Summary. The liver appears to be the main site of the conversion of pteroglyglutamic acid (PGA) to citrovorum factor (CF) in the body. Other tissues had very limited capacity to form CF, with the exception of bone marrow which, relative to the small amount present in this tissue, formed an appreciable quantity of the factor. The hepatic tissue of rats which had been depleted of folic acid and CF was flooded with CF within one hour following the intraperitoneal injection of PGA. Homogenates of rat or chick liver, when incubated with PGA under an atmosphere of nitrogen, were capable of forming CF. Little CF was found when similar preparations were incubated under oxygen. The yield of CF was consistently increased by the presence of ascorbate in the incubation mixture.

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Hemagglutination with Certain Arthropod-Borne Viruses.* (20299)

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Specific hemagglutination (HA) with neurotropic viruses was first described by Hallauer(1) for the Columbia SK and Columbia MM viruses, by Lahelle and Horsfall(2) for the GD VII virus, and by Olitsky and Yager (3) not only for the Columbia SK and Columbia MM, but also for the Mengo and encephalomyocarditis viruses. Other reports have followed, largely confirming these findings; but departures from them were recorded by Sabin and Buescher(4) and Sabin(5), who included several other encephalitis viruses. The former(4) reported success with Japanese B encephalitis, West Nile disease, and Russian Far Eastern encephalitis viruses; the latter(5) reported success not only with these agents, but in addition with St. Louis encephalitis and Western equine encephalitis viruses.

Recently Sweet, Chanock, and Sabin(6) showed the presence of hemagglutinins associated with 2 immunologically distinct types of dengue virus, Hawaiian and New Guinea C. The method of preparation of viral extracts, the pH range needed for positive reactions, and the conditions under which serum inhibition of HA took place were found to be rigid and exacting and varied from virus to virus. MacDonald(7), in a recent step forward, readily prepared specific HA extracts from the brains of newborn mice infected with Murray Valley encephalitis virus. Prior studies in this laboratory have shown the superiority of the brains of newborn mice as source material for the preparation of complement fixation antigens. It was therefore

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planned to use such mice‡ in an attempt to simplify the HA test for certain arthropod-borne viruses. As the following will show, a method was devised which is relatively simple and which gives readily reproducible results.

The method is essentially that employed for the preparation of complement fixation antigens from mouse brain treated with acetone and ether(8). This antigen as such is the viral extract used in the HA test.

Viruses. The viruses—and their strains—under investigation and the number of passages in mice and other animals for each were: Anopheles A(9): Adult mice, 166; plus newborn mice, 2. Dengue—Hawaiian: Adult mice, 76; plus newborn mice, 4. Dengue—New Guinea B: Adult mice, 43; plus newborn mice, 6. Eastern equine encephalitis: Adult mice, unknown (hundreds); plus newborn mice, 2. Ilhéus (10): Adult mice, 25; plus newborn mice, 2. Japanese B encephalitis—Nakayama: Adult mice, 100 to 200; plus newborn mice, 8. Ntaya (11): Adult mice, 10; plus chick embryos, 7; and newborn mice, 4. Poliomyelitis§—Type 2, MEF 1: Adult mice, 20; plus newborn mice, 137. Russian Far Eastern encephalitis—No. 1: Adult mice, unknown number of passages in Russia; adult mice in this laboratory, 10 to 20; plus newborn mice, 1. West Nile—original: Adult mice, 30; plus newborn mice, 1. West Nile—Egypt 101: Chick embryos, 5; plus adult mice, 0; and newborn mice, 5 to 8. Yellow fever—Asibi: Adult mice, 0; plus monkeys, unknown; and newborn mice, 2.

Preparation of brain extracts. Two hundredths ml of the virus, a 10^{-1} suspension of infected brain tissue, was inoculated intracerebrally into 2- to 4-day-old mice. When signs of infection were noticeable, the etherized mice were sacrificed by exsanguination. The brains were frozen rapidly with solid CO_2-alcohol mixture and stored at —25°C until used. Extracts were prepared by treatment of the brains with acetone and ethyl ether as already described(8)—essentially, 2 extractions with acetone, one with a mixture of equal parts of acetone and ether, and 2 with ether. The residue, after drying, was resuspended in 0.9% sodium chloride in distilled water; the suspension represented 1 g tissue in 2 ml diluent. The suspensions were kept overnight at 4°C, then centrifuged at about 9,000 G for one hour. In the end the extract represented a 1:3 dilution of original brain tissue. Dilutions shown in the tables are those of the original brain tissue when introduced into the test tubes. Controls comprised similarly prepared extracts of normal mouse brain. Preparations were stored at —25°C. Repeated freezing and thawing 3 or 4 times apparently did not significantly affect the hemagglutinin titers; 6 or 8 times did, however, cause a definite reduction. One of the preparations (Japanese B encephalitis virus) was lyophilized and this led to a moderate loss of titer.

Erythrocytes. Sheep and human O red blood cells were not generally satisfactory, but cells from one-day-old chicks were employed successfully. They were prepared as described by Sabin(5), *i.e.,* collected in Alsever's solution, and when needed were washed 3 times in physiological saline solution. Of a 0.25% suspension of the washed cells in saline, 0.5 ml was added to each tube in the tests.

Tests. Tubes were kept in an ice bath at 4° to 8°C, the optimal temperature, although tests could also be carried out at room temperature. To each tube was added 0.5 ml of extract in increasing 4-fold dilutions in physiological saline, pH 6.8 or 7, with phosphate buffer, M 0.02. The cells were then added. The tubes were shaken and kept at 4°C for one to 2 hours at which time readings according to Salk's pattern(12) were made. Generally speaking, the lowest dilutions gave a coarse type of agglutination and the moderate and high dilutions gave the shield type.

Results. Two or more different lots of extract of each virus were tested, and each lot was used repeatedly. A summary of the results is given in Table I.

It will be seen that Anopheles A, Eastern equine encephalitis, Type 2 poliomyelitis, Russian Far Eastern encephalitis, and West Nile (original) viruses failed to show agglu-

‡ Unless otherwise specified, all brain tissue mentioned hereafter will refer to the brains of newborn mice.

§ Although not known to be an "arthropod-borne" virus, the poliomyelitis virus is included here because of other properties common to the group.

TABLE I. Hemagglutination with Viruses in Mouse Brain Treated with Acetone and Ether.

Virus	Strain	Titer (expressed as reciprocal of dilution of brain tissue)
Anopheles A		0*
Dengue	Hawaiian	49152
"	New Guinea B	3072
Eastern equine encephalitis		0
Ilhéus		49152
Japanese B encephalitis	Nakayama	196608
Ntaya		3072
Poliomyelitis	Type 2, MEF 1	0
Russian Far Eastern encephalitis		0
West Nile	Original	0
"	Egypt 101	49152
Yellow fever	Asibi	12288
Normal tissue		0

* 0 = dilution <1:12.

tination. It is to be noted that none of the normal brain preparations gave positive results, nor did any positive viral extracts reveal inhibition of hemagglutination in low dilutions. Only when extracts were added undiluted, *i.e.*, dilution of brain tissue 1:3, was there often inhibition, but this was not evident in the lowest dilution employed in the tests, 1:12.

Specific inhibition. In order to establish the specificity of the reactions, known immune sera were tested for inhibition against several of the viruses under study. Prior to their use in the tests, the sera were treated in the manner described by Sabin and Buescher(4) and Sabin(5) in order to eliminate nonspecific inhibitors. Thus far, only monkey and mouse sera have been used; studies are currently being carried out with human sera. The animal sera were prepared as follows: They were heated at 56°C for 30 minutes, then diluted 1:10 in physiological saline. Twelve volumes of acetone were added. After low-speed centrifugation, the sediment was re-extracted with 12 volumes of acetone, centrifuged, dried, and resuspended in physiological saline in a proportion to yield a 1:10 dilution of serum. The HA inhibition tests were carried out with serial 2-fold dilutions of serum in saline containing phosphate buffer M 0.02 at pH 6.8; the first dilution of serum was 1:10 and the volume was 0.25 ml. The tubes containing the serum dilutions were then placed in an ice water bath at 4° to 8°C and the antigen added. This consisted of 0.25 ml of the brain extract diluted in such a way as to provide 16 hemagglutinating units in that volume. Finally, 0.5 ml of the 0.25% suspension of chick erythrocytes was added, and the tubes were kept in the cold room at 4°C.

Table II shows the results of tests with

TABLE II. Inhibition of Hemagglutination with 16 Agglutinating Units.

Virus	Serum	Serum dilutions 10	20	40	80	160	320	640	1280
West Nile, Egypt 101 strain	Monkey 49-60, pre-infection	+	+	+	+	+	+	+	+
	convalescent	0	0	0	0	0	±	+	+
	Monkey 49-69, pre-infection	+	+	+	+	+	+	+	+
	convalescent	0	0	0	0	±	+	+	+
Dengue, Hawaiian strain	Monkey 49-75, pre-infection	+	+	+	+	+	+	+	+
	convalescent	0	0	0	0	0	0	±	+
	Mouse, normal	±	+	+	+	+	+	+	+
	" hyperimmune	0	0	0	0	0	0	0	+
Japanese B encephalitis	Monkey 49-35, pre-immunization	+	+	+	+	+	+	+	+
	hyperimmune	0	0	0	±	+	+	+	+
	Mouse, normal	+	+	+	+	+	+	+	+
	" JBE, hyperimmune	0	0	0	0	0	±	+	+
	" EEE "	+	+	+	+	+	+	+	+

+ = agglutination; 0 = no agglutination; ± = questionable agglutination.

West Nile (Egypt 101 strain), dengue (Hawaiian strain), and Japanese B encephalitis viruses. The sera from monkeys infected or hyperimmunized with the respective viruses inhibited HA, while the control samples of serum taken prior to infection or immunization failed to do so. Furthermore, it can be seen that 2 different pools of normal mouse serum did not affect HA with dengue (Hawaiian strain) or Japanese B encephalitis viruses; nor did an Eastern equine encephalitis hyperimmune mouse serum inhibit HA with Japanese B encephalitis virus. The hyperimmune homotypic mouse sera, however, inhibited agglutination in dilutions from 1:160 (for Japanese B encephalitis) to 1:640 (for dengue, Hawaiian strain).

Summary. A simple, easily reproducible method for preparation of high-titer hemagglutinins with several arthropod-borne viruses is described. The method is based on the use of brains from newborn mice infected with virus as the source material, and on treatment with acetone and ether for the elimination of nonspecific factors. With this method, stable specific HA has been found associated with the following viruses: dengue (Hawaiian type), dengue (New Guinea B type), Ilhéus, Japanese B encephalitis, Ntaya, West Nile (Egypt 101 strain), and yellow fever (Asibi strain). As yet, no agglutination has been uncovered associated with Anopheles A, Eastern equine encephalitis, poliomyelitis (Type 2, MEF 1 strain), Russia Far Eastern encephalitis, and West Nile (original strain) viruses.

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Failure of Rutin and Catechin* to Affect Vascular Lesions of Experimental Hypertension or Hypersensitivity.† (20300)

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In a previous communication, one of the authors, (J.L.O.), in collaboration with several others, reported the effectiveness of rutin in decreasing the gross hemorrhage in experimental malignant hypertension in dogs(1). Shortly after that work was completed, Moss, Beiler and Martin(2) reported the effectiveness of another flavonol, catechin, in preventing anaphylactic shock in guinea pigs. Because we were interested in both hypersensitivity arteritis and malignant hypertension, it was decided to test the effectiveness of each of these two compounds in hypersensitivity arteritis and also in the arteritis of malignant hypertension.

Methods. Seven rabbits and 6 dogs were given a solution of rutin in 0.1 N NaOH with

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