cyclic derivatives of sulfanilamide, even if closely related, are soluble in one and the same solution almost to the extent of their separate solubilities. Based on this observation, the toxicity and antibacterial activity of sulfadiazine-sulfathiazole mixtures was investigated. It was found that the acute and chronic toxicity of this drug combination for albino rats is strikingly low if compared with the effect of similar or identical total concentrations of either sulfathiazole or sulfadiazine alone. Evidence derived from chemical analyses and post-mortem examinations pointed to a diminution of intrarenal obstruction from drug precipitate as the chief reason for the low toxicity of the mixture. *In vitro* antibacterial studies showed that the effectiveness of the mixture corresponded largely to the total concentration of free sulfonamide. On the basis of these observations, the substitution at the bedside of sulfathiazole or sulfadiazine by combinations of these two compounds was suggested, because their application might decrease the danger of renal complications without interfering with the antibacterial efficacy. Clinical trials are in progress.

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Poliomyelitis Virus in the Blood Stream in the Experimental Disease.*

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Although most attempts to demonstrate poliomyelitis virus in the blood of experimentally-infected monkeys have been unsuccessful, four positive tests have been reported,[1,2,3,4] 3 of which were described more than 30 years ago. In all 4 instances the blood was drawn soon after the onset of paralysis. Furthermore the virus has long been sought in human blood but with the exception of a single instance, in spite of many trials,[5] such tests have been negative.[6]

The present data are placed on record to indicate some of the conditions under which poliomyelitis virus has been detected in the blood of monkeys during a recent series of experiments. In addition the virus has been looked for in the colon contents of many of these monkeys and the results of these tests are also presented.

Methods. Rhesus (*Macaca mulatta*) and capuchin (*Cebus capucina*) monkeys were used. The monkeys used as donors had been infected by the intracerebral, intracutaneous, and other routes. Three strains were used—all in their early passages. These strains originated in human stools of poliomyelitic patients from Texas (1942), Hartford (1942), and various sections of the country (1940-1941). The history of these strains in their early passages through monkeys has been described.[7]

Blood was collected from the heart and no attempt was made to prevent clotting. The whole blood was either stored on dry ice or it

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

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TABLE I.
Poliomyelitis Virus in Monkey Blood and Colon Contents.

Inoculum				Donor monkey No.‡	Result of inoculation		Day blood and colon contents collected	Results of virus test in		
Strain-Generation*		Material	Route†		Onset of fever§	Onset of paralysis‖		Blood¶	Colon contents¶	Spinal cord¶
Hartford	i	Stool	IC	R-2259	7	10	11	+	—	
"	i + ii	Spinal cord	IQ	R-2300	0	8	9	+	+	+
"	iii + iv	" "	IC, IP IN, IQ SQ	C-2324	9	11	11	+	—	+
"	ii + iii	Stool	IC	R-2370	10	11	12	+		
"	ii	Blood	IC	R-2274	11	13	15	—		
"	i + ii	Spinal cord	IQ	R-2299	12	13	13	—	—	
"	ii + iii	Stool	IC	R-2335	0	19	19	—	—	
U.S.	i	Spinal cord	IC	R-2338	14	16	16	—		
Texas	i	" "	IC, IP IQ, SQ	C-2356	7	10	11	—	+	+
"	o	Stool	IC	R-2289	7	10	11	—	—	+

* o = original human material was inoculated; i = material from monkey representing the first generation of the virus in monkeys was inoculated, etc.

† IC = intracerebral; IQ = intracutaneous; IN = intranasal; SQ = subcutaneous; IP = intraperitoneal.

‡ R = rhesus (*Macaca mulatta*) monkey; C = capuchin (*Cebus capucina*) monkey.

§ 7 = fever occurred 7 days after inoculation; 0 = no fever observed.

‖ 10 = paralysis first observed 10 days after inoculation.

¶ + = positive test for poliomyelitis virus; — = negative test for poliomyelitis virus. One monkey was used for each test.

was immediately prepared for inoculation. To the volume of blood drawn—30 to 90 cc—enough water was added to make a total volume of 120 cc; the hemolyzed blood was mixed in a Waring blendor for about 5 minutes. It was then centrifuged at 3000 RPM for 20 minutes. 90 cc of the supernatant fluid was subjected to ultracentrifugation at 39,000 RPM for 60 minutes. The gelatinous pellets were taken up in 2 to 3 cc of water, spun lightly in the laboratory centrifuge, and 1 to 2 cc inoculated intracerebrally into a rhesus monkey. Colon contents, taken at autopsy immediately after the blood was drawn, were also prepared by ultracentrifugation as outlined in a previous paper.[8] These samples were also inoculated intracerebrally, one monkey being used for each test. Spinal cord was passed as a 10% suspension and was inoculated intracerebrally and intraperitoneally.

The criterion for a positive test (*i.e.*, demonstration of virus in blood, colon contents, or spinal cord) was the production in the test animals of typical histological lesions, viz., neuronophagia and neuronal necrosis associated with perivascular and interstitial cellular infiltration.

Results. In this series of experiments (Table I) virus was found in the blood stream of 4 out of 7 monkeys infected with the Hartford strain and in none out of 3 monkeys infected with other strains. Two of the monkeys with viremia had been inoculated *intracerebrally* with the stools of experimentally infected monkeys whereas the other 2 had been inoculated with infected spinal cord. Of the latter 2 monkeys, one, a rhesus, had received an *intracutaneous* inoculation and the other, a capuchin, had received multiple inoculations by intracerebral as well as by other routes. The colon contents of 3 of these 4 monkeys were also tested for virus and only one yielded a positive result. The spinal cords of 2 of these monkeys were passed successfully.

Tests were carried out on the colon contents of 4 of the monkeys yielding negative blood tests; one was positive.

The specimens recorded as positive in Table

[8] Melnick, J. L., *J. Exp. Med.*, 1943, **77**, 195.

I produced paralysis in the test monkeys in all cases but one. This latter monkey had the non-paralytic form of the disease as evidenced by a negative clinical course coupled with poliomyelitic lesions in its spinal cord.

Comment. The attempt will not be made to determine the significance of finding virus in the blood of 4 of 10 paralyzed monkeys. There is no reason to suspect that this represents an extra-neural pathway for the primary distribution of virus in the body; it may well be the direct result of the overflow or of the breakdown of virus-laden nerve cells. The distribution of lesions[9] and of virus[10] only in certain regions of the central nervous system both in the experimental and human disease would lead one to favor the view that virus in the blood does not play a predominant role in determining the location of virus within the central nervous system, once the latter has gained access to this tissue. In these experiments the presence of *detectable* amounts of virus in the blood stream was neither a prerequisite nor a predisposing factor for the appearance of *detectable* amounts of virus in the colon contents during the acute disease.

All of the positive results were found in monkeys inoculated with one strain (Hartford) of poliomyelitis virus. Three tests on two other strains were negative. This finding may recall the fact that certain strains, usually in their early passages, are more infectious by the cutaneous route than are others.[11,12]

Summary. Of 10 monkeys, paralyzed after being inoculated with poliomyelitis virus of recent human origin, three rhesus and one capuchin monkey were found to have the virus in the blood stream. All of the positive tests in the blood were due to a single strain. The colon contents of three of these latter monkeys were examined for virus and it was found in only one instance. Of 4 monkeys in whose blood virus could not be detected, one was found to have virus in its colon contents.

[9] Howe, H. A., and Bodian, D., *Neural Mechanisms in Poliomyelitis*, New York, 1942.

[10] Sabin, A. B., and Ward, R., *J. Exp. Med.*, 1941, **73**, 771.

[11] Trask, J. D., and Paul, J. R., *J. Bact.*, 1936, **31**, 527.

[12] Trask, J. D., and Paul, J. R., *Science*, 1938, **87**, 44.

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Effect of Aspergillic Acid on Equine Encephalomyelitis Virus.*

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During the current outbreak of equine encephalomyelitis among horses in the Southwest, a few human cases occurred.[1] This prompted study on treatment of the disease, since no effective therapeutic agent is available. Although antiserum may be effective in the treatment of horses when administered during the early stages of the disease, such therapy is of little value in man because the central nervous system lesions are usually too far advanced at the time when the diagnosis of encephalitis is made. A study of the effect of aspergillic acid on a neurotropic virus seemed of interest because, to our knowledge, no reports have appeared in the literature concerning the effect of this substance on viruses, despite its efficacy on a wide variety of bacteria.[2] Also, there are indications that this drug diffuses rapidly into the central

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[1] Sulkin, S. E., and Ashworth, C. T., to be published.

[2] Jones, H., Rake, G., and Hamre, D. M., *J. Bact.*, 1944, **45**, 461.