Adsorption *in vitro* of Poliomyelitis Virus on Human Erythrocytes.* (20321)

C. W. JUNGEBLUT,† B. HOFMAN, JR., AND J. D. VERLINDE.

From the Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, and the Institute for Preventive Medicine, Leiden, Netherlands.

Among the neurotropic viruses adsorptive or hemagglutinating reactions have been described for lymphocytic choriomeningitis virus (1), Japanese B encephalitis virus(2), Theiler's mouse encephalomyelitis virus(3), and Col-SK virus(4). Little is known of the mechanism of interaction between these viruses and the red cell, except for Col-SK virus where the controlling factors have been analyzed in some detail(5-11). Classical poliomyelitis virus has given so far no evidence, through hemagglutination, of reacting *in vitro* with red cells. Yet viremia in poliomyelitis has been repeatedly demonstrated in experimentally infected animals(12-15) as well as in the human disease(16-18). Moreover, certain electronmicrographic alterations of the erythrocytes have been interpreted as indicating the presence of virus particles on the cellular surface(19,20). It became of interest, therefore, to investigate whether human poliomyelitis virus is capable of entering into specific interaction *in vitro* with red cells, as determined by viral adsorption.

Experimental. The virus chosen was the Leon strain of poliomyelitis virus.‡ Virus dilutions and cell suspensions were prepared with sterile potassium-veronal buffer(11). In preliminary experiments, 1 cc of virus dilutions of 1:100 was combined with 1 cc of 20% suspensions of washed human (O) or rabbit red cells and the mixtures were kept in the icebox (4°C) for several hours (4-16 hr). Following cold-centrifugation (15 minutes at 3000 R.P.M.) the red cells were separated from the supernatant fluids and the cellular sediments were resuspended in 2 cc of buffer solution or were laked by adding 2 cc of distilled water. Aliquots of the red cell preparations and of their corresponding supernatant fluids were tested for the presence of active virus by intracerebral injection into pairs of rhesus monkeys. Control animals received the test dose of virus which had been combined with buffer solution. The results of several experiments are summarized in Table I.

Results. These experiments show that active virus was uniformly present in the red cell sediment and in the supernatant fluid when human erythrocytes were used. When rabbit erythrocytes were used, virus was found on the red cells in one instance only but was regularly present in the supernatant fluid. Since the potency of Leon virus, as determined in control animals, reached ID_{50} values as high as 10^{-7}, it is evident that the red cells had contacted excessive amounts of virus in the original combination.

Another experiment was set up to study the quantitative aspects of the partition of virus between red cells and supernatant fluid in the original combination. Mixtures consisting of 2 cc of a 1:100 dilution of Leon virus and 2 cc of 20% suspensions of human or rabbit red cells were held in the icebox overnight and the virus content of the supernatant fluid was assayed by titration in monkeys. A control tube which contained 2 cc of a 1:100 dilution of virus in combination with 2 cc of buffer solution was included. The results are given in Table II.

It appears that the amount of free virus which could be detected in the supernatant decanted from the human red cell combination was at least 5 times less than that recovered from the supernatant decanted from the rabbit red cell combination. Since the latter equalled the titer found in the control, the human red cells had evidently removed an appreciable quantity of virus. No such re-

* Aided by grants from the Sister Elizabeth Kenny Foundation and the Dr. Simon Baruch Foundation.

† Fulbright scholar in the Netherlands, 1951-1952.

‡ We are indebted to Drs. H. A. Howe and D. M. Horstman for sending us samples of the Leon strain for experiments in Leiden and in New York.

TABLE I. Infectivity of Cells, before and after Washing, and of Their Corresponding Supernatant Fluids in Virus-Red Cell Combinations.

		Cell-virus combinations							
		Unwashed		1 × washed		2 × washed		3 × washed	
Red cells	Virus	Cells	S*	Cells	S	Cells	S	Cells	S
Human 20%	Leon 10^{-2}	7/8	7/7	1/4	0/2	1/4	0/2	9/10	0/7
Rabbit "	"	1/3	4/4	0/2	0/2			0/3	0/3

Virus controls: $10^{-2.3} = 7/7$; $10^{-3} = 3/4$; $10^{-4} = 6/6$; $10^{-7} = 2/4$; $10^{-7.33} = 0/3$; $10^{-8} = 0/2$

* S = Supernatant.
Denominator = No. of monkeys inj.; numerator = No. of monkeys paralysed.

TABLE II. Titration of Supernatant Fluid from Original Virus-Red Cell Combination.

Virus red cell combination		Titration of supernatant fluid		
Red cells	Virus	$10^{-2.3}$	10^{-3}	10^{-5}
Human 20%	Leon 10^{-2}	3/3	0/4	0/2
Rabbit "	"	3/3	2/2	0/2
Buffer control	"	3/3	2/2	0/2

Denominator = No. of monkeys inj.
Numerator = " " " paralysed.

action could be demonstrated with the rabbit red cells.

While the above results suggested that partial adsorption of virus had occurred on the human red cells, a possibility could not be dismissed that the cells had been contaminated by free virus in the ambient fluid. Further experiments were, therefore, carried out in which human or rabbit red cells, which had contacted virus, were washed repeatedly with large amounts of buffer solution. The washed cells and their corresponding supernatant fluids were then tested for the presence of active virus in the usual manner. Chilled reagents and glassware were used exclusively so as to minimize the chances of possible elution of the virus. Washing was accomplished by resuspending 0.2 cc of centrifuged, packed cells in 9.8 cc of buffer solution, agitating the suspension, and then recentrifuging it to obtain a new sediment. This procedure resulted in a 50-fold dilution of the contents in the original combination; by repeating the washing 3 successive times, a cumulative final dilution of 1:25,000,000 was obtained which was in excess of the verified endpoint of potency of the virus. The results obtained in these experiments are also recorded in Table I.

It will be seen that active virus was no longer demonstrable in any of the supernatant fluids decanted from either human or rabbit red cells after the first, second or third cycle of washing. On the other hand, the washed human cells were definitely infectious in several instances whereas similarly washed rabbit cells were non-infectious.

Summary and conclusions. When poliomyelitis virus was combined with human O erythrocytes at 4°C, active virus was present in the cellular sediment as well as in the supernatant fluid. Upon titration, the supernatant was found to contain less free virus than could be demonstrated in the supernatant from combinations of virus and rabbit erythrocytes, or in a buffer control. Human erythrocytes which, after contact with virus, had been washed repeatedly carried active virus whereas the corresponding supernatant was non-infectious. The described facts suggest that poliomyelitis virus may be adsorbed *in vitro* onto human erythrocytes.

1. Shwartzman, G., *J. Immunol.*, 1944, v48, 111.
2. Sabin, A. B., and Buescher, E. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 222.
3. Lahelle, O., and Horsfall, F. L., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 713; Lahelle, O., and Ward, T. J., *J. Immunol.*, 1951, v67, 75; Morris, M. C., *J. Immunol.*, 1952, v68, 97; 1953, v70, 37.
4. Hallauer, C., 4th Internat. Congr. Microbiology, July 1947, Copenhagen 1949, p. 257; Olitsky, P. K., and Yager, R. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 719.
5. Verlinde, J. D., and DeBaan, P., *Ann. Inst. Past.*, 1949, v77, 632.
6. Jungeblut, C. W., *Bull. N. Y. Acad. Med.*, 1950, v26, 571.
7. Jungeblut, C. W., Horvath, B., and Knox, A., *Arch. Ped.*, 1952, v69, 321.
8. Hallauer, C., *Arch. Virusforschg.*, 1951, v4, 224.
9. Bremer, A., *Ann. Inst. Past.*, 1951, v80, 40.
10. Vivell, O. and Mauer, R. Z., *Immun. Forschg.*,

1952, v109, 246.

11. Horvath, B., and Jungeblut, C. W., *J. Immunol.*, 1952, v68, 627.

12. Smith, M. G., PROC. SOC. EXP. BIOL. AND MED., 1943, v52, 88.

13. Verlinde, J. D., and Beem, B., *Ant. v. Leeuwenhoek,* 1952, v18, 1.

14. Horstman, D. M., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 417.

15. Bodian, D., *Am. J. Hyg.,* 1952, v55, 414.

16. Ward, R., Horstman, D. M., and Melnick, J. L., *J. Clin. Invest.,* 1946, v25, 284.

17. Koprowski, H., Norton, T. W., and McDermott, W., *U. S. Publ. Health Rep.,* 1947, v62, 1467.

18. Jungeblut, C. W., and Huenekens, E. J., to be published.

19. Mulé, F., *Istituto Superiore di Sanità, Roma,* 1952, v15, 220, 226, 230.

20. Reagan, R. L., Day, W. C., Harmon, M. P., and Brueckner, A. L., *Am. J. Path.,* 1953, v29, 175.

Received September 8, 1952. P.S.E.B.M., 1953, v83.

Increased Plasma 17-ketosteroid Concentration in Congenital Adrenal Hyperplasia. (20322)

LYTT I. GARDNER. (With the technical assistance of Mrs. Sally Merrick.)

From the Department of Pediatrics, State University of New York Medical School, Syracuse.

Although there have been numerous studies of urinary 17-ketosteroid excretion in congenital adrenal hyperplasia(1-4), no data appear to be available on plasma concentration of 17-ketosteroids. Previous attempts to measure endogenous 17-ketosteroids in peripheral blood have resulted either in loss of the steroid during fractionation of plasma(5) or in overestimation of the material extracted(6). A method has recently been developed permitting estimation of endogenously produced 17-ketosteroids in peripheral plasma(7). Accordingly the plasma of normal adults and of patients with congenital adrenal hyperplasia has been investigated.

Methods. Oxalated or heparinized venous blood was collected. Ether extracts from acid-hydrolyzed whole plasma (10% HCl) were made and chromatographed on magnesia-silica gel columns, after which the residues were subjected to a micro-Zimmermann reaction(7). Optical density measurements were related to a calibration curve made with dehydroepiandrosterone acetate and the results expressed as μg 17-ketosteroids per 100 ml plasma. The spectrophotometric curves were done on the Zimmermann color of the residues prepared as described above, using a Beckman DU spectrophotometer (light path 1.0 cm, final vol. 0.5 ml). Recoveries of pure androsterone added to plasma samples carried through the extraction procedure varied between 85 and 100%. No 17-ketosteroids could be detected in the plasma of a gonadectomized-adrenalectomized adult. Prepubertal children were found to have plasma values of <3 μg %.

Results. Fig. 1 shows spectrophotometric curves of the Zimmermann reaction done on plasma steroid residues of a normal man age 35 years, and a girl with untreated congenital adrenal hyperplasia age 13 years. The maxima at approximately 520 mμ are noteworthy,

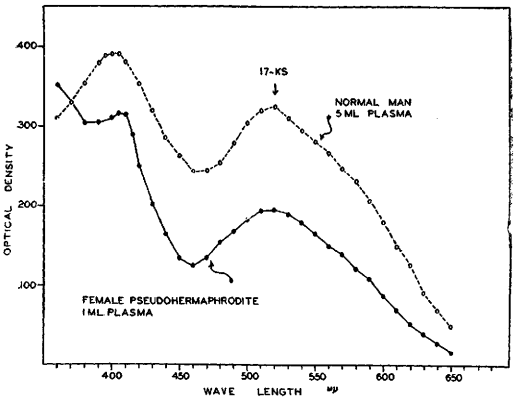

FIG. 1. Spectrophotometric curves of the Zimmermann reaction on plasma residues from a man age 35 years and a girl with congenital adrenal hyperplasia age 13 years (case Gr).