjusted to pH 7.4

* Supported by research grant from Nat. Inst. Health, U.S.P.H.S.

† Present address: Dept. of Pediatrics, Beth Israel Hospital, Boston, Mass.

with $NaHCO_3$. The final suspension, containing $5\text{-}14 \times 10^6$ nucleated cells/ml and an uncounted number of red cells, was delivered in 1 ml aliquots to 125×16 mm screw cap test tubes. *Virus.* ECHO 9 virus, Hill strain, was furnished by Dr. Herbert A. Wenner. A stock prepared in primary rhesus monkey kidney cell (MKC) cultures had an infectivity titer of 6.25 log $TCD_{50/0.1\ ml}$. *Viral Assay.* All infectivity titers were determined in MKC cultures, which were prepared and initially nourished in Hanks' balanced salt solution (BSS) containing 0.5% lactalbumin hydrolysate (LAH) and 2-10% calf serum. Prior to use cultures were refed with Eagle's basal medium (4,5) prepared with BSS. Penicillin, streptomycin and mycostatin were added to both media. Serial 10-fold dilutions of inoculated blood cell suspensions were prepared in BSS with 0.5% LAH and inoculated in 0.1 ml aliquots into 3 cultures per dilution. Cultures were kept stationary at 36-37°C, observed for cytopathic effect and discarded after 2 weeks. Titers were calculated by the Reed-Muench method and expressed as the log $TCD_{50/0.1\ ml}$ of inoculum.

Experimental. Propagation of ECHO 9 virus in the suspended blood cells was demonstrated in the following representative experiment (Table I). A 1 ml cell suspension inoculated with 0.1 ml of a 100-fold dilution of stock virus was incubated in a roller wheel at 35-36°C. At 2-3 day intervals 0.1 ml aliquots were transferred to fresh cell suspensions and at the same time the infectivity titer was determined for a similar aliquot. After 5 such subcultures, assuming no viral inactivation at 35-36°C over an 11 day period, total dilution alone would result in the extinction of non-multiplying virus in the original inoculum. However, at this stage the actual log infectivity titer was 4.25, demonstrating that viral propagation had occurred. It was then shown in 6 of 7 successive experiments that multiplication of virus occurred during single passage in blood cell suspensions. In contrast, neither medium 199 plus 20% calf serum without leucocytes, rapidly frozen and thawed leucocytes nor suspensions comprising only red blood cells supported ECHO 9 multiplication. This emphasizes the dependence of viral multiplication on the presence of intact leucocytes. In similar experiments the virus failed to multiply in rabbit blood cell suspensions during single or multiple passages.

TABLE I. Infectivity Titers of ECHO 9 Virus during 5 Serial Passages in Human Blood Cell Suspensions over an 11 Day Period.

Passage Level	Day of Exp.	Max. Infectivity Titers of Suspensions (log $TCD_{50/0.1\ ml}$) Estimated*	Observed
0	0	3.25	—
1	2	2.25	1.25
2	4	1.25	2.25
3	7	.25	>2.25†
4	9	< .25	3.50
5	11	< .25	4.25

* Estimated titers, in absence of viral multiplication, are based on dilution and the assumption that there is no loss due to thermal inactivation. Zero day titer is the calculated infectivity titer of the original suspension immediately after inoculation with diluted stock virus. Titer of stock virus was 6.25 log $TCD_{50/0.1\ ml}$. All titers were determined in MKC cultures.

† Endpoint not reached at final dilution tested.

Experiments were performed to determine from which fraction of the blood cell suspension virus was recoverable. An inoculated suspension was centrifuged after 3 days incubation. Cell-free supernatant fluid yielded approximately 100-fold less infective virus than did whole blood cell suspensions when simultaneous assays were performed in MKC cultures. It was not determined whether virus associated with cells was primarily intracellular or surface attached.

After 2-3 days incubation neither infected nor non-infected leucocytes appeared to attach to glass and the majority of cells maintained their characteristic morphologic appearance when stained with Wright's or hematoxylin and eosin stains. The appearance of degenerating leucocytes in inoculated and uninoculated suspensions was similar. Mitotic cells were not observed. Study of numerous preparations stained with fluorescent antibody or acridine orange failed to indicate which cell of the leucocyte series supported viral multiplication.

Discussion. It was shown previously that human white blood cells *in vitro* supported propagation of measles virus (2). Demonstration of the ability of human leucocytes *in vitro* to support the growth of ECHO 9 virus provides one more example of the susceptibility of these cells to viral infection and reinforces the possibility that leucocytic infection may

occur *in vivo;* further, it suggests that in studies of viremia leucocytes should not be excluded from specimens to be tested.

The observations with ECHO 9 and measles viruses do not justify the assumption that leucocytes are universally susceptible to viruses which are known to produce viremia. In the case of infection with type 1 poliovirus, viremia has been well documented(8). However, experiments similar to those now reported and conducted recently in this laboratory have not provided evidence that type 1 poliovirus multiplies in leucocytes.

Whereas leucopenia is associated with measles, in human infection due to ECHO 9 virus the peripheral white count is usually within normal limits or slightly diminished and may rarely be elevated early in the illness (6,7); consequently, although it is suggested that both viruses may infect leucocytes *in vivo*, it is not clear that such infection influences leucocyte survival.

Summary. Propagation of ECHO 9 virus in suspensions of human blood leucocytes has been described.

1. Yoshioka, I., Horstmann, D. M., *New Eng. J. Med.*, 1960, v262, 3.

2. Berg, R. B., Rosenthal, M. S., PROC. SOC. EXP. BIOL. & MED., 1961, v106, 581.

3. Melnick, J. L., *Diagnostic Procedures for Virus and Rickettsial Diseases*, 2nd Ed., Am. Pub. Health Assn., 1956, 145.

4. Eagle, H., *Science*, 1955, v122, 501.

5. ———, Omaya, V. I., Levy, M., Freeman, A. E., *J. Biol. Chem.*, 1957, v226, 191.

6. Kibrick, S., Enders, J. F., *New Eng. J Med.*, 1958, v259, 482

7. Melnick, J. L., *Viral and Rickettsial Infections of Man*, 3rd Ed., Lippincott, 1959, 564.

8. Horstmann, D. M., McCollum, R. W., Mascola, A. D., *J. Exp. Med.*, 1954, v99, 355.

Received September 5, 1961 P.S.E.B.M., 1961, v108

Demonstration of Serological Specificity in the Reaction of Individual Gamma Globulins with Rheumatoid Factors by Hemagglutination.* (27054)

WALLACE V. EPSTEIN (Introduced by Ernest Jawetz)

The Allergy Clinic and Rheumatic Disease Group, Department of Medicine, University of California School of Medicine, San Francisco

Two general serological methods utilizing human gamma globulin for detection of rheumatoid factors in human serum are now being used. Human gamma globulin isolated by the Cohn alcohol fractionation method (Fraction II) from pooled plasma may be coated on latex particles(1) or may be attached to red blood cells by means of a tannic acid bond(2). Although the sensitivity of the several techniques utilizing pooled gamma globulin vary, they are all characterized by an absence of specificity for the several types of rheumatoid factor as defined by the second method in general use. The Gm system defines the rheumatoid factors by means of human Rh_0 positive red cells coated with selected incomplete anti-D sera(3). The rheumatoid factors are defined as anti-Gm factors of differing types depending on the Gm characteristics of the anti-D coat. Rheumatoid sera of the Gm (a+) type may be negative by this test or may show a marked prozone reaction. Harboe (4) has reported that such sera become positive and lose their prozone reaction when the euglobulin fraction is studied free of the coexisting specific inhibitor contained in the serum pseudoglobulin fraction.

Past efforts to utilize 7S gamma globulin isolated by physical and chemical means from individuals as a cell coating material to detect the several types of rheumatoid factors as defined by the Gm system have been unsuccessful (5,6,7). Isolated gamma globulin fractions from individuals have been either nonreactive or when partially denatured by heating or

* Supported by grants from Nat. Inst. Health and Northern California Chapter of Arthritis and Rheumatism Foundation.