

Effect of Heat on the Agent of Homologous Serum Hepatitis.* (20599)

RODERICK MURRAY AND WILLIAM C. L. DIEFENBACH.
(Introduced by Sara E. Branham.)

From the Laboratory of Biologics Control, National Microbiological Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.

The etiological agents of serum hepatitis and of infectious hepatitis are unusually resistant to chemical and physical methods of inactivation(1). The occurrence of numerous cases of homologous serum hepatitis following inoculation of certain lots of yellow fever vaccine to which human serum had been added has demonstrated that the agent of homologous serum hepatitis can survive heating at 56°C for 30 to 60 minutes(2,3). The human serum which was used in the manufacture of early lots of yellow fever vaccine was routinely "inactivated" by heating at 56°C for one hour. Actual practice, however, probably fell somewhat short of this(2). Oliphant, Gilliam, and Larson(4) showed that a sample of dried yellow fever vaccine from one of the incriminated lots was still capable of producing hepatitis after a further period of heating at 56°C for 30 minutes. Gellis, Neefe, Stokes *et al.* have demonstrated that a mixture consisting of 40 ml of normal serum albumin and 10 ml of proved icterogenic plasma failed to produce serum hepatitis when inoculated in 10 ml amounts into 5 volunteers after it had been heated at 60°C for 10 hours (5). The difference between the amounts of heat applied in heating at 56°C for one hour and at 60°C for 10 hours is considerable, and there is no indication of the factor of safety involved in heating under the latter conditions as is required in the manufacture of Normal Serum Albumin (Human)(6). Because of this, and the fact that heated plasma might be

clinically useful were the amount of heat applied sufficient to inactivate the virus of serum hepatitis without serious damage to the proteins, it was decided to investigate the effect of heating at 60°C for 4 hours and for 2 hours by means of inoculation studies in human volunteers. Preliminary work had shown that heating at 60°C for 4 or more hours caused serious injury to the plasma as evidenced, among other things, by the occurrence of turbidity and the formation of a precipitate(7).

Effect of heating infected pool plasma at 60°C. The present study was part of an extensive program concerned with the safety of blood and blood products with respect to viral hepatitis, the general conduct of which is being reported elsewhere(8). Material from a single large pool of infected plasma, referred to as infected pool plasma, was used in all the studies on the safety of plasma and its derivatives conducted by our group.

Samples of infected pool plasma were subjected to controlled heating as follows: Four 10 ml bottles closed by means of sleeve stoppers and containing 7 ml of infected material were suspended in a water bath so that each bottle was completely immersed. The water was kept constantly agitated by means of a mechanical stirrer. Two bottles were removed after 2 hours of such heating, while the remaining 2 were removed after 4 hours. During this period of time, the temperature of the bath was maintained between 59.2 and 60.4°C. Two thermometers were used. A fifth similar bottle was kept at room temperature during the heating process. This served as a control. All bottles were shell-frozen by means of dry ice and alcohol immediately after heating and subsequently stored at -20°C until the time approached for administration to volunteer subjects. At this time the samples were transported to the institution where they were to be used in a Dewar

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TABLE I. Effect of Heating Infected Pool Plasma at 60°C.

Duration of heating, hr	No. inoc.	Cases of hepatitis		Total	Incubation period (days)
		With jaundice	Without jaundice		
2	10	3	1*	4	70, 70,* 126, 147
4	10	3	2†	5	84,† 87, 88, 91,† 97
Not heated	5	1	1	2	77, 77*

* Case of hepatitis showing clinical signs and symptoms but no jaundice.

† Cases showing only abnormal laboratory findings.

flask under dry ice refrigeration. Some of the dry ice was still present when the flask was opened at the time of the inoculations. Two groups of 10 volunteers who had been carefully screened by a series of liver function tests were inoculated with heated material. One group received 1.0 ml of the plasma which had been heated for 2 hours at 60°C and the other 1.0 ml of that which had been heated for 4 hours at 60°C. Members of a third group of 5 men were each given 1.0 ml of the control material. All volunteers were kept under close observation for 6 months. The results are summarized in Table I.

It will be seen that 3 cases of hepatitis with jaundice and one showing clinical signs and symptoms of hepatitis but with no visible jaundice occurred in the group of 10 men inoculated with plasma which had been heated for 2 hours. Three cases of hepatitis with jaundice and 2 cases showing abnormal laboratory findings occurred in the group which received the plasma which had been heated for 4 hours. One case of hepatitis with jaundice and one case of hepatitis without jaundice occurred in the group which received the control material.

Experience with untreated infected pool plasma used as control material in this and

other studies indicates that out of 55 subjects who received this material 22 (40%) developed hepatitis with jaundice, while 3 (5.5%) developed hepatitis without jaundice. Four additional subjects in these control groups showed laboratory findings suggestive of hepatitis.

Conclusions. Samples of plasma known to contain the agent of homologous serum hepatitis retained their ability to produce hepatitis even after being heated at 60°C for 4 hours.

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