cultures of *Vibrio cholerae,* which destroyed the receptors for influenza virus. $ZnSO_4$ in minute amounts inhibited the hemagglutinin of the Japanese B virus but not that of influenza virus. The inactive zinc-hemagglutinin complex was reactivated by dilution or removal of zinc with H_2S. The inhibitor present in normal human serum was removed by shaking with chloroform, while the specific hemagglutination inhibiting antibody was unaffected, thus providing a simple test for diagnosis of infection with the Japanese B virus. Specific hemagglutinins, with properties different from that of the Japanese B virus, have also been found for the West Nile and Russian Spring-Summer encephalitis viruses.

Addendum. Since this paper was submitted for publication, work carried out in association with Drs. J. Warren and M. Weil of the Army Medical School, indicated that prolonged passage of the Nakayama strain of Japanese B encephalitis virus in mice resulted in complete loss of its hemagglutinating activity. Tests on 6 additional strains of Japanese B encephalitis virus, with relatively few passages in mice, revealed that all possessed a hemagglutinin similar to that described here for the Nakayama, Okinawa and Korea strains. Since the SLE, WEE, and EEE strains of virus we had used, had all had hundreds of mouse passages for many years, we repeated the work with freshly isolated strains of these viruses and have succeeded in demonstrating a specific hemagglutinin for chick and sheep cells at pH 6.5 to 7.0 with 3 strains of SLE, but thus far not with WEE or EEE. This work will be reported in detail in forthcoming publications.

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A Method for the Determination of Sodium Ferrocyanide at Low Concentrations in Body Fluids.* (17862)

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Gersh's(1) histochemical studies in the mammalian kidney on the distribution of ferrocyanide established its exclusive glomerular excretion. Renal clearances of ferrocyanide have been measured in the dog with plasma concentrations from 60-200 mg%. In one study the ferrocyanide was determined as Prussian blue(2); in a second as ferricyanide(3). These methods are accurate only at these high plasma concentrations. When human subjects were used, nephrotoxic reactions were encountered even with plasma concentrations of 3.3 to 43.6 mg%. The analyses were performed by hydrolysis and recovery of HCN(4). This time consuming method has lowered the working range of ferrocyanide concentrations, but in order to avoid the possible danger of toxic reactions, we have devised an accurate method sensitive enough to measure sodium ferrocyanide concentrations of 5-15 mg%. By the acid mixture described below, ferrocyanide is oxidized to ferricyanide and allowed to react with ferrous sulfate to form Turnbull's blue. The per cent transmittance is measured at 700 millimicra in the Coleman, Jr. spectrophotometer. The calibration curves show that the reaction follows Beer's law, and do not vary significantly from day to day. The color is

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TABLE I.
Recovery of Various Concentrations of Sodium Ferrocyanide Added to Serum and Urine.

Amt $Na_4Fe(CN)_6$ added to serum and urine mg%	No. of single determinations	Mean recovery mg%	Stand. Dev. from mean, mg%
5	14	4.96	± 0.10
10	15	9.96	± 0.28
15	15	14.88	± 0.38
20	13	20.03	± 0.38

stable within 1% transmission for at least one hour after the addition of the ferrous sulfate.

Reagents. 1. *Sodium ferrocyanide.* Recrystallized in 95% ethyl alcohol. Standard solutions of 5, 10, 15, 20 and 25 mg% are prepared from this salt. 2. *Acid mixture.* 35 ml concentrated nitric acid and 40 ml 60% perchloric acid are added to 425 ml distilled water. 3. *Trichloroacetic acid.* 20%. 4. *Ferrous sulfate solution.* $FeSO_4 \cdot 7H_2O$, fine crystal, c.p. is added to saturation in 1N H_2SO_4. One part of this is added to 5 parts of the duponal solution and 7-10 drops of concentrated hydrochloric acid are added per 100 ml of this mixture. If the ferrous sulfate-duponal solution becomes turbid, it should be filtered; the solution remains clear after this filtration. 5. *Duponal solution.* Approximately 25 mg of duponal per liter of distilled water.

Procedure. Two ml of ferrocyanide standard, serum or urine, in proper dilution, are pipetted into a 20 x 150 mm ignition tube and 6 ml of distilled water added and thoroughly mixed with a footed stirring rod. Eight ml of acid mixture are then added with gentle stirring and the mixture allowed to stand 10-30 minutes with occasional stirring. After the addition of 2 ml of 20% trichloroacetic and further stirring, the tube is stoppered and centrifuged at 2500 r.p.m. for 15 minutes. Two ml of water treated in the same way provide a reagent blank. One ml of the ferrous sulfate-duponal solution is added to a 5 ml aliquot of the supernatant solution and the intensity of the blue color is then determined with a Coleman, Jr. spectrophotometer at a wave length of 700 millimicra. The reagent blank is set at 100% transmission.

Results. Recoveries of added sodium ferrocyanide to serum and urine in amounts of 5, 10, 15 and 20 mg%, give mean recoveries of 4.96, 9.96, 14.88 and 20.03 mg% respectively with standard deviations ranging from ±0.10 to ±0.38.

Conclusion. The above described procedure is a satisfactory method for measuring the sodium ferrocyanide concentration of body fluids at concentrations of 5 mg% or more.

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Sickle Cell Anemia, Blood Viscosity and Sodium Tetrathionate.* (17863)

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A decrease in blood viscosity, caused by the intravenous injection of sodium tetrathionate, has been reported by Theis and Freeland(1). This decrease apparently is accompanied by an increase in oxygen satura-

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