Serum Hepatitis in U. S. Troops in Germany.* (18351)

ALFRED S. EVANS. (Introduced by Francis G. Blake.)
From the Section of Preventive Medicine, Yale University School of Medicine.

Acute hepatitis continues to have a high prevalence among U. S. troops stationed in Germany(1). The institution of effective control measures has been made difficult by an inability to determine by clinical observations or by laboratory tests, the extent to which such cases represent naturally occurring infectious hepatitis (IH), or homologous serum jaundice (SH) or both. The purpose of this paper is to call attention to the proved existence of serum hepatitis in at least one instance among such personnel, as established by experiments in human volunteers.

The experiments involving the use of volunteers reported in this paper represent a continuation of similar studies on viral hepatitis begun in 1944 by Havens(2-5). The current study was initiated in the summer of 1949 with a view to obtaining a sample of icterogenic serum of the short incubation (IH) type for use in attempts to propagate this agent in developing chick embryos or in tissue culture. It was a surprise, therefore, when the positive sample obtained from a U.S. soldier in Germany, turned out to be the long incubation type. Because of its bearing on the control of this disease in our troops, this finding is reported.

Materials and Methods. Inoculum: Plasma samples were obtained from 2 soldiers (W.M. and J.W.), who contracted acute hepatitis in Germany†. There had been no reason to suggest clinically that their illness was serum hepatitis. The blood had been drawn on the fourth and second day of their illnesses respectively, as shown in Table I. The plasma samples had been frozen in a CO_2 dry ice box for 42 days and had been transported to this country in the frozen state, following which they were thawed, heated at 56° for 30 minutes, and tested for bacteriologic sterility.

Subjects. Volunteers, aged 21 to 28 years, were males in good physical condition and without histories of prior hepatitis. They were isolated for a period of 40 days following inoculation and observed clinically each week for a period of 10-15 weeks. *Method of inoculation*: Each sample was administered as follows: 0.5 ml subcutaneously, 0.1 ml intradermally, and 0.5 orally in 30 ml of skim milk. *Laboratory work*: Multiple tests of liver function were carried out at weekly intervals, or more often, for a period of at least 3 months.

Results. Three volunteers were inoculated with sample W.M., and 3 other volunteers with sample J.W. The results are shown in Table II. With sample W.M., none of the volunteers developed appreciable evidence of hepatitis. Minor symptoms, however, were encountered about 3 weeks after inoculation. One volunteer (A.B.) experienced anorexia, nausea, "dark urine," and tenderness over the liver unaccompanied by altered liver function tests, including bromsulfalein excretion, and at the same time a second volunteer (R.H.) had diarrhea but no other symptoms.

With serum J. W., all 3 volunteers eventually developed definite clinical and laboratory

* Representing work done under the auspices of the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of The Surgeon General, U. S. Army, Washington, D.C.

Appreciation is expressed to Mr. Allen Shank, Warden, and Dr. Leon A. Witkin, Physician, Federal Correctional Institution, Danbury, Conn., for their cooperation in this study.

1. Paul, J. R., and Gardner, H. T., *Am. J. Med.*, 1950, v8, 565.

2. Havens, W. P., Ward, R., Drill, V. A., and Paul, J. R., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 206

3. Havens, . P., Jr., PROC. SOC. EXP. BIOL. AND MED., 1945, v58, 203.

4. Havens, W. P., Jr., PROC. SOC. EXP BIOL. AND MED., 1945, v59, 148.

5. Havens, W. P., Jr., *J. Exp. Med.*, 1946, v83, 251.

† These specimens were received from the Hepatitis Research Team at the 98th General Hospital, Germany. Acknowledgement is made to Dr. J. W. Colbert and Dr. Dorothy M. Horstmann for this material.

TABLE I. Data on Patients Whose Blood Was Used in Transmission Experiments.

Patient	Age	History of inj., 1949	Character of hepatitis: Onset: 1949	Character of hepatitis: Onset: Type	Character of hepatitis: Severity of illness	Inoculum obtained 1949
W.M.	23	Penicillin treatment, August Dental treatment, July	26 Sept.	Insidious	Mild	30 Sept.
J.W.	30	Sutures for cut on leg, August Dental extraction, August	28 Sept.	"	"	30 Sept.

TABLE II. Results of Transmission Experiments in Human Volunteers.

Inoculum from donor	Method of inoculation	Volunteer	Experimental hepatitis: Early findings: Day after inoc.	Experimental hepatitis: Early findings: Type	Experimental hepatitis: Late findings: Jaundice	Experimental hepatitis: Late findings: Day after inoc. when clinical jaundice appeared	Experimental hepatitis: Late findings: Highest bilirubin mg/100 ml	Final result
W.M.	Oral and P	A.B.	17	Anorexia, "dark urine," tender liver	None			0
		R.H.	17	Diarrhea	None			0
		G.F.		None	None			0
J.W.	Oral and P	F.T.		None	Yes	53	2.96	+
		B.S.	18	Anorexia, tender liver, slight fever	Yes	68	9.96	+
		C.H.	19	Malaise, tender liver, slight fever	Yes	87	7.97	+

P = Parenteral inoculation by intradermal and subcut. inj.

evidences of jaundice with incubation periods of 53, 68, and 87 days, respectively. Two of these subjects (B.S. and C.H.) had also experienced early symptoms occurring about 3 weeks after inoculation and consisting of malaise, slight fever, back or epigastric discomfort, and tenderness over the liver without enlargement; one subject complained of early anorexia and nausea. In one of these volunteers (C.H.), similar symptoms recurred on the 73rd day after inoculation and persisted through the occurrence of definite jaundice on the 87th day. The preliminary symptoms in these 2 volunteers were not associated with alterations in liver function tests except for slight increases in thymol turbidity units in the serum of one. Elevation of values for thymol turbidity and 1-2 + cephalin cholesterol flocculation tests, however, preceded by several weeks the onset of clinical jaundice in both these volunteers.

A second experiment, details of which will not be given here, was later carried out in the hope of obtaining plasma containing the agent of I.H. In an attempt to avoid the production of serum hepatitis in these volunteers, the inoculum was given by the *oral* route alone, since I.H. is readily transmitted in this way while S.H. has been induced experimentally *only* by parenteral inoculation (6) with 2 possible exceptions(7,8). Three samples of plasma from 3 soldiers in the early stages of acute viral hepatitis were tested in 3 volunteers each by the oral route alone. None of these 9 volunteers developed clinical jaun-

6. Havens, W. P., Jr., *Medicine*, 1948, v27, 279.

7. Findley, G. M., and Martin, N. H., *Lancet*, 1943, v1, 678.

8. MacCallum, F. O., and Bauer, D. J., *Lancet*, 1944, v1, 622.

dice although transient symptoms and minor deviations in liver function tests did occur in 2 out of 3 volunteers in each group from 41 to 64 days after oral administration of the plasma and in one volunteer a serum bilirubin of 2.16 mg/100 ml was observed on the 44th day. The failure to demonstrate the agent of I.H. in these 3 acute phase blood samples can not be accepted as unequivocal evidence that it was not present since the number of volunteers employed was small. The results obtained lend some weight to those of experiment 1, *i.e.*, that serum hepatitis is at least in part responsible for jaundice in U.S. troops in post-war Germany.

Discussion. Our knowledge of the viruses of I.H. and S.H. is extremely limited and to date has been accumulated almost entirely from work with volunteers. The previous experiments have been well reviewed in a recent report by Havens(6).

The results of the present experiment establish the existence of serum hepatitis, or at least "long incubation period" hepatitis, in U.S. troops stationed in Germany in 1949 where viral hepatitis has been endemic and highly prevalent among U.S. soldiers(1). There is no evidence presented by this or other published studies which indicates the extent to which serum hepatitis may be present among these troops although there is considerable circumstantial evidence from other sources which would suggest that serum hepatitis is common(1).

The soldier whose plasma contained an icterogenic agent of the long incubation period type had been exposed in the 2 months preceding his jaundice to dental instruments for a tooth extraction, as well as to suture needles, either of which may have been contaminated with icterogenic blood. Possible transmission of this disease by dental equipment as well as by surgical instruments should thus be borne in mind.

The occurrence of clinical symptoms and of tenderness over the liver was noted 17-20 days after inoculation in 2 of 3 volunteers who developed definite jaundice at a much later date and similar findings were also noted in 2 of 3 volunteers who never developed definite jaundice. These early changes in the course of serum hepatitis have previously been noted in volunteers studied by Neefe *et al.*(9) and it is known that the causative agent is present in the blood of such volunteers during the first months after inoculation, as demonstrated by Paul, *et al.* and others(6).

Summary. The presence of serum hepatitis (SH), or at least of long incubation period hepatitis, among the hundreds of cases of acute hepatitis which have been occurring annually in U.S. troops stationed in Germany has been established by human volunteer experiments using as the inoculum acute phase plasma obtained from a soldier who had contracted hepatitis in that area. In one experiment, one of 2 sera so tested produced clinical jaundice in 3 of 3 volunteers following incubation periods of 53, 68 and 87 days, respectively. Symptoms suggesting mild hepatitis occurred about 3 weeks after inoculation in 2 of these same 3 volunteers.

9. Neefe, J. R., Stokes, J., Jr., Reinhold, J. G., and Lukens, F. D. W., *J. Clin. Invest.*, 1944, v23, 836.

Received November 15, 1950. P.S.E.B.M., 1950, v75.

Retardation of Tumor Growth in Mice by Oral Administration of Methyl Androstenediol and Methyl Testosterone. (18352)

E. J. FOLEY. (Introduced by R. Tislow.)

From the Biological Research Laboratories, Schering Corporation, Bloomfield, N. J.

The retardation of growth of transmissible tumors of mice by steroid hormones has been described by several investigators(1-4), and favorable results in the treatment of metastatic breast cancer in women by the parenteral injection of methyl androstenediol have been reported by Homburger, Kasdon and Fishman(5). The present report presents ex-