PERIOD OF INFECTIVITY OF PATIENTS WITH EXPERIMENTALLY INDUCED INFECTIOUS HEPATITIS*

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Considerable new information has been made available by the experimental transmission of infectious hepatitis[1] to human volunteers. It has been found that the etiologic agent is filtrable (1–3), resistant to a temperature of 56°C. for at least 30 minutes (1), and is transmissable to man in serial passage (1). This agent is believed to be a virus. It has been detected in the stool and serum of patients in the pre-icteric and early icteric phases of the disease and may be transmitted to human volunteers by feeding or by parenteral inoculation (1–11). Reports of the infectivity of urine (2, 4, 6) and nasopharyngeal washings (6) of patients in the acute phase of the disease have been contradictory and evidence for the positive results has been meagre. Recent attempts by Neefe and Stokes to recover "virus" from such materials obtained during an epidemic of infectious hepatitis in a children's camp have been unsuccessful (3).

Most of the experiments in the transmission of infectious hepatitis to human volunteers have employed infectious material obtained from patients in the acute phase of the disease but little is known as to the limits of the infective period; namely, when the etiologic agent appears in the blood or stool, or how long it remains there. The importance of the latter is evident in view of experimental evidence that the *intestinal-oral* circuit may be one of the methods of spread of the naturally occurring disease. As already mentioned the data on these points are scant. Voegt (4) has reported that the duodenal contents of patients obtained on the 24th and 30th days of the naturally occurring disease (before jaundice) are infectious when fed to human volunteers. Neefe and Stokes (10) found that stools obtained 3 weeks after the disappearance of jaundice in human volunteers convalescent from experimentally induced infectious hepatitis were not infectious when fed to other human volunteers.

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[1] In this paper a distinction has been made between infectious hepatitis and homologous serum jaundice.

There is as yet no information available concerning the existence of ambulatory intestinal carriers of this disease or whether patients with relapse have "virus" present in their blood or stool.

It is the purpose of this paper to report the results of experiments on the determination of: (1) the period of infectivity of patients with experimentally induced infectious hepatitis; (2) the infectivity of urine and nasopharyngeal washings of patients in the pre-icteric phase of experimentally induced infectious hepatitis.

Materials and Methods

Subjects.—34 male human volunteers varying in age from 19 to 34 years were employed as experimental subjects. These men were housed in two segreated groups in New Haven, Connecticut, during the two 4 month periods of the experiments (February 14 to October 31, 1945). They were instructed to take certain precautions which included scrupulous personal hygiene with careful cleansing of the hands after defecation or urination. None of the men inoculated had any contact with the kitchen or preparation of the food. All meals were eaten at the dormitory and none of the inoculated subjects ate in public or private houses. Water or soft drinks were allowed outside only if obtained in paper containers which were destroyed by the subject. No one who was not a part of the experiment was allowed to eat at the dormitory. During this period there were a small number of cases of infectious hepatitis in the city of New Haven although there was no evidence that any contact cases developed among members of the experimental group. The number of subjects employed in testing various materials is necessarily small due to the difficulty in obtaining human volunteers.

Virus.—The strain of virus used in this laboratory was originally obtained from the stool of a U. S. Army soldier (BE) who contracted *epidemic infectious hepatitis* in Sicily in September, 1943. It has been through four passages in human volunteers to date. This agent (1) is filtrable through an L2 Chamberland filter and withstands heating to 56°C. for at least 30 minutes. It has produced the disease in 27 out of 40 human volunteers (including this experiment) inoculated parenterally or orally with incubation periods ranging from 15 to 34 days.

Observation of Experimental Subjects.—All subjects were followed for periods of 110 to 115 days after inoculation. Each man was seen by the author at least three times a week after inoculation and questioned as to symptoms. All subjects were instructed to report at once the occurrence of any symptoms, which were then investigated. Those volunteers who contracted infectious hepatitis were hospitalized in the Isolation Pavilion of the New Haven Hospital under our supervision.

Laboratory Observations.—The following tests of liver function were performed at weekly intervals on all volunteers who received the material to be tested throughout the two periods of the experiments (4 months each). Those volunteers who reported symptoms or sickened had more frequent determinations made: (*a*) quantitative total serum bilirubin,[2] (*b*) cephalin-cholesterol flocculation, (Hanger) (13), (*c*) bromsulfalein dye retention test,[3] (*d*) thymol turbidity test (14), (*e*) determination of urobilinogen (15), and bilirubin (16) in urine. Normal values for each volunteer were established before inoculation. Only volunteers who had symptoms and signs of infectious hepatitis in conjunction with consistently abnormal brom-

[2] According to the method of Malloy and Evelyn (12) using the photo-electric colorimeter.

[3] 10 per cent retention in the blood 30 minutes after the intravenous injection of 5 mg. bromsulfalein per kg. of body weight was considered the maximum normal level.

sulfalein dye retention and cephalin-cholesterol flocculation were regarded as positive cases. In this study all positive cases had clinical jaundice.

Experiment 1

Methods and Materials.—Materials for testing employed in this experiment were serum and stools obtained at various times during the incubation period and course of disease of one human volunteer (ZI) with experimentally induced (by feeding) infectious hepatitis as follows:

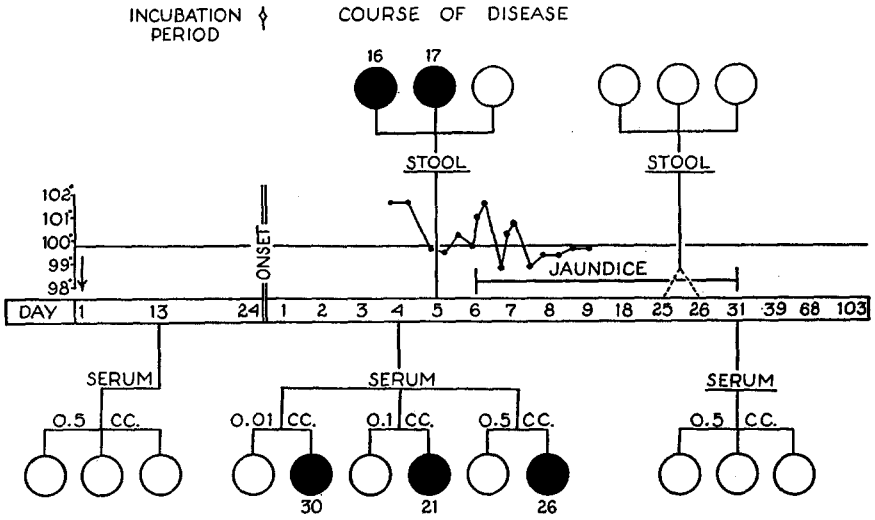

FIG. 1. Illustration of results of administration to human volunteers of serum and stool obtained during the midincubation period (13th day), acute (4th to 5th day), and convalescent phases (25th, 26th, 31st days), of experimentally induced infectious hepatitis in one patient (ZI). The arrow indicates the time of infection of patient (ZI) and the subsequent incubation period, onset and course of disease, and convalescence are indicated through the 103rd day. Rectal temperatures are recorded. Open circles indicate volunteers who were inoculated and failed to contract infectious hepatitis; black circles indicate volunteers who contracted infectious hepatitis. All sera were inoculated parenterally and all stools were fed. The figure adjacent to each black circle represents the length of incubation period in days.

serum was collected from ZI half way through the incubation period, on the 4th day of disease, and again on the 31st day of disease. Stools were collected on the 5th day of disease and again on the 25th and 26th days of disease. Both serum and stools were stored at dry-ice box temperature for a period of 4 to 5 months and were thawed at room temperature before use. The serum was sterile and the stools contained no pathogenic bacteria. The serum was diluted with sterile 10/M buffered sodium phosphate before use and the stool was administered in a double gelatin capsule.

18 volunteers were fed or inoculated with the serum or stools to be tested. The results are indicated in Fig. 1. It is apparent that serum collected midway through the incubation period of patient ZI was not infectious for 3 human

volunteers in the amount inoculated. Serum and stool, however, obtained 4 and 5 days after onset of disease and 1 and 2 days before the appearance of jaundice in the patient (ZI) contained enough virus to produce infectious hepatitis with jaundice in 5 out of 9 human volunteers. Serum and stools obtained in the convalescent phase, 25 to 31 days after onset of disease, were not infectious in the amount administered.

In Table I are recorded certain salient facts concerning the duration and height of fever, duration of jaundice, maximum measured level of total serum bilirubin, and severity of disease in the 5 patients who contracted infectious

TABLE I

Duration and Height of Fever and Jaundice and Severity of Illness in 5 Human Volunteers with Experimentally Induced Infectious Hepatitis

Volunteer	Inoculum	Source*	Amount	Route	Duration			Maximum		Severity
					Incubation period	Fever	Jaundice	Fever‡	Serum Bilirubin	
					days	*days*	*days*	*°F.*	*mg. per cent*	
KL	S	4th day disease (ZI)	0.01 cc.	P	30	5	24	104.4	8.6	+++
SN	"	" " " "	0.1 "	"	21	11	12	103.8	4.4	++
BK	"	" " " "	0.5 "	"	26	6	23	104.8	6.7	+++
LG	F	5th day disease (ZI)	1 gm.	O	16	8	16	102.8	8.3	++
WE	"	" " " "	1 "	"	17	7	14	101.8	4.1	+

S = serum; F = feces; P = parenteral; O = oral.

* *Cf.* Fig. 1.

‡ Rectal temperatures are recorded.

hepatitis. The onset of illness in each of these men was accompanied by vague generalized complaints of malaise and fatigue with the abrupt appearance of fever and more severe constitutional symptoms 2 to 4 days later. Recovery was uneventful and complete in all 5 patients.

3 of the 6 volunteers who received acute phase serum in amounts ranging from 0.01 cc. of 0.5 cc. contracted infectious hepatitis with jaundice. The length of incubation period was apparently not influenced by size of dose and no particular relationship between severity of disease and amount of inoculum was demonstrated.

Experiment 2

Although it was demonstrated that the stool of a single patient with experimentally induced infectious hepatitis was no longer infectious to human volunteers 25 and 26 days after onset, it appeared desirable to test pooled specimens of feces from several patients during the convalescent period in an attempt to detect the appearance of a possible convalescent carrier state. At

the same time, it also seemed desirable to test the infectivity of urine and nasopharyngeal washings of patients in the acute phase of infectious hepatitis when virus could be demonstrated to be present in the blood and feces.

Materials and Methods.—Materials employed in this experiment consisted of the following pools of specimens obtained during the acute (1st to 8th day) stage of experimentally induced (by feeding) infectious hepatitis of 5 human volunteers: serum, urine, stools, nasopharyngeal washings. A pool of stools from the convalescent phase (26th to 29th day) from the same

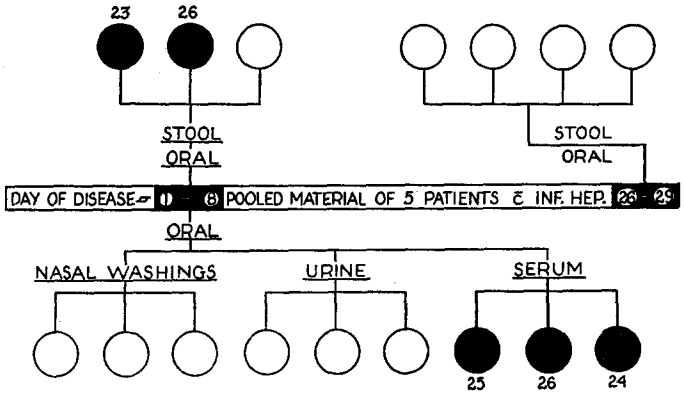

FIG. 2. Illustration of results of administration to human volunteers of pools of serum, stool, urine, and nasopharyngeal washings obtained in the acute phase (1st to 8th day) and a pool of stools obtained in the convalescent phase (26th to 29th day) of 5 patients with experimentally induced infectious hepatitis. Open circles indicate volunteers who were inoculated and failed to contract infectious hepatitis; black circles indicate volunteers who contracted infectious hepatitis. The figure adjacent to each black circle represents the length of incubation period in days.

patients was also made. The various acute phase specimens were always collected from the same patient on the same day and all had been stored at dry-ice box temperature for periods ranging from 7 to 12 months. The nasopharyngeal washings had been made originally with sterile 10/M buffered sodium phosphate. 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: (*a*) serum, (*b*) urine, and (*c*) nasopharyngeal washings were heated to 56°C. for 30 minutes in a water bath, cultured, and stored overnight in the ice box; (*d*) 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 ranging from 6.8 to 7.2. The filtrates were then heated to 56°C. for 30 minutes in a water bath, cultured, and stored overnight in the ice box. All material was sterile. Before administration the various materials were diluted in 30 cc. of tap water.

16 volunteers were fed the stool filtrates, serum, urine, and nasopharyngeal washings to be tested. The nasopharyngeal washings were also given intranasally. The results are indicated in Fig. 2. Both acute phase serum and

stool were infectious as might be expected, producing hepatitis in 5 of 6 volunteers. Urine and nasopharyngeal washings, obtained on the same days as the stools and sera which were demonstrated to be infectious, apparently did not contain virus in sufficient amount to produce clinical disease or deflection of liver function tests in the subjects tested. Convalescent phase stools were again negative. It is of interest that the only man who failed to contract the disease after receiving infectious acute phase stool, gave the history that his father and sibling had had infectious jaundice when he was a child. Although he had not been sick, it is quite possible that he had had subclinical infection and obtained immunity. In Table II are recorded certain salient

TABLE II

Duration and Height of Fever and Jaundice and Severity of Illness in 5 Human Volunteers with Experimentally Induced Infectious Hepatitis

Volunteer	Inoculum	Source*	Amount	Route	Duration			Maximum		Severity
					Incubation period	Fever	Jaundice	Fever‡	Serum bilirubin	
			cc.		*days*	*days*	*days*	*°F.*	*mg. per cent*	
BT	F	1st—8th day disease (5 patients)	10	O	23	6	9	102.6	3.0	+
KY	"	" "	10	"	26	9	31	101.6	11.0	++++
VW	S	" "	0.5	"	25	14	24	103.5	14.0	++++
CK	"	" "	0.5	"	26	8	27	102.8	23.0	++++
SD	"	" "	0.5	"	24	6	8	103.4	1.4	++

S = serum; F = feces; O = oral.

* *Cf.* Fig. 2.

‡ Rectal temperatures are recorded.

Feces were administered as Seitz filtrates of 10 per cent suspensions.

facts about the duration and height of fever, duration of jaundice, maximum measured level of total serum bilirubin, and severity of disease in the 5 volunteers who contracted infectious hepatitis. While the incubation periods are quite similar there was a wide variation in severity and duration of disease.

DISCUSSION

It has been pointed out in this paper that although serum and feces of patients in the acute phase of either naturally occurring or experimentally induced (by feeding) infectious hepatitis are infectious when administered to human volunteers, little else is known as to when or how long the etiologic agent is in the blood or feces. It is obvious that for the final determination of the ex-

istence and limits of a carrier state, pooled specimens from many patients in various stages of convalescence as well as patients with relapse of disease must be tested. In a single patient in whom we have demonstrated "virus" in the serum and feces in the acute phase of disease, there was insufficient "virus" in serum obtained midway through the incubation period or in serum and stools obtained in the convalescent period to produce apparent infection when fed or inoculated into human volunteers.

It may be pointed out here that the failure to find "virus" in a patient during the midincubation period of infectious hepatitis, if confirmed by further instances, will constitute a difference between this condition and homologous serum jaundice in which the presence of "virus" has been demonstrated in serum obtained a third and two-thirds the way through the incubation period of the latter disease in human volunteers (9, 21).

Pooled specimens of feces obtained from 5 other patients in the convalescent phase of infectious hepatitis also failed to produce the disease although acute phase feces and serum from the same patients were proven to be infectious. In contrast to the reports of others, urine (2, 4) and nasopharyngeal washings (6) obtained in the acute phase of the disease, when virus was demonstrable in the serum and stools, were not infectious. These facts suggest: (1) that invasion of the peripheral blood may be a relatively late phenomenon in the incubation period of infectious hepatitis; (2) that in convalescence "virus" disappears, perhaps as a result of some form of neutralization which may occur by the 4th week of the disease. Although little is known about the actual existence of antibodies in infectious hepatitis, it is evident from the demonstration by Stokes and Neefe (17) and others (18, 19) of the prophylactic protection against infectious hepatitis conferred by the administration of human gamma globulin, that certain neutralizing substances may exist in the blood of the adult population. Whether such substances develop spontaneously or following subclinical or clinical attacks of the disease is unknown. The lack of appreciable differences in length of incubation period in human volunteers receiving widely different amounts of one strain of "virus" parenterally suggests that size of dose within this range plays little part and that differences of strain and individual host susceptibility may be important in influencing the different lengths of incubation period reported by various laboratories. In further support of this concept are data from previous experiments (1, 20, 21) in this laboratory, which have indicated that route of inoculation does not influence the length of incubation period when the same strain of "virus" is fed or inoculated parenterally.

SUMMARY

1. Serum and stools obtained in the pre-icteric phase of one patient, and pooled specimens of the same materials from 5 patients with experimentally

induced (by feeding) infectious hepatitis produced the disease in 10 out of 15 human volunteers following feeding or parenteral inoculation.

2. Pooled specimens of urine and nasopharyngeal washings from 5 patients, obtained in the acute phase of infectious hepatitis when virus was proven to be in the stool and serum, were not demonstrably infectious when fed and given intranasally to 6 volunteers.

3. Serum obtained in the midincubation period of one patient with experimentally induced infectious hepatitis failed to produce apparent infection when inoculated parenterally into 3 human volunteers. This is in contrast to the situation in homologous serum jaundice in which "virus" has been demonstrated in the sera of volunteers during the incubation period.

4. Serum and stools obtained from one patient and pooled specimens of stools from 5 patients 25 to 31 days after onset of experimental infectious hepatitis failed to produce apparent infection in 10 human volunteers.

5. No appreciable difference was detected in length of incubation period following the parenteral administration of widely different amounts of the same strain of "virus."

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