

or bovine serum albumins showed vigorous secondary-type responses in both 19S macroglobulin and 7S gamma globulin antibody production. The data indicate that capacity for a secondary response is not confined to 7S gamma globulin synthesis.

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### Parainfluenza 2 Virus: Increase in Hemagglutinin Titer on Treatment With Tween-80 and Ether.\* (30711)

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Treatment of myxoviruses with ether results in loss of infectivity and the release of hemagglutinin (HA) (1,2,5,7). The amount of recoverable hemagglutinin measured as guinea pig red cell agglutinin titer varies for different viruses. Compared with the HA titer of the virus preparation prior to ether treatment, there is a slight increase for Newcastle disease virus and a marked fall for parainfluenza 1 and mumps viruses(8). On the other hand, treatment with a detergent and ether results in moderate elevation of the HA titer for influenza A & B(4), Sendai and Newcastle disease viruses (fowl red cells)(9) and a marked elevation for measles virus(6).

Because of the frequent use of parainfluenza and mumps viruses in serodiagnosis by hemagglutination inhibition (HI) titration, these viruses were treated with a detergent (Tween-80) and ether in an attempt to enhance their HA titer. The results are shown in Table I. This paper reports in detail stud-

TABLE I. Tween 80-Ether (T-E) Treatment of Myxoviruses.

Virus	HA titer before T-E treatment	HA titer after T-E treatment	Rise in titer
Uninfected monkey kidney cells	<1:2	<1:2	—
Parainfluenza 1	1:8	1:8	0
" 3	1:64	1:128	× 2
Mumps	1:128	1:512	× 4
Parainfluenza 2	1:16	1:512	× 32

ies done on parainfluenza 2, because of the marked enhancement of HA titer as shown in Table I.

Parainfluenza 2 virus was obtained from Drs. Helen Coates and Robert M. Chanock (Parainfluenza 2 (CA) MK<sub>5</sub>MH<sub>3</sub>—9-27-63—Bethesda). In our laboratory it was passed once in primary rhesus monkey kidney cell monolayers (Flow Laboratories) maintained at 37°C in medium 199 in Earle's BSS without serum. On the sixth day, the virus was harvested by scraping the cell sheet down and triturating vigorously. Tween-80 was added to a final concentration of 0.125% and mixed well by shaking at room temperature for 5

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TABLE II. Tween-80-Ether (T-E) Treatment of Parainfluenza 2.

Virus	HA titer before T-E treatment	HA titer after T-E treatment	HA titer after storage at 4°C for 8 wk
Uninfected monkey kidney cells	<1:2	<1:2	—
Parainfluenza 2 MK <sub>5</sub> MH <sub>3</sub> MK <sub>1</sub>	1:16*	1:512†	1:512
" 2 MK <sub>5</sub> MH <sub>3</sub> MK <sub>1</sub>	1:64	1:1024	1:1024

\* Infectivity +.

† Infectivity 0.

minutes. Then ether was added to a final concentration of 33.3% by volume and mixed well with repeated shaking at 4°C for 15 minutes. This mixture was centrifuged at 3,000 r.p.m. for 20 minutes and the aqueous phase was withdrawn into an open container and ether was allowed to evaporate. The final volume obtained was approximately the same as the original volume. This experiment was also performed after two additional passages of the virus in primary monkey kidney tissue culture. Uninfected monkey kidney cells were treated simultaneously as control.

HA titers were performed using guinea pig red cells, as described by Chanock and Johnson(3). Infectivity was determined by inoculation of 0.1 ml aliquots into primary monkey kidney tissue culture tubes in duplicate, and overlaying with guinea pig red cells on the fifth and tenth days, as previously described (3). Presence of hemadsorption was the criterion for infectivity. HA titers of the Tween-80- and ether-treated hemagglutinin were repeated after 8 weeks of storage at 4°C. These results are recorded in Table II.

On both occasions there was a marked increase in HA titer with Tween and ether treatment. Storage at 4°C for 8 weeks did not alter this titer.

same was true for parainfluenza 2, simultaneous HI titers were done using both untreated and treated antigens. HI tests were done as described by Chanock and Johnson, but without heat inactivation of the sera, and using 0.25 ml of guinea pig red cells(3). Three examples are shown in Table III.

Thus the HI titers were slightly higher with the Tween-ether-treated antigen. Also the end points appeared more clearly defined compared with those done with the untreated antigen.

Further studies on the properties and morphology of the hemagglutinin are in progress.

*Summary.* Treatment with Tween-80 and ether resulted in a marked increase in hemagglutination (HA) titer of parainfluenza 2 virus, a slight increase for parainfluenza 3 and mumps viruses and no increase for parainfluenza 1. This parainfluenza 2 hemagglutinin had no infectivity, and the HA titer remained unaltered at 4°C for 8 weeks. The hemagglutination inhibiting antibody titers of sera tested with this antigen were slightly higher than the titers obtained with untreated antigen.

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TABLE III. HI Titers of Sera.

No.	HI using untreated antigen	HI using T-E treated antigen
1	1:32	1:64
2	1:16	1:32
3*	1:64	1:256

\* Guinea pig parainfluenza 2 antiserum.

Norrby demonstrated a 2-fold increase in serum HI titers when Tween- and ether-treated measles antigens was substituted for untreated material(6). To test whether the

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### Plasma Hematin-Binding and Clearance in the Rhesus Monkey. (30712)

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The binding of hematin to human serum proteins was suggested by Heilmeyer in 1933 on the basis of spectroscopic evidence(1). In 1934 Fairley and Bromfield described a new heme pigment in the plasma of a malaria patient(2), and Fairley later characterized the heme-albumin complex or methemalbumin more fully with studies both *in vivo* and *in vitro*(3-5). In recent years binding of hematin by human serum globulin, as well as by albumin, has been described. This heme-binding globulin has been reported to be an  $\alpha_2$ -globulin(6,7) or a  $\beta$ -globulin(8-10). The quantitative and qualitative patterns of hematin-binding by proteins in serum from many lower animals differ significantly from the pattern in human serum(6,7,11). However, in monkeys Fairley observed rapid formation of methemalbumin in plasma when hematin was injected intravenously(5), and studies of plasma heme pigments after hemolytic transfusion reactions in rhesus monkeys demonstrated patterns of hemoglobin-binding and metabolism similar to those in humans(12). In the latter studies significant amounts of methemalbumin were found in the plasma of monkeys after intravascular hemolysis. These facts suggested that the monkey would provide a useful model for the investigation of hematin-binding and metabolism.

The present studies were undertaken to compare protein binding of hematin by human and rhesus monkey plasma and to trace

the disappearance of heme-proteins from the plasma of monkeys after intravenous injection of hematin.

*Methods.* These studies were performed on female rhesus monkeys (*Macaca mulatta*) maintained on a standard monkey chow diet. Hemin was prepared from human erythrocytes by the method of Labbe and Nishida (13) and was recrystallized once. For intravenous administration hemin was dissolved just prior to use in 1% sodium carbonate (pH 11) at a concentration of 5 mg/ml. This alkaline hematin solution was freed of bacteria by filtration through a Millipore® filter† with pore diameter of 0.45  $\mu$ . In most experiments the animals were anesthetized for hematin injection with phencytidine,‡ 7.5 to 10.0 mg intramuscularly. In Experiments 1C and 2B anesthetic was not used. Hematin was injected into the saphenous vein. Blood samples were taken periodically without anesthesia from the saphenous vein, or in a few instances the femoral vein, through a #19 needle into a plastic syringe. The blood was placed in a siliconized tube, centrifuged immediately, and the plasma removed. Plasma samples were frozen and studies performed within a few days after collection. Six hematin-injection experiments were performed in three monkeys (Table I). It will be noted that 2 or 3 weeks elapsed between experiments on the same animal. In Experiment 1C the monkey was given an injection of hematin on each of the 3 days prior to the day of the actual experiment. In Experiment 3A Fe<sup>59</sup>-hematin was administered.

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