Pathology, and G. G. Robertson, Department of Anatomy, B.U.C.M.

1. Greene, H. S. N., *J. Exp. Med.*, 1941, v73, 461, 475.

2. Struble, E. C., and Bellows, J. G., *J.A.M.A.*, 1944, v125, 685.

3. Sorsby, A., and Ungar, J., *Brit. Med. J.*, 1946, v2, 723.

4. Ward, J. E., Newton, B. L., and Gardner, H. L., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 869.

5. Greene, H. S. N., *Cancer Res.*, 1947, v7, 491.

Received January 13, 1953. P.S.E.B.M., 1953, v82.

Poliomyelitis Virus in Human Blood During the "Minor Illness" and the Asymptomatic Infection.* (20139)

DOROTHY M. HORSTMANN AND ROBERT W. MCCOLLUM.

From the Section of Preventive Medicine, Yale University School of Medicine, New Haven, Conn.

Viremia in human poliomyelitis has been reported previously from this laboratory, in one child out of 111 tested(1). The positive result was obtained with blood drawn from a 9-year-old girl 6 hours after the onset of symptoms in an abortive attack of poliomyelitis. At the time, these results were considered evidence that viremia is of rare occurrence in the human disease, and probably not of importance in its pathogenesis. Subsequently, it became known that antibodies are already present at the time of onset of CNS signs both in patients(2,3) and in primates orally infected in the laboratory(4,5). Thus our negative results had to be reinterpreted, for it is clear that if viremia occurs, it must do so early, either in the incubation period or *minor illness* phase, and in 110 of our patients we had probably looked for it too late. The possibility of viremia in the experimental disease had been recently restudied in laboratory primates, and 2 reports(6,7) indicate that the presence of virus in the blood is a regular feature after oral and other routes(8) of administration. In cynomolgus monkeys and in chimpanzees, viremia occurs as early as between the 3rd and 8th days after its ingestion or cutaneous injection, and as long as a week *before* the appearance of paralysis. In reinvestigating viremia in human poliomyelitis, therefore, efforts have been concentrated on obtaining specimens from contacts of cases, *i.e.*, children who might be in the incubation period, and from children with the minor illness syndrome in the midst of an epidemic. The present report deals with the findings in one infected family, in which 3 of the 4 children had minor illnesses during a poliomyelitis epidemic in their community.†

Procedure. Family epidemiology. The W. family resides in the small town of Burbank, Ohio, where an epidemic of poliomyelitis occurred during the summer of 1952(9). The family had had no known contact with a frank case of the disease, but beginning June 20, 21, and 22, the 3 older children aged 10, 4, and 3, developed mild, febrile illnesses, while the youngest, aged 2, remained well. Slight fever, anorexia, nausea and vomiting, headache, and sore throat, in various combinations were the complaints. The oldest child complained also of soreness of the calves of the legs. The father, aged 37, had noted a headache on June 19, but this had disappeared and there were no associated symptoms. The mother remained well throughout. All of the illnesses were compatible in their symptomatology

* Aided by a grant from the National Foundation for Infantile Paralysis.

† These specimens were collected by one of us (R.W.Mc.) in Wayne County, Ohio. Our thanks are due to Dr. E. E. Kleinschmidt, Health Commissioner of the Wayne & Medina General Health District and to Dr. Fred Wentworth, Chief of the Communicable Disease Division, Ohio State Department of Health and their staffs, and to Mr. Edward Tulley, Chairman of the Summit County Chapter of the National Foundation for Infantile Paralysis, for their generous cooperation. We are also indebted to Mr. Claude Reed, Miss Ilah Kaufman, and Mrs. Berttina Orsborne, for assistance in the collection of specimens.

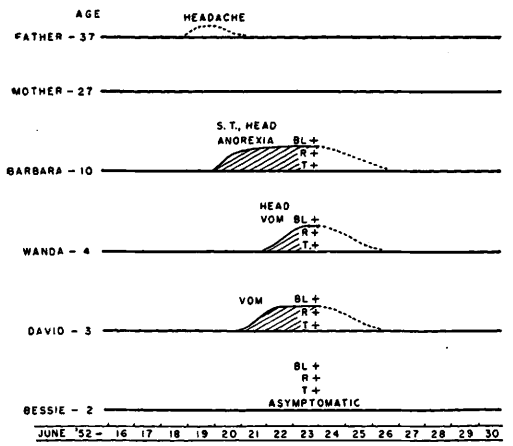

FIG. 1. Diagram of a family outbreak of poliomyelitis in which 3 cases of the minor illness occurred. Shaded areas indicate period of fever. S.T., sore throat; head., headache; vom., vomiting. + refers to results of tests for virus in blood (Bl), rectal swabs (R), and throat swabs (T).

with abortive poliomyelitis(10). They were of short duration, and none was followed by any sequellae. A schematic diagram of the illnesses appears in Fig. 1. *Collection of specimens.* On June 23, at the height of the family epidemic, throat swabs, rectal swabs and blood specimens were collected by one of us (R.W.Mc.) from the 4 children. Throat and rectal swabs were preserved in 50% sterile glycerine, and frozen on dry ice. The bloods were collected in "vacutainer" tubes containing potassium oxalate, and were centrifuged for 10 minutes at 3000 rpm to layer the plasma and red cells before being frozen on dry ice. All materials were transported to New Haven in the frozen state, and kept frozen until tested. *Testing of specimens.* Both tissue culture tubes and rhesus monkeys (*M. mulatta*) were used in testing blood specimens, while tissue culture alone was employed for the testing of throat and rectal swabs. The roller tube method, with monkey testicular tissue was used(11), except in a few tests in which monkey kidney tubes were employed. With the exception of one blood specimen (Bessie, aged 2), which was tested before and after ultracentrifugation at 30,000 rpm for one hour, the plasma and/or whole blood was inoculated directly into a minimum of 4 tubes. If no degeneration occurred, the 8- and 12-day harvests of all 4 tubes were pooled and a 2nd passage using 2-5 tubes was made before the test was considered negative. Several tests were carried out with each blood, returning each time to the original specimen. For monkey inoculation, 0.6 ml of plasma, or plasma and red cells mixed, was inoculated intracerebrally, 0.3 ml being directed into either side of the thalamus. The injection was repeated after 3-4 weeks, if the animal remained well. All monkeys showing any suggestive signs of poliomyelitis were sacrificed for histological examination. *Identification of virus strains.* Tissue culture neutralization tests(11) were carried out on all strains isolated. A series of tubes was set up with equal parts of the unknown virus harvested from a tissue culture passage, and Type 1, Type 2, and Type 3, hyperimmune monkey serum, diluted 1:5. A control tube contained virus and balanced salt solution in equal parts. The mixtures were incubated at room temperature for one hour before being inoculated into 2 tubes each. The test was read at 4 and 8 days, and complete inhibition of fibroblastic degeneration by serum of one type only was taken to indicate specific neutralization of the virus.

Results. Poliomyelitis virus was isolated from the blood of all 4 of the children—from the asymptomatic youngest child, as well as from the 3 older ones who had symptoms. All 4 also had virus in throat and rectal swabs. A summary of the results correlated with the clinical course, is indicated in Fig. 1, and the details of the virus isolations are given in Table I. All strains isolated proved to be Type 1 (Brunhilde).

The amount of virus present in the positive blood specimens must have been minimal for it was insufficient to produce poliomyelitis on direct intracerebral inoculation into rhesus monkeys. On first passage in testicular tissue roller tubes, the results were only suggestive, but on 2nd and 3rd passages, characteristic fibroblastic degeneration of increasing severity occurred. Third tissue culture passage from 2 of the specimens was inoculated into rhesus monkeys, and produced typical poliomyelitis, clinically and histologically. One blood specimen (from Bessie, aged 2) was positive in only one of 3 tests using testicular tubes; after ultracentrifugation it yielded a strongly

TABLE I. The Isolation of Type 1 Poliomyelitis Virus from blood, throat and rectal swabs of 4 children in one family.

	Age	Date onset	Specimens collected 23/6/52	Tests for poliomyelitis virus: Direct monkey inoculation	Tissue culture passage: 1st	2nd	3rd	Monkey inoc., 3rd T.C. passage
B	10	20/6/52	Blood	0/1*	2/3	4/4	2/2‡	1/1
				0/2†	2/3	4/4		
			Throat swab		2/2	2/2	2/2‡	
			Rectal swab		1/2	2/2	2/2‡	
W	4	22/6	Blood	0/2	0/4	0/2		
					3/5	3/3	2/2‡	1/1
					1/3	1/2		
			Throat swab		2/2	2/2	2/2‡	
			Rectal swab		2/2	2/2	2/2‡	
D	3	21/6	Blood	0/2	0/4	0/2		
					0/4	0/3		
					0/5	1/5	2/2‡	
					3/10K	2/2‡K		
			Throat swab		4/4	2/2‡		
			Rectal swab		2/2	2/2	2/2‡	
Be	2		Blood	0/2	0/4	0/2	0/2	
					0/4	0/4		
					1/6	5/6		
					9/10‡§K	2/2K		
			Throat swab		2/2	2/2	2/2‡	
			Rectal swab		2/2	2/2	2/2‡	

* Denominator, No. inoculated; numerator, No. positive.
† These animals received 0.3 cc intracerebrally once only.
‡ Indicates specific neutralization by Type 1 antiserum.
§ Specimen ultracentrifuged before being tested in kidney tissue tubes.
K indicates kidney tissue roller tubes.

positive result in kidney tissue tubes (Table I).

Discussion. The isolation of poliomyelitis virus from the blood of an asymptomatic child and from 3 children with symptoms of the *minor illness* confirms our earlier observation from this laboratory of viremia in a child with abortive poliomyelitis. As with the earlier case(1) all of the children were excreting virus in the stools at the time it was found in the blood. Evidence is thus accumulating that viremia can occur in the human infection as it does in chimpanzees and cynomolgus monkeys, and, as with the latter, it is *not* associated with the clinical appearance of CNS signs, but occurs several days earlier, in association with the minor illness, or in the asymptomatic infection, probably several days after exposure. Whether or not viremia can occur also in the incubation period of the single or double phase illness has not yet been demonstrated. In any event, the presence of viremia early in the course of *human*—as well as *experimental* infection—supports earlier hypotheses(12-14) of rapid virus multiplication outside the CNS. Where this multiplication occurs, whether in neural or non-neural elements, is not as yet certain. Bodian(15) believes that primary multiplication somewhere in the intestinal mucosa, followed by discharge of virus into the blood stream, which carries it to the CNS, is the most likely sequence of events. Faber *et al.*(16) on the other hand, are of the opinion that the source of circulating virus in orally infected cynomolgus monkeys is early multiplication in peripheral ganglia; they discount the possibility of multiplication or spread in non-neural tissues. The problem can only be resolved by demonstrating directly the early presence or absence of virus in various neural and non-neural tissues after various routes of administration.

Summary. Poliomyelitis virus has been isolated from the blood of 4 children in one family during a poliomyelitis epidemic in Ohio in 1952. Three of the children had characteristic clinical pictures of the *minor illness* or abortive poliomyelitis, and one was asympto-

matic. All were found to have virus in the throat and rectal swabs as well as in the blood. None went on to develop signs or symptoms of the *major illness,* either paralytic or non-paralytic.

1. Ward, R., Horstmann, D. M., and Melnick, J. L., *J. Clin. Invest.,* 1946, v25, 284.
2. Hammon, W. McD., and Roberts, E. C., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 256.
3. Steigman, A. J., and Sabin, A. B., *J. Exp. Med.,* 1949, v90, 349.
4. VonMagnus, H., Separatum, *Acta Pathologica,* 1950, v27, 222.
5. Horstmann, D. M., and Melnick, J. L., *J. Exp. Med.,* 1950, v91, 573.
6. Horstmann, D. M., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 417.
7. Bodian, D., *Am. J. Hyg.,* 1952, v55, 414.
8. Horstmann, D. M., unpublished observations.
9. Kleinschmidt, E. E., Abbot, M., and Kauffman, I., *Pub. Health Rep.,* 1952, v67, 1109.
10. Paul, J. R., Sallinger, R., and Trask, J. D., *J.A.M.A.,* 1932, v98, 2262.
11. Ledinko, N., Riordan, J. T., and Melnick, J. L., *Am. J. Hyg.,* 1952, v55, 323.
12. Draper, G., Acute Poliomyelitis, 1917, P. Blakiston's Sons & Co.
13. Sabin, A. B., *J. Mt. Sinai Hosp.,* 1944, v11, 185.
14. Horstmann, D. M., and Paul, J. R., *J.A.M.A.,* 1947, v135, 11.
15. Bodian, D., *Am. J. Pub. Health,* 1952, v42, 1388.
16. Faber, H. K., Silberberg, R. J., and Dong, L., *J. Exp. Med.,* 1952, v97, 69.

Standards for Hepatic and Hematologic Tests in Monkeys: Observations During Experiments with Hepatitis and Mononucleosis.* (20140)

ALFRED S. EVANS,† BRIGITTE K. EVANS, AND VIKTORIA STURTZ.
(Introduced by C. V. Seastone.)
From the Hepatitis Research Center, 98th General Hospital, U. S. Army, Munich, Germany.

The paucity of available data on the values for liver function tests and blood counts in monkeys has suggested that publication of such information might be useful to other laboratories interested in investigating chemical or infectious agents suspected or capable of producing hepatic damage in monkeys.

The observations recorded in the present report were obtained during the course of unsuccessful attempts to transmit viral hepatitis and infectious mononucleosis to monkeys. It had been our hope that administration of adrenocorticotropic hormone (ACTH), cortisone, or urethane might induce susceptibility to these diseases in a laboratory animal otherwise resistant. Under the conditions of these experiments, this did not occur.

Materials and methods. Twenty normal monkeys (16 rhesus and 4 cynomologous) were bled from the femoral vein prior to inoculation and the following tests of liver function carried out by standard methods; total and one minute serum bilirubin, thymol turbidity, cephalin cholesterol flocculation, and alkaline phosphatase. Later in the study bromsulfalein dye retention (5 mg/kg) was determined 45 minutes after inoculation. Leucocyte counts were done in duplicate and differential counts were done by enumeration of 200 cells on Giemsa stained glass slides. Fourteen of these twenty monkeys were then inoculated with materials obtained from the patients in the active stage of characteristic viral hepatitis. Except for one sample of pooled plasma which had produced serum hepatitis in 10 or 20 human recipients(1),‡ it was not known whether SH or IH virus was represented. In addition to the hepatitis materials, 8 of these monkeys received injections of cortisone, 3 received ACTH, and 1 was fed

* Representing work supported in part by the Virus and Rickettsial Disease Commission, Armed Forces Epidemiological Board, Office of the Surgeon General, U. S. Army.

† Present address: Dept. of Preventive Medicine and Microbiology, University of Wisconsin.

‡ Appreciation is expressed to Dr. A. W. Wright, Albany, N. Y., for this material.