PERIOD OF INFECTIVITY OF PATIENTS WITH HOMOLOGOUS SERUM JAUNDICE AND ROUTES OF INFECTION IN THIS DISEASE*

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(Received for publication, March 5, 1946)

In a preceding paper (1) the period of infectivity in infectious hepatitis has been studied. In the present paper the same type of study has been made in cases of homologous serum jaundice. Primarily it is important that a distinction be made between these two conditions. This distinction is not easy for it is quite possible that serum jaundice and infectious hepatitis may not only be closely related but confused, in that the former may occasionally represent the artificial production of the latter. Nevertheless, while the artificial production of infectious hepatitis may be true in some instances, it cannot yet be said that all serum jaundice belongs in this category. Thus it is the belief expressed in previous papers from this laboratory (2, 3) and elsewhere (4–6) that the two conditions may actually be different. Neefe, Stokes, and Gellis (7) have recently summarized certain of the differences and similarities between them. At present however, the definition of homologous serum jaundice is an artificial one: it may be described arbitrarily as a form of hepatitis ordinarily produced by the parenteral inoculation of human blood products obtained from an individual who, though not apparently ill, is carrying the causative icterogenic agent in his blood (2). Large numbers of cases have been produced in individuals who have been injected with human immune serum (8–10), with vaccines containing human serum (11–13), and with whole blood or plasma (14–16).

Considerable new information has been made available by the experimental transmission of homologous serum jaundice to human volunteers (2, 5, 7, 17, 18). It has been found that the etiologic agent (in serum) is filtrable, resistant to a temperature of 56°C. for at least 60 minutes, and is transmissible to man in serial passage. This agent is believed to be a virus and like the agent of infectious hepatitis it has not been transmitted to laboratory animals or grown

* These investigations represent work done for the Commission on Neurotropic Virus Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army.

Acknowledgment is made to Ralph H. Walker, Warden, and in particular to Dr. F. E. Priddy, Senior Physician, and to the Connecticut State Prison, Wethersfield, Connecticut, for valuable assistance and for certain facilities in the use of their institution.

in tissue culture (13, 17, 19). It has been detected in the serum of patients early in the incubation period (2) as well as in the acute phase of the disease and in practically all known instances has been transmitted to man by *parenteral* inoculation. Reports of the infectivity of nasopharyngeal washings of patients in the acute phase of disease have appeared but the evidence for positive results has been meagre (19). Recent attempts by Neefe and Stokes (20) to recover virus from the stools of patients in the acute phase of homologous serum jaundice have been unsuccessful. Moreover, with the exception of one case of experimental infection (18) of a human volunteer by *feeding* infectious serum, this disease has not been transmitted experimentally by the oral route.[1]

Experiments in the transmission of homologous serum jaundice to human volunteers have employed serum obtained from patients during the incubation period as well as the acute phase of the disease. Thus it has already been demonstrated in this laboratory that virus may be present in the circulating blood of an apparently well human volunteer 34 days after experimental inoculation with a strain of homologous serum jaundice and 60 days before onset of his disease (2). Little is known, however, as to the limits of the infective period. Oliphant (17) has reported that the blood of patients convalescent from serum jaundice was no longer infectious 2½ months after disappearance of jaundice when inoculated into human volunteers.

It is the purpose of the following experiment to determine: (1) the period of infectivity of patients with experimentally induced homologous serum jaundice; (2) the infectivity of stools of patients in the acute phase of serum jaundice; and (3) whether serum jaundice can be produced in human volunteers by ingestion of serum known to be infectious.

Materials and Methods

Subjects.—29 male human volunteers varying in age from 21 to 41 years were employed as experimental subjects; they were all housed in the same institution. All subjects were followed for 135 days after inoculation and were instructed to report at once the occurrence of any symptoms, which were then investigated.

Virus.—The strain of homologous serum jaundice virus used in this laboratory was originally derived from a serum pool collected by Lieutenant Colonel A. B. Sabin from British and

[1] During early transmission experiments in this laboratory (21) a mixed pool of serum obtained from 3 patients with homologous serum jaundice and 1 patient with infectious hepatitis, produced hepatitis in 3 human volunteers following ingestion. This is believed to represent a transmission of infectious hepatitis rather than homologous serum jaundice, for the following reasons: (1) repeated attempts to infect human volunteers by feeding infectious material similar to the homologous serum jaundice component of the pool have failed, while parenteral inoculation of the same material has been successful in transmitting this disease; (2) feeding infectious material similar to the infectious hepatitis component of the pool has regularly produced this disease in human volunteers; (3)patients convalescent from homologous serum jaundice induced by parenteral inoculation of that component of the pool are not immune to reinoculation with the infectious hepatitis component of the pool (3).

American troops in the course of studies on sand-fly fever in Egypt (22). The donors, who were ill with sand-fly fever when the blood was drawn, showed no signs at that time of jaundice and the icterogenic capacity of the blood was a chance finding.[2] This icterogenic agent or virus has now been through four passages in human volunteers. It withstands heating to 56°C. for at least 30 minutes. It has produced clinical jaundice in 14 out of 31 human volunteers, inoculated parenterally, with incubation periods ranging from 56 to 134 days. Experimental subjects convalescent from this type of hepatitis produced by this agent are not immune to experimental re-infection as induced by the strain of infectious hepatitis used in this laboratory (3).

Laboratory Observations.—All volunteers were inoculated on the same day. Starting 60 days after inoculation the following tests of liver function were performed every 2 weeks (or more often) until the end of the period of observation: (1) quantitative total serum bilirubin;[3] (2) cephalin-cholesterol flocculation (Hanger) (23); and (3) thymol turbidity test (24). If the cephalin flocculation or thymol turbidity test became positive, bromsulfalein dye retention[4] was estimated. Only volunteers who had symptoms and signs of homologous serum jaundice in conjunction with consistently abnormal cephalin-cholesterol flocculation and/or bromsulfalein dye retention were regarded as positive cases. In this study all positive cases had clinical jaundice.

EXPERIMENTAL

Materials for testing employed in this experiment were pools of serum and stools obtained at various times during the incubation period and course of disease of 3 volunteers with experimentally induced homologous serum jaundice. These men had contracted homologous serum jaundice experimentally 56, 66, and 70 (average 64) days respectively after parenteral inoculation of the strain of homologous serum jaundice used in this laboratory. All 3 patients had clinical jaundice with a mild course of disease (21). Serum was collected from each man: (*a*) three-fourths through the *incubation period*, (*b*) during the *first week of disease*, (*c*) and in the *convalescent period* 28 to 32 days after onset. Stools were collected in the *first week of disease*. Acute phase stools and sera were collected from the same patient on the same day. All specimens were stored at dry-ice box temperature for a period of 14 to 15 months. Before use all material was thawed at room temperature. Equal amounts of the same material from each patient were pooled and treated in the following manner: *serum* was heated to 56°C. for 30 minutes in a water bath to eliminate bacteria; *stools* were ground with sterile alundum and suspended in enough sterile 10/M buffered sodium phosphate to make a 10 per cent suspension. This was centrifuged at 1500 R.P.M. for 15 minutes at room temperature to remove coarse particles. The supernate was removed and centrifuged at 6500 R.P.M. for 30 minutes at room temperature. This fairly clear supernate was removed and filtered through a Seitz EK filter at a pH of 7.0. The filtrate was then heated to 56°C. for 30 minutes in a water bath.[5] Both serum and filtrates of stool were sterile. Before administration the serum was diluted 1:3 with sterile 10/M buffered sodium phosphate and the stool filtrates were diluted with 30 cc. of tap water.

[2] Of the 11 men whose blood went into this pool, 10 were followed for a period of 4 months. None of them reported having had jaundice. The eleventh man had left the country and could not be traced.

[3] According to the method of Malloy and Evelyn (25) using the photoelectric colorimeter.

[4] 10 per cent retention of dye in the blood 30 minutes after the intravenous injection of 5 mg./kg. of body weight was considered maximum normal.

[5] Stool extracts are prepared (heating and filtration) in this manner in order to eliminate bacteria. This treatment does not eliminate the virus of infectious hepatitis from stools (1).

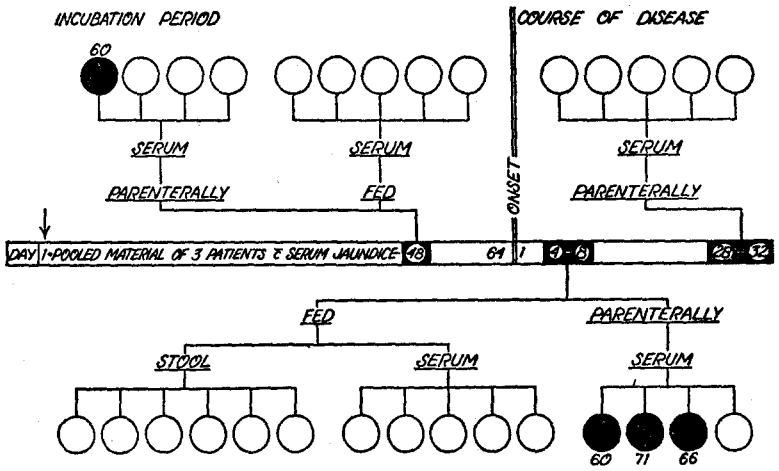

FIG. 1. Illustration of results of administration to human volunteers of pools of serum and stool obtained three-fourths through (48th day) the incubation period and during the acute (4 to 8th day) and convalescent (28 to 32nd day) phases of 3 patients with experimentally induced homologous serum jaundice. The horizontal bar in the middle of the diagram indicates the general course of the 3 patients who were donors of the material to be tested. Incubation periods of the donors were 56, 66, and 70 (average 64) days respectively. Serum was obtained from each man three-fourths through his respective incubation period (average 48 days). Open circles indicate volunteers who were inoculated and failed to contract the disease; black circles indicate volunteers who contracted homologous serum jaundice. The figure adjacent to the black circle represents the length of incubation period in days.

TABLE I

Data on 4 Human Volunteers with Homologous Serum Jaundice Experimentally Induced by Parenteral Inoculation of 0.16 Ml. of Icterogenic Serum

Volunteer	Source of inoculum*	Duration days			Maximum		Severity
		Incubation period	Fever	Jaundice	Fever	Serum bilirubin	
		days	*days*	*days*	*°F.*	*mg. per cent*	
KY	Incubation period (3 patients)	60	8	30	101.0	17	+++
HY	4th–8th day disease (3 patients)	71	0	10	98.6	3.9	+
KI	" " " " " "	60	7	26	99.6	6.3	++
BK	" " " " " "	66	14	60	99.4	40	++++

* *Cf.* Fig. 1.

The small number of tests that can be performed in experimentation of this type, limit the statistical value of the results. Nevertheless, the positive results are significant and the negative ones less so. In brief the results were as follows: 29 human volunteers were fed or inoculated with the stool filtrates or serum to be tested. Serum obtained both during the incubation period and during the first week of disease (and heated as above described) was infectious on sub- and intracutaneous inoculation, producing the disease in 4 out of 8 human volunteers. The same serum when fed to 10 other volunteers failed to produce clinical disease or deflection of liver function tests. Acute phase stools when fed and convalescent phase serum when inoculated sub- and intracutaneously were apparently not infectious (Fig. 1). Certain salient facts about the clinical course of disease of the 4 patients who contracted serum jaundice experimentally are recorded in Table I.

DISCUSSION

The results are based upon a single experiment performed with one strain of homologous serum jaundice. One should not generalize too far about these results. However, in so far as the experiment goes, it substantiates the assumption that certain differences exist between homologous serum jaundice and infectious hepatitis. These differences are apparent in relation to the period of infectivity of the blood, the effective route of inoculation, and the presence or absence of the virus in the feces.

Attention has already been drawn in this paper to an earlier observation (2) from this same laboratory, in which the serum of a human volunteer inoculated experimentally with the virus of homologous serum jaundice was found to contain virus 34 days later and 60 days before onset of his disease. Little else has been known as to when the virus appears in the blood or how long it remains there.

In the present communication virus has been demonstrated experimentally from a serum pool collected from 3 patients three-fourths through their respective 56, 66, and 70 day incubation periods and again during the acute phase of disease. There was insufficient virus in the convalescent phase serum of these same patients to produce infection when inoculated into human volunteers.

It may be pointed out here that the presence of virus in the serum both one-third and three-fourths through the long incubation period of patients with homologous serum jaundice, may, if substantiated by further experiments, constitute a difference between this condition and infectious hepatitis in which one attempt to demonstrate virus midway through the short incubation period of a single patient has been unsuccessful (1). This difference might be based on the fact that the incubation period of serum jaundice is generally so much longer than that of infectious hepatitis. It is possible that for the first 20 to 30

days of the incubation period the blood stream might be free of virus in both diseases but that in serum jaundice there is a subsequent long (30 to 150 day) period of viremia preceding the development of hepatitis. This has a bearing on the choice of donors for transfusions since an apparently healthy person may carry the virus of homologous serum jaundice in the blood for a long period and thereby constitute a menace.

Pooled specimens of feces obtained from 3 patients in the acute phase of homologous serum jaundice when virus was demonstrated to be present in the serum failed to produce the disease when fed to volunteers. Moreover, serum from the incubation period and acute phase of disease, which was proven to be infectious by patenteral inoculation, failed to produce disease when ingested. This is another difference between our two strains of homologous serum jaundice and infectious hepatitis. In the latter condition virus has been found in the stools and also the disease has been produced experimentally by feeding infectious serum. These facts suggest: (1) that invasion of the blood occurs during the long incubation period of homologous serum jaundice; (2) that in convalescence virus has not been demonstrated, and its absence may be the result of some form of neutralization which may occur by the 4th week of disease; (3) the failure to demonstrate virus in the stools of patients with homologous serum jaundice as well as the failure to transmit this disease by feeding known infectious material suggests that the intestinal-oral route is not of importance in the spread of homologous serum jaundice. This constitutes another difference experimentally, at least, from infectious hepatitis.

SUMMARY

1. Pooled specimens of serum obtained from 3 human volunteers three-fourths through their respective 56, 66, and 70 day incubation periods of homologous serum jaundice produced the disease in 1 out of 4 human volunteers following parenteral inoculation.

2. Serum specimens obtained from these same 3 patients during the acute, pre-icteric phase of their homologous serum jaundice produced the disease in 3 out of 4 human volunteers following parenteral inoculation.

3. These same sera, proven to be infectious by parenteral inoculation, failed to produce disease when ingested by 10 other human volunteers.

4. Pooled specimens of serum obtained in the convalescent phase (28 to 32 days after onset) of these 3 patients failed to produce apparent infection when inoculated parenterally into 5 human volunteers.

5. Pooled specimens of feces of 3 patients obtained in the acute phase of homologous serum jaundice, when virus was proven to be in the serum, were not demonstrably infectious when fed to 6 volunteers.

6. These findings are slightly different from those encountered in a similar study with infectious material from cases of infectious hepatitis.

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