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 Horstmann, D. M., unpublished observations.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.

TABLE I. Results of Liver Function Tests and Blood Counts in 20 Normal Monkeys.

Name of test	No. of tests	Unit	Result			
			Mean	Range	S.D.	±2 S.D.
Total serum bilirubin	25	mg %	.10	.08– .18	.033	.04– .17
1′ serum bilirubin	25	mg %	.06	.03– .12	.007	.04– .07
Thymol turbidity	25	unit	.93	.25– 2.00	.40	.14– 1.72
Cephalin flocculation	25	48 hr	0	0	0	
Alkaline phosphatase	14	unit	17.46	8.2 –25.1	4.49	8.5 –26.5
Leucocyte count	24	×1000*	13.4	7.7 –20.7	3.36	6.7 –20.1
Lymphocytes	24	%	63.0	31 –85	12.20	38.0 –87.0
Neutrophiles	24	%	35.2	14.0 –67.0	12.50	10.0 –60.0
Eosinophiles	24	%	1.7	0 – 7.0	1.88	0 – 5.46

* No. of cells/mm³.
S.D. = Stand. dev. ±2 S.D. includes 95% of the observations.

urethane in milk. Six other monkeys received materials by various routes obtained from patients early in the course of infectious mononucleosis in conjunction with injections of ACTH. Following inoculation with these various materials, the monkeys were bled weekly or oftener, for liver function studies and blood counts over a period of 75 to 116 days in the hepatitis experiments and for approximately 50 days in the infectious mononucleosis experiments.

Results. Normal values. A statistical analysis§ of the values for liver function tests, leucocyte counts and differential blood counts in 20 normal monkeys prior to inoculation is shown in Table I. The highest total and one minute serum bilirubin values observed were 0.18 and 0.12 mg % respectively. The thymol turbidity value never exceeded 2.0 units and the cephalin cholesterol flocculation test was consistently negative. In contrast to these results, which are in a much lower range than is considered normal for man, the alkaline phosphatase was comparatively very high with an average value of 17.42 units. Later in the study the bromsulfalein dye excretion test was carried out 35 times in 14 monkeys. The average BSP retention at 45 minutes was 1.58 mg % with a range from 0 to 2.5 mg %. During the course of the experiments described in the next section, 185 additional determinations of serum bilirubin, cephalin-cholesterol flocculation and thymol turbidity were made. Except for 3 slight increases in serum bilirubin and in thymol turbidity all results fell within the normal limits shown in Table I. *Leucocyte counts* in normal monkeys varied from 7700 to 20000 per cubic mm with a mean of 13400 per cubic mm and the differential counts showed similar variation with a range in lymphocyte percentages from 31 to 85. Considerable variation was encountered in blood counts of individual monkeys from day to day.

Experiments with viral hepatitis and infectious mononucleosis. The design and results of experiments attempting the transmission of viral hepatitis to monkeys are summarized in Table II. In most instances cortisone, ACTH, or urethane was administered in conjunction with materials from patients with viral hepatitis in the hope of altering host-virus relationships. The results of these studies were essentially negative; during a period of 75 to 141 days no monkey became clinically ill, showed significant fever, or had more than slight and transient changes in liver function tests.‖ Thus, as shown in Table II, monkeys RH 3 and RH 28 both had increases in total serum bilirubin (TSB) to 0.22 mg %, one on the 10th day after inoculation of pooled hepatitis sera, and the other 72 days after the initiation of a 5-day period of inoculation with liver suspension from a fatal case of hepatitis. Both of these monkeys also received cortisone. Monkey 886 had rises in TSB to 0.28 and 0.32 mg % on the 25th and 53rd day respectively after the initiation of a series of repeated injections of liver suspension from 2 fatal cases of hepatitis. None of these 3 monkeys with slight increases in TSB had alterations in other liver function tests. Three other monkeys had

§ Appreciation is expressed to Major W. A. Haendiges, 98th General Hospital, for the statistical analysis.

‖ Monkey 889 died on 205th day after inoculation of uncertain cause.

TABLE II. Summary of Attempts to Produce Viral Hepatitis in Monkeys.

Monkey No.	Hepatitis inoculum: Type	Hepatitis inoculum: Route and method	Other inoculum: Type	Other inoculum: Inoc. schedule*	Other inoculum: Daily dose (mg)	Other inoculum: Total dose (mg)	Length of observations (days)†	Result
CY 001 RH 2	Pooled early sera	Multiple parenteral	Cortisone	—2 to + 5	50	350	75	Rise in TSB in RH2 to 0.22 mg % on 10th day
30	Proved S.H. sera	IV and SQ	"	—2 to +14	25	400	141	Negative
889	Duodenal juice Stool suspension	Parenteral Oral	"	—1 to + 1	25	75	103	"
27	Liver biopsy susp. Stool suspension	Parenteral Oral	"	—1 to + 1	25	75	103	"
26	Liver biopsy susp., then convalescent sera 3 wk later	Parenteral	"	—1 to 0	25	50	117	"
28 29	Liver susp. of fatal cases	IP daily for 5 days	"	—2 to +14	25	400	139	Rise in TSB in RH28 to 0.22 mg % on 72nd day
15 7 CY 004	Pool of early sera. Pool of early duodenal juice, both repeated once	Parenteral Gastric tube	ACTH	—2 to +19	40	840	115	Negative
RH 884	Proved S.H. sera	IV and SQ	Urethane	—3 to +57	500 (oral)	30500	141	"
886 887	Liver susp. of 2 fatal cases followed in 10 days by pooled acute phase sera	IP daily for 4 days IV	0				116	Rise in TSB to 0.28 and 0.32 mg % in RH 886 on 25th and 53rd day respectively

* Minus and plus refer to days before and after inoculation of hepatitis materials respectively; 0 day is the day of inoculation.

† Period during which liver function tests were done. Monkeys were observed clinically for 6 months.

IV = Intravenous; SQ = Subcutaneous; IP = Intraperitoneal; CY = Cynomologous; RH = Rhesus monkey; SH = Serum hepatitis. It is not known whether the other materials represented SH or IH.

slight and isolated increases in thymol turbidity above 2.0 units, the highest being 3.25 units.

None of the 6 monkeys inoculated with materials from patients with infectious mononucleosis in conjunction with ACTH administration developed clinical, hematological, or serological evidence of this disease and in none were alterations in liver function tests seen that exceeded the normal limits shown in Table I.

Discussion. This paper has reported unsuccessful attempts to transmit viral hepatitis to monkeys. Inocula consisted of sera, liver biopsy material or stool samples obtained from patients early in the course of viral hepatitis or of liver suspensions from fatal cases of this disease. These materials were given either alone or in conjunction with the administration of ACTH, cortisone, or urethane. Liver function tests performed weekly over a period of 75 to 141 days remained within normal limits. It is conceivable but unlikely that the incubation period might be longer than this in monkeys. Similar unsuccessful attempts were made to transmit infectious mononucleosis to monkeys receiving simultaneous injections of ACTH. The ability of adrenal hormones to increase the severity of certain infections in susceptible hosts is now well established as emphasized in a recent review(3), but in accord with the results of this paper it has been difficult to induce susceptibility to an infection with these hormones in a species naturally resistant.

In conjunction with these experiments an analysis was made of the results of liver function tests carried out in 20 normal monkeys prior to inoculation. With the exception of the alkaline phosphatase, all the results fell within a narrow range and the standard deviation was small. The reliability of these results as standards for normal is further supported by the fact that 182 of 185 additional tests of liver function carried out in the course of the experiments described were also within this normal range. It should be emphasized that the mean value, as well as the range of values, for each of these liver tests in monkeys represents very low figures as compared to values for these tests in normal humans. For this reason, human standards must not be used to interpret liver function tests in monkeys. This is particularly true of the total serum bilirubin in which the upper limit of normal in the monkey is only ¼ of that of normal man. Thus an abnormal serum bilirubin, elevated as much as 4 times above normal in a monkey might be overlooked if interpretation were based on human standards.

An exception to these low values was found to be the alkaline phosphatase. Results for this test in normal monkeys were consistently higher than seen in normal man. This may depend on a more actively metabolizing osseous system rather than on liver function *per se*.

Leucocyte counts and differential blood counts in normal monkeys were found to have great variation not only within the species but in individual monkeys. Similar findings have been reported by other investigators as summarized by Wintrobe(2).

Summary. 1. The inoculation of materials from patients with viral hepatitis or infectious mononucleosis into monkeys either alone or in conjunction with adrenocorticotropic hormone, cortisone, or urethane failed to result in evidence of successful transmission of these diseases. 2. Normal values for liver function tests, leucocyte counts, and differential blood counts in monkeys are recorded.

1. James, G., Korns, R. F., and Wright, A. W., *J.A.M.A.*, 1950, v144, 228.

2. Wintrobe, M. M., Clinical Hematology, Lea & Febiger, 1951, p. 999.

3. Kass, E. H., and Finland, M., *New England J. Med.*, 1951, v224, 464.

Received February 5, 1953. P.S.E.B.M., 1953, v82.