Clinical Studies

Homologous Serum Hepatitis and Infectious (Epidemic) Hepatitis*

Studies in Volunteers Bearing on Immunological and Other Characteristics of the Etiological Agents

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In a previous report,¹ the pertinent literature was reviewed and the results of preliminary studies suggesting antigenic and other differences in the etiological agents of homologous serum hepatitis and infectious (epidemic) hepatitis were described. The present report deals with additional studies which confirm and extend the preliminary observations.

MATERIALS, METHODS AND GENERAL PROCEDURE

A. Etiological Agents. The hepatitis viruses used in these studies were obtained from three different immediate sources:

1. Virus SH. This virus was present in the pool of mumps convalescent plasma that has been described in previous reports as plasma A.¹,² It probably is the same virus that, as a result of its presence in certain lots of yellow fever vaccine, was responsible for a large outbreak of hepatitis in the United States Army in 1942.¹ This virus (in plasma A) consistently produced acute hepatitis in volunteers two to four and one-half months after its parenteral injection. As this syndrome is characteristic of that described as homologous serum hepatitis or jaundice,¹,²,³ the causative agent will be referred to here as virus SH (virus, serum hepatitis). The following human biological materials related to this virus were used for the studies in volunteers:

(a) Plasma A: The origin and preparation of this pool of mumps convalescent plasma and the probable relationship between the hepatitis agent it contained and that in the icterogenic lots of army yellow fever vaccine have been described in detail elsewhere.¹,³ (b) Feces Pools 1, 2, and 3 FSH: These preparations also have been described in detail in a previous report.⁴ Briefly, these pools were composed of feces specimens obtained from six volunteers during various stages of acute hepatitis that had been induced by parenteral injection of virus SH (plasma A). In addition to the crude preparations, a bacteriologically sterile Seitz filtrate of a mixture of feces pools 2 and 3 FSH was employed in one of the present studies. (c) Nasopharyngeal washing pool 1

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NPSH: This consisted of a pool of the saline washings from the nose, pharynx and throats obtained from two volunteers on the fifth and thirteenth day, respectively, of acute hepatitis due to parenterally injected virus SH (in plasma A). The washings were frozen immediately after collection and were stored at \(-20^\circ\text{C.}\) until used. (d) Urine pool 1 USH: This consisted of a pool of four urine specimens collected (without preservative) between 10:00 P.M. and 8:00 A.M. on each of four successive nights from one volunteer from the ninth to the thirteenth day of acute hepatitis induced by the parenteral injection of virus SH (in plasma A). Each specimen was frozen immediately after collection and the four specimens were stored at \(-20^\circ\text{C.}\) until used. Prior to use, the specimens were thawed and equal quantities from each were pooled to form pool 1 USH.

2. Virus I. H., Pa. This virus was responsible for an epidemic of infectious hepatitis that occurred during the summer of 1944 at a civilian summer camp in Pennsylvania.\(^5\) In this epidemic, the virus was transmitted to the majority of the persons by contaminated drinking water. In volunteers, this virus consistently produced acute hepatitis eighteen to thirty-seven days after its entry by the oral route. As the syndrome was typical of that referred to as infectious hepatitis and the virus was encountered in Pennsylvania, it will be referred to herein as virus I. H., (Pa.). The following materials related to this virus were used for the studies in volunteers: (a) Feces pool 1 FIH: This preparation consisted of feces specimens obtained from four persons during the early stages of acute infectious hepatitis contracted at the summer camp mentioned above. The origin and preparation of this pool and a Seitz filtrate derived from it have been discussed in detail in a previous report.\(^5\) (b) Feces pool 3 FIH: This has been described in an earlier report\(^4\) and consisted of a pool of single specimens of feces obtained from two volunteers approximately three weeks (thirty-third and forty-third days of disease, respectively) after the disappearance of jaundice due to orally administered virus I. H., (Pa.). The pool was prepared in the same manner as pool 1 FIH and was stored at \(-20^\circ\text{C.}\) until used. (c) Feces pool 7–8 FIH: This consisted of a pool of feces specimens obtained from each of four volunteers during the early stages of infectious hepatitis induced by the parenteral injection of virus I. H., Pa. (pool 2 SIH). The preparation contained portions of each of the following specimens: (1) two specimens obtained from one volunteer during the week prior to the onset of hepatitis; (2) thirteen specimens obtained from the four volunteers during the first week of the disease; (3) six specimens from three of the volunteers during the second week of the disease; (4) four specimens obtained from two of the men during the third week of the disease. The pool was prepared in the same manner as that described elsewhere in connection with the preparation of feces pool 1 FIH.\(^5\) (d) Serum pool 1 SIH: The origin and preparation of this pool have been described in detail elsewhere.\(^5\) This pool consisted of eight sera obtained during the first or second weeks of the disease from eight patients with infectious hepatitis acquired at the summer camp and due to virus I. H., (Pa.). (e) Serum pool 2 SIH: This pool, which has been described in detail elsewhere,\(^5\) consisted of thirty-nine serum specimens obtained from four volunteers before and after the onset of hepatitis induced by the oral administration of feces pool 1 FIH (containing virus I. H., Pa.). (f) Nasopharyngeal washings, pools 1 and 2 NPIH: These pools were prepared from nasopharyngeal washings obtained from patients with hepatitis due to virus I. H., Pa. who had acquired the disease during the summer camp epi-
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demic. The two pools included specimens from twenty-six patients collected at all stages of the disease. The details concerning the specimens and preparation of the pools have been described elsewhere.5 (g) Urine pool 1 UIH: This consisted of portions of urine specimens collected from thirty-eight patients with hepatitis due to virus I. H., Pa. who had acquired the disease during the summer camp epidemic. The details concerning the specimens and preparation of the pool have been presented elsewhere.5

3. Virus I. H., S. Feces containing this virus were provided for these studies by Dr. W. P. Havens and Dr. J. R. Paul. The origin and properties of this virus have been described in detail in a recent report by Havens.6 The virus originally was obtained from the feces of a United States Army soldier who acquired epidemic infectious hepatitis in Sicily in September, 1943. This virus, in feces or serum, produced the disease in human volunteers (Havens) inoculated orally or parenterally after incubation periods of fifteen to thirty-four days. As the characteristics of the disease associated with this virus correspond to the syndrome described as infectious hepatitis1 and apparently are similar to those associated with virus I. H., Pa., the virus will be referred to herein as virus I. H., (S.). The material used in the present study, feces pool 1, FIH, (S.), consisted of pooled feces specimens obtained from volunteers with hepatitis due to oral inoculation with this virus by Dr. Havens.

B. Dosage of Infectious Materials. As the hepatitis viruses cannot as yet be isolated in pure form, the quantity administered can be estimated only in terms of the quantity of material used. In some of the various experiments reported herein, the dosages have not been listed as the quantities used were known to be infective from results of other experiments previously reported or from the results in control subjects reported herein. In the experiments on the effect of route of entry on the incidence of hepatitis, comparable doses were given by the parenteral and oral routes. Specific mention of dosage will be made in those experiments in which the dosage may have influenced the results obtained.

C. General Procedure. The general conduct of the transmission experiments in humans has been detailed in previous reports.1,2,4,6,7,8 The term "normal" will be used herein to refer to volunteers who had no past history of recognized hepatic disease, no present history, physical signs or laboratory evidences of existing hepatic disturbance, and who had no previous inoculations with infectious materials. Subjects under thirty-five years of age were selected. A group of hepatic studies was carried out two or more times weekly before and after their inoculation with infectious, or potentially infectious, materials. The hepatic studies included the total and prompt direct-reacting serum bilirubin determinations, urine bilirubin and urobilinogen studies, cephalin cholesterol flocculation, thymol, and colloidal gold tests, total serum protein, albumin and globulin determinations, total and esterified serum cholesterol analyses and the bromsulphalein test.5,8 Clinical and laboratory observations were continued for at least six months after inoculation irrespective of whether or not the subjects developed hepatitis. Volunteers who were inoculated with materials related to virus I. H., Pa., or I. H., S., were isolated for two to four months after the date of inoculation. Volunteers inoculated with materials related to virus SH were not isolated. All syringes and needles used in these studies were carefully cleaned and autoclaved for fifteen to thirty minutes after each use. Control subjects living under the same conditions as those inoculated were observed and sub-
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D. Time Relationships in Immunity Studies. The interval of time between an immunizing infection and the challenge inoculation used to test for the presence of immunity is of importance in the evaluation of the results. The length of this interval depends on whether it is measured from the time of onset, recovery, or from some other stage of the immunizing infection. In respect to hepatitis, the time at which antibodies begin to appear cannot as yet be determined. It is probable, however, that they are present long before recovery from hepatitis is complete. Furthermore, the time of recovery often is difficult to determine, as objective clinical evidences may disappear while laboratory evidence of hepatic disturbance persists or clinical manifestations may persist after the subsidence of laboratory evidence of hepatic disturbance. For these reasons, the interval between the immunizing infection and the challenge inoculation was measured from the time the diagnosis of hepatitis (initial immunizing infection) was definitely established to the time of the challenge inoculation.

E. Diagnosis. The diagnosis of hepatitis with jaundice was made in volunteers who developed the typical clinical and laboratory manifestations of the disease associated with visible jaundice and with a total serum bilirubin concentration higher than 2.0 mg. per 100 ml. The diagnosis of hepatitis with subclinical jaundice was made in those with typical symptoms and signs of hepatitis associated with a significant increase in the total serum bilirubin concentration above the preinoculation level for that volunteer and above the upper limit of the normal range (1.4 mg. per 100 ml.) but not exceeding 2.0 mg. per 100 ml. The diagnosis of hepatitis without jaundice was made in those who developed, after the usual incubation period, symptoms and signs suggestive of hepatitis and definitely significant laboratory evidences of hepatic disturbance but without elevation of the total serum bilirubin concentration above the upper limit of normal (1.4 mg. per 100 ml.). In such cases, an elevation of the total serum bilirubin concentration above the preinoculation range for the person concerned but not above the upper limit of the normal range (1.4 mg.) frequently was noted. Only those cases in which the authors were confident of the diagnosis are included in this category and they are therefore listed as hepatitis in the results. The diagnosis of questionable hepatitis was made in those who developed questionably significant, mild abnormalities in the responses of one or more of the various hepatic tests and who had no symptoms or signs suggestive of hepatitis. In such cases, the findings possibly could have been due to a very mild hepatitis but also probably could be explained by other non-specific disturbances. Cases falling in this group are mentioned in the results but are not listed as hepatitis. In none of the cases were the manifestations clinically significant or sufficient to interfere with routine duties.

STUDIES ON VIRUS S. H. (TABLE I)

1. Effect of Route of Entry of Virus SH. (a) Parenteral route: Twenty-five normal volunteers have been injected parenterally with virus SH (in plasma A). Of these, eighteen (72 per cent) developed acute hepatitis after two to four and one-half months, the disease being associated with visible jaundice in fourteen (78 per cent) and with subclinical jaundice in four (22 per cent). One additional person showed slight abnormalities in the results of the hepatic laboratory tests but these were not considered sufficient to warrant a diagnosis of hepatitis. Parenteral doses of 1, 2, 5, 9, 10, 12 and 250 cc. of plasma A induced hepatitis. The 2 cc. parenteral dose was
used most commonly. The twenty-five men were inoculated in groups of three to five men between October, 1943, and November, 1945. As no difference in incidence or severity of the disease was observed in the groups inoculated at different times, it appears that the virus retained its original activity after three and one-half years of residence in frozen plasma. The results in this group have been used, in the interpretation of the results of other experiments to be described, as an index of the expected incidence of hepatitis (72 per cent) in normal volunteers of this age group following parenteral injection of this agent. (b) Oral route: Virus SH (in 4 to 10 cc. doses of plasma A) was administered orally to ten apparently normal volunteers. During the subsequent period of observation (six to twelve months), none developed clinical or laboratory evidence suggestive of hepatic disturbance. Virus SH (plasma A, 4 cc.) also was administered orally to four volunteers who, during the preceding six months, had recovered from an induced attack of infectious hepatitis due to virus I. H., (Pa.).

None of these men developed clinical or laboratory evidences of hepatic disturbance during a six-month period of observation.

The results show that virus SH was highly effective in producing obvious hepatitis when it was injected parenterally. In contrast, it was relatively ineffective when entry was by the oral-intestinal route, no signs of active infection being detected. Not including the negative results in the four men who previously had had infectious hepatitis, the difference in incidence of active hepatitis with the parenteral and oral routes of entry is statistically significant.
the probability of the difference occurring as a result of chance being once in 2,000 trials (Chi square = 12.08). The results also suggest that a previous infection with virus I. H., Pa. did not increase the susceptibility to orally administered virus SH. This observation is of interest because of the results of a previous study suggesting that a previous attack of hepatitis due to virus SH was followed by greater than normal susceptibility to parenterally injected virus I. H., (Pa.).

2. Presence of Virus SH in Human Biological Materials. The results described above show that virus SH may be present in the blood. Attempts to demonstrate this virus in the feces of patients with hepatitis due to this agent have been described previously in part. Feces pools 1, 2, or 3 FSH were administered orally to nineteen apparently normal subjects and subsequently, the Seitz filtrate, obtained from a mixture of feces pools 2 and 3 FSH, was injected parenterally into five apparently normal subjects. None of those receiving the feces preparations orally or parenterally developed clinical or laboratory evidences of hepatitis during a six-month period of observation. One of the five injected parenterally showed slight abnormalities of uncertain significance in the results of certain hepatic tests but these alone were not sufficient to be regarded as adequate evidence of hepatitis and they were not supported by recognizable clinical manifestations.

In view of the failure of plasma A, which was known to contain virus SH, to induce apparent hepatitis when administered orally, the failure of feces from patients with hepatitis due to virus SH to induce apparent infection when administered orally provides no evidence concerning the presence or absence of the virus in the feces. The negative results thus could be explained either by an absence of the virus in the feces or, if present, by the relative ineffectiveness of the virus when administered orally. However, the negative results on subjects inoculated parenterally with the Seitz filtrate from the feces pools strongly suggest that the virus was not present in the feces preparations used herein, at least not in quantities sufficient to be detected by this method of testing. The results thus provide considerable evidence that virus SH was not present in detectable amounts in the feces of patients with active hepatitis due to parenterally injected virus SH.

The attempts to demonstrate virus SH in the nasopharyngeal washings and urine of patients with active hepatitis due to this virus have been too limited to warrant any conclusions. It has seemed desirable, however, to record (briefly) the results obtained to date. Nasopharyngeal washings pool 1 NPSH (see materials), after thawing, was sprayed into the nose and throat of each of four volunteers. A total of 4 to 5 cc. was administered, the material accumulating in the pharynx being swallowed. None of these men developed significant evidence of hepatitis during a six-month period of observation. Fifty cc. of urine pool 1 USH were given orally to the only volunteer available at the time. He showed no evidence of hepatitis during a ten-month period of observation. These results provide no conclusive evidence regarding the presence or absence of virus SH in the nasopharyngeal secretions and urine for the following reasons: (a) The pools did not include specimens from sufficient cases of this type of hepatitis; (b) the pools did not include specimens obtained during the earliest stages of the disease; (c) too few volunteers were available for an adequate test of the materials described; (d) even though a greater number of specimens representing all stages of the disease from a greater number of persons with this type of hepatitis had been tested, negative results would not have indicated an absence of
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this virus in these materials since they were not tested by the parenteral route and the studies described above have indicated that this agent may not be effective by the oral-intestinal route. Thus no conclusions can be drawn from these studies with nasopharyngeal washings and urine obtained from patients with hepatitis due to virus SH.

3. Characteristics of Hepatitis Induced by Parenterally Injected Virus SH. The date of onset of hepatitis induced by this virus frequently was difficult to determine. In some cases, transient episodes characterized by mild symptoms and laboratory evidence of mild hepatic disturbance occurred prior to onset of clinically recognizable acute hepatitis. The onset of acute hepatitis leading up to the development of jaundice often was insidious and the initial symptoms often were so mild that their significance might have been overlooked had they not been associated with laboratory evidence of significant and progressively increasing hepatic disturbance. In some cases, laboratory evidence of hepatic disturbance was obtained before the onset of symptoms. None of the eighteen cases had elevations of temperature exceeding 100°F. (oral) at the onset. For these reasons it often was difficult to determine the exact incubation period, the day of onset frequently being indistinct. Thus it has seemed preferable in referring to the interval between inoculation and the occurrence of hepatitis to use the interval between inoculation and the development of jaundice (first significant elevation of serum bilirubin). Using this criterion, the interval from inoculation to jaundice in the eighteen cases of hepatitis due to parenteral injection of virus SH varied from two to four and one-half months. This interval apparently was not significantly influenced by the various doses of virus SH (in plasma) used in these studies.

4. Resistance to Reinfection with Virus SH (Homologous Immunity) Following Recovery from Previous Infection with This Virus. Nine volunteers who had recovered from hepatitis induced by parenteral injection of virus SH subsequently were reinoculated parenterally with this virus. The results have been reported in part in a preliminary report. In respect to the severity of the first attack, three had overt jaundice of moderate degree, two had overt jaundice of mild degree, two had subclinical jaundice with symptoms of moderate degree, and two had very transient subclinical jaundice with only mild symptoms. The interval in months from the initial attack of hepatitis to the challenge inoculation of the nine volunteers was two and three-fourths, four and one-fourth, four and one-fourth, five and three-fourths, five and one-fourth, five and three-fourths, eight, nine and three-fourths, and eleven and three-fourths months, respectively. Following the challenge inoculation, none of the nine volunteers developed incapacitating symptoms or jaundice although five showed transient mild symptoms and/or laboratory findings suggestive of mild hepatic disturbance after one and one-half, two, two, three and four and one-half months, respectively. Although the findings in these five cases were suggestive of mild hepatic disturbance presumably related to the challenge inoculation with virus SH, the manifestations were not sufficient to interfere with the usual activities of the men and a diagnosis of hepatitis could not be established with certainty. None of the other four men (challenge inoculations with virus SH received after intervals of two and three-fourths, eight, nine and three-fourths, and eleven and three-fourths months) showed any manifestations, clinical or laboratory, suggestive of hepatitis. Thus clinically significant hepatitis was not observed in any of the nine men following a second parenteral inoculation with virus SH. As the expected
incidence of clinically significant hepatitis in apparently normal volunteers inoculated parenterally with virus SH for the first time is 72 per cent, the failure of any of the nine men tested to develop clinically significant hepatitis or jaundice provides strong evidence that the previous infection with virus SH was followed by complete, or nearly complete, resistance to the same virus for the periods of time involved in this investigation.

**STUDIES ON VIRUS I. H., PA. TABLE II**

1. Effect of Route of Entry. (a) Oral route—

_Feces pool 1 FIH_ has been administered orally to thirty-three apparently normal volunteers. Twenty-four (73 per cent) developed typical infectious hepatitis, the interval from inoculation to onset ranging from seventeen to twenty-seven days. Twenty-two (91 per cent) of the twenty-four cases of hepatitis developed overt jaundice, and two had hepatitis without jaundice. Another developed suggestive symptoms and signs but had no laboratory findings to support a diagnosis of definite hepatitis. The _Seitz filtrate of feces pool 1 FIH_ was administered orally to three volunteers and all three (100 per cent) developed typical infectious hepatitis with jaundice,

<table>
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<th>Material</th>
<th>Volunteers</th>
<th>Route</th>
<th>No Hepatitis</th>
<th>Questionable Hepatitis</th>
<th>Definite Hepatitis</th>
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<td>0</td>
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<tr>
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<td>3</td>
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<td>Parenteral</td>
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<td>0</td>
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<tr>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1</td>
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<tr>
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*Results of transmission experiments with materials containing or related to virus I. H., (Pa.). See legend of Table I for explanation of volunteers designated "previous S.H." and "previous I.H."
the onset occurring after intervals of twenty-eight, thirty, and thirty-two days, respectively. Serum pool 2 SIH was given orally to three volunteers and, after intervals of twenty-six and thirty-three days, respectively, two (66 per cent) developed typical infectious hepatitis with jaundice.

The results show that feces pool 1 FIH, the Seitz filtrate obtained from it, and serum pool 2 SIH contained virus I. H., Pa. and that the agent was filtrable. Combining the 3 groups, a total of thirty-nine men have received this virus orally. Twenty-nine (74 per cent) developed unquestionable infectious hepatitis, the interval from inoculation to onset ranging from seventeen to thirty-three days. The expected incidence of hepatitis in apparently normal persons of this age group following oral inoculation with virus I. H., Pa. thus would be in the neighborhood of 74 per cent (minimum 66 per cent).

The subjects receiving feces pool 1 FIH, the Seitz filtrate, and serum pool 2 SIH were inoculated in groups of three to six men over an interval of fourteen months. During this time the materials were stored in the frozen state at approximately minus 20°C. No apparent difference was observed in the incidence or severity of the disease in groups inoculated soon after collection of the materials as compared with those inoculated after storage of the material in the frozen state for periods up to fourteen months. Thus virus I. H., Pa. apparently retained its activity in spite of residence for at least fourteen months in the frozen feces preparations.

The studies concerning feces pool 1 FIH cited above and certain others reported elsewhere or as yet unpublished suggest some relationship between the quantity administered orally and the incubation period of the disease. In experiments involving different doses of feces pool 1 FIH (virus I. H., Pa.), the results have suggested that a decrease of the quantity administered orally below a certain critical level (less than 1.8 cc. of feces pool 1 FIH) resulted in a prolongation of the incubation period up to thirty-seven days. (b) Parenteral route: The bacteriologically sterile Seitz filtrate of feces pool 1 FIH (1 cc.) was injected subcutaneously into three apparently normal volunteers. None developed clinical or laboratory evidences of hepatitis during a six-month period of observation. Serum pool 2 SIH (2.1 to 3 cc.) was injected parenterally into six apparently normal volunteers. After an interval of thirty-five days, one developed infectious hepatitis with subclinical jaundice (maximum total serum bilirubin concentration 1.8 mg. per 100 cc.). Sometime between the thirty-seventh and fifty-second day, another developed weakly positive thymol flocculation and colloidal gold tests but he had no symptoms or physical signs suggestive of any illness at any time and multiple other hepatic tests revealed no laboratory evidences suggestive of hepatic disturbance. If this represented a very mild infection, it apparently was not of clinical significance as he continued his usual physical activities, including moderately strenuous work and exercise, throughout the post-inoculation period. The other four showed no clinical or laboratory evidence of hepatitis during a six-month period of observation.

Thus, of nine apparently normal men inoculated parenterally with materials known to contain virus I. H., Pa., only one developed definite hepatitis and another may have had a subclinical infection. Using un­questionable hepatitis as the criterion, the incidence of hepatitis in this group of apparently normal persons inoculated parenterally with virus I. H., Pa. was eleven per cent and this one case was relatively mild. As the minimum incidence following oral administration of the same infectious materials (Seitz filtrate pool 1 FIH, serum pool
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In addition to the nine men who received parenterally the materials known to contain virus I. H., Pa., three other volunteers were injected parenterally with serum pool 1 SIH. In none of the three volunteers developed clinical or laboratory evidences of hepatitis during a six-month period of observation. Unfortunately, volunteers were not available for a test of this material by the oral route so that the presence of the virus in this serum pool was not definitely established. As described in a previous report, this pool consisted of portions of eight serum specimens collected from six patients during the first week and from two patients during the second week of hepatitis due to virus I. H., (Pa.). Based on previous experience and that of others, the virus should have been present in this pool. However, the significance of the negative results of the parenteral inoculations with this material (pool 1 SIH) is uncertain due to the lack of definite evidence that the virus was present.

In view of the apparent relative resistance of normal persons to parenterally injected virus I. H., Pa., it is of particular interest that three of four men who had recovered from hepatitis due to virus SH (and then had shown resistance to reinfection with virus SH) developed hepatitis twenty-eight, twenty-eight and thirty-seven days, respectively, after parenteral inoculation with virus I. H., Pa. (serum pool 2 SIH, 2 cc.). The fourth man also developed similar symptoms and signs starting on the thirty-fifth day but the only significant laboratory evidence of hepatitis was the development, and persistence for one month, of a 4+ cephalin cholesterol flocculation test. It appears probable that these manifestations also were due to mild hepatitis. The incidence in this group thus was 75 to 100 per cent depending on the interpretation of the fourth case. The severity of the disease in these three (or four) cases was mild. One developed overt jaundice (maximum total serum bilirubin, 4.0 mg. per 100 ml.), two developed subclinical jaundice, and the fourth (questionable case) had no jaundice.

2. Presence of Virus I. H., Pa. in Human Biological Materials. Pools of serum, feces, urine and nasopharyngeal washings obtained from patients with hepatitis due to virus I. H., Pa. (oral-intestinal route of entry) have been tested for the presence of the agent as measured by their effects in apparently normal human volunteers. As described herein and in a previous report, serum (pool 2 SIH) and feces (pool 1 FIH) obtained during the active stages of the disease contained the virus whereas urine (pool 1 UIH) and nasopharyngeal washings (pools 1 and 2 NPIH) administered by the oral-intestinal route failed to induce the disease, suggesting that the virus was not
present in these materials, at least in quantities demonstrable by tests in volunteers.

In a preliminary study concerning the duration of the intestinal carrier state reported elsewhere, four single specimens of feces obtained from two volunteers approximately three weeks (thirty-third and forty-third day of disease, respectively) after the disappearance of jaundice due to orally administered virus I. H., Pa. were pooled and the pool (3 FIH) was administered orally to seven volunteers. None developed any evidences of hepatitis during a six-month period of observation suggesting that the virus was not present in these specimens.

At the present time, pools of feces and serum specimens obtained at frequent intervals between the third and tenth months of the disease from three volunteers with active chronic non-icteric hepatitis following acute hepatitis induced by orally administered virus I. H., Pa. are being tested for the presence of the agent. The pooled serum and feces preparations from these cases were administered to separate groups consisting of five apparently normal volunteers. During the seventy days which have elapsed to date, no definite evidences of hepatitis have been detected. The details and final results of this study will be reported later.

As feces specimens obtained from patients with hepatitis due to parenterally injected virus SH apparently did not contain the virus, it seemed important to determine if the feces of patients with hepatitis due to parenterally injected virus I. H., Pa. contained this virus. For this reason, feces pool 7–8 FIH (consisting of specimens obtained at frequent intervals from four volunteers during the early stages of hepatitis induced by parenteral injection of virus I. H., Pa.) was administered orally to five apparently normal volunteers. During the subsequent six-month period of observation, none of the five developed a clinically apparent attack of hepatitis or jaundice. Two of the five, however, presented laboratory evidences suggestive of mild hepatic disturbance starting after thirty days and persisting intermittently for approximately six weeks. The laboratory findings consisted of positive cephalin cholesterol flocculation tests (3 to 4+), weakly positive thymol tests and intermittent urobilinogenuria. Because of the timing and as no other cause was apparent, it seems probable that these manifestations were due to mild subclinical hepatitis. However, the results of this study must be regarded as inconclusive.

3. Characteristics of Hepatitis Induced by Virus I. H., Pa. In contrast to the onset of hepatitis due to virus SH, the onset of hepatitis due to virus I. H., Pa. usually was abrupt and suggestive of the onset of a generalized infection. There rarely was any difficulty in determining the day of onset of the disease. Symptoms and signs usually preceded significant laboratory evidence of hepatic disturbance by twenty-four to seventy-two hours. Fever was observed during the first days of the disease in thirty-three of thirty-four cases and in all of these, the temperature exceeded 100°F. (oral) at some time during the preicteric stage of the disease. This occurred irrespective of the route of inoculation (oral or parenteral). A febrile onset with the temperature exceeding 100°F. (oral) also was observed in four of five cases of hepatitis without overt jaundice due to this agent. One or more frank chills were not uncommon the first few days and chilly sensations were noted by nearly all of the patients. In the cases who received virus I. H., Pa. orally, the interval from inoculation to onset ranged from seventeen to thirty-three days and that from inoculation to jaundice ranged from twenty-two to thirty-seven days. In those who were injected parenterally with virus I. H., Pa., the interval from inoculation to onset ranged from twenty-eight to thirty-seven days. The interval from inoculation to jaundice in the
one of the five who developed overt jaundice was thirty-six days.

4. Resistance to Reinfection with Virus I. H., Pa. Following Recovery from Previous Infection with This Virus. Four volunteers who had recovered from infectious hepatitis with overt jaundice due to virus I. H., Pa. were challenged by oral inoculation with the same virus (in feces pool 1 FIH) five, seven and one-half, eight and nine months, respectively, after the onset of the previous attack of hepatitis. None of these men developed clinical or laboratory evidences of hepatitis during a six-month period of observation. Eight additional volunteers who had recovered from infectious hepatitis due to virus I. H., Pa., but who did not develop overt jaundice during the course of the disease, also were challenged by oral inoculation with the same virus (in feces pool 1 FIH) one, two, four, four and one-half, four and three-fourths, five and seven months after the onset of the previous attack of hepatitis. None of these men developed clinical manifestations or jaundice. Except for one man (challenged two months after the onset of the previous infection) who showed abnormal urobilinogenuria on the twenty-fourth and thirty-eighth days after the challenge inoculation, none showed evidence of hepatic disturbance detectable by the multiple tests used. Thus, none of twelve volunteers, challenged by oral inoculation with virus I. H., Pa. two to nine months after a previous infection due to the same agent, again developed significant evidence of hepatitis. As the incidence of hepatitis in normal groups of persons of this age group inoculated orally with virus I. H., Pa. (in feces pool 1 FIH) has averaged 74 per cent, these data provide significant evidence that an infection with virus I. H., Pa. was followed by resistance to reinfection by the same virus for at least the time periods covered in this experiment.

STUDIES OF HETEROLOGOUS IMMUNITY FOLLOWING INFECTIONS WITH VIRUSES S. H. AND I. H., PA. TABLE III

1. Studies on Susceptibility to Virus I. H., Pa., Following Infection with Virus S. H. These studies have been described in a previous report.1 Five men resistant to parenterally injected virus SH, as demonstrated by challenge inoculation, were challenged (one orally, feces pool 1 FIH; four parenterally, serum pool 2 SIH) with virus I. H., (Pa.). Four of the five developed unquestionable acute hepatitis after intervals of twenty-five (oral inoculation), twenty-eight, twenty-eight and thirty-seven days. The fifth man, after thirty-five days, developed a mild illness associated with positive cephalin cholesterol flocculation tests which probably, but not unquestionably, also represented a mild type of hepatitis. Thus, these men with demonstrated resistance to reinfection with virus SH were not resistant to infection with virus I. H., (Pa.). In view of the previously cited evidence suggesting that normal volunteers were relatively resistant to parenterally injected virus I. H., Pa. (11 per cent incidence of very mild hepatitis), the occurrence of clinically significant hepatitis in three and possibly all of the four men of this group who were inoculated parenterally suggests that the previous infection with virus SH was followed by an increased, rather than a decreased, susceptibility to virus I. H., (Pa.). To summarize the results of this experiment, five men, following an infection with virus SH, first were found to have complete, or nearly complete, resistance to reinfection with virus SH and then were found to be susceptible to infection with virus I. H., (Pa.). The results apparently indicate that resistance against virus SH developed as a result of the first attack of hepatitis due to that virus. This afforded protection against reinfection
with the same agent, but did not afford protection against virus I. H., (Pa.).
Following recovery from the attack of hepatitis due to virus I. H., Pa., and five to eight months after the onset of that attack, four of the five men cited above again were challenged by oral inoculation with virus I. H., Pa. (feces pool 1 F1H). None of the four developed clinical or laboratory evidences of hepatitis during a six-month period of observation, and at the termination of the experiment, all appeared to be in good general condition without any clinical or laboratory findings suggestive of residual hepatic disturbance. Liver biopsies (surgical excision at laparotomy) were obtained from the four men, who had had four inoculations with infectious material and two attacks of hepatitis, just prior to the termination of the experiment. At the time of biopsy, no gross abnormalities in the size or appearance of the liver were apparent. The reports on the microscopic examinations of the biopsy specimens, which are not yet available but will be described in detail in a subsequent report, should provide interesting information concerning the completeness of recovery from multiple attacks of virus hepatitis.

2. Studies on Susceptibility to Virus SH Following Infection with Virus I. H., Pa. After apparently complete recovery from hepa-

### Table III*

<table>
<thead>
<tr>
<th>Volunteers</th>
<th>Challenge Inoculation</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Virus</td>
</tr>
<tr>
<td>Previous infection with Virus S.H.</td>
<td>9</td>
<td>S.H.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I.H., Pa.</td>
</tr>
<tr>
<td>Previous infection with Virus I.H., Pa.</td>
<td>12</td>
<td>I.H., Pa.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I.H., S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.H.</td>
</tr>
<tr>
<td>Previous parenteral inoculation with Virus I.H., Pa., without subsequent apparent infection</td>
<td>9</td>
<td>I.H., Pa.</td>
</tr>
</tbody>
</table>

*Results of studies pertaining to homologous and heterologous immunity following apparent or possible inapparent infections with viruses SH and I. H., (Pa.). Expected average incidence of hepatitis in normal persons (no previous inoculations or history of hepatitis) of this age group inoculated with these viruses for the first time were as follows: Virus SH parenteral, 72 per cent; Virus I. H., Pa. oral, 74 per cent, parenteral, 11 per cent; Virus I. H., S. oral, 50 per cent on basis of small control group.
The data indicating that resistance produced by, and affording protection against, virus SH did not protect against virus I. H., Pa., and that resistance produced by, and affording protection against, virus I. H., Pa., did not protect against virus SH provide strong evidence of an antigenic difference in the two viruses. These observations indicate that at least two viruses, which may be different viruses or only different strains of the same virus, are concerned in the problem of virus hepatitis.

3. Resistance to Virus I. H., S., Following Infection with Virus I. H., Pa. In view of the apparent antigenic difference between virus SH and virus I. H., Pa., it appeared desirable to investigate the antigenic relationship between the infectious hepatitis virus obtained in Pennsylvania (virus I. H., Pa.) and that obtained from a patient who acquired infectious hepatitis in Sicily (virus I. H., S.). Four men who apparently had completely recovered from hepatitis due to virus I. H., Pa., and one who had failed to develop hepatitis after oral inoculation with this virus (feces pool 1 FIH) were inoculated orally with feces pool I. H., S., five and one-half to eight months after the onset of the attack of hepatitis caused by virus I. H., (Pa.). None of these five men developed clinical or laboratory evidences of hepatitis during a six-month period of observation. One (50 per cent) of the only two normal volunteers available at the time as controls for this experiment developed acute hepatitis twenty-five days after oral inoculation with feces pool I. H., (S.). The second volunteer showed laboratory findings suggestive of mild hepatic disturbance starting twenty-eight days after inoculation, but these were not sufficient to warrant a definite diagnosis of hepatitis. Although the groups are too small for the results to be of definite significance, they suggest that the previous infection with virus I. H., Pa. produced antibodies that were effective in protecting against virus I. H., (S.). This in turn suggests an antigenic similarity in viruses I. H., Pa. obtained in Pennsylvania and virus I. H., S. obtained in Sicily.

4. Observations Bearing on the Relationship between the Virus Responsible for So-called "Catarrhal Jaundice" and Viruses I. H., Pa. and SH. Although there appears to be no basis for differentiation between so-called "catarrhal jaundice" and infectious hepatitis, the following observations are of interest: (a) F.D.W.L. developed a disease in 1932 that was diagnosed as "catarrhal jaundice" by Dr. T. Grier Miller, Clinical Professor of Medicine at the School of Medicine of the University of Pennsylvania. In 1942, during an attack of mumps, he received an intravenous infusion of 250 cc. of plasma A (before this plasma was known to contain virus SH). Seventy-six days later he developed acute hepatitis associated with intense jaundice that persisted for three months. This series of events showed that an attack of "catarrhal jaundice" did not result in immunity effective against virus SH ten years later. (b) Volunteer H. J. C. developed in 1939, while a student at Yale University, a disease that was diagnosed as "catarrhal jaundice." In 1943, he was injected parenterally with virus SH and after sixty-seven days developed acute hepatitis. Subsequently, after recovery, he was reinoculated parenterally with virus SH and developed no definite indications of hepatitis during a six-month observation period. During the following year he received two inoculations (six months apart) with virus I. H., Pa., one parenteral and one oral. He showed no signs of hepatitis following either of these inoculations. Of particular interest is the fact that he was the only one of a group of six men subjected to the same series of four inoculations (two with virus SH, two with virus I. H., Pa.) who failed to develop hepatitis as a result of the first inoculation (third of the series) with virus.
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I. H., (Pa.). He also was the only one of the six men who, previous to participation in these experiments, had had a recognizable attack of hepatitis (“catarrhal jaundice” in 1939). This series of events suggests that he may have been protected from virus I. H., Pa. as a result of immunity acquired from the previous “catarrhal jaundice” rather than as a result of the previous infection with virus SH. The results also show that an attack of “catarrhal jaundice” did not produce immunity effective against virus SH injected parenterally four years later but may have produced immunity effective against virus I. H., Pa. five years later. (c) Subject M. W. reported an attack of jaundice in childhood at the age of six (1927) that was diagnosed as “catarrhal jaundice.” In 1945, at six-month intervals, he received two inoculations with virus I. H., Pa. orally. He did not develop significant clinical manifestations or other definite evidence of acute hepatitis as a result of either inoculation. He did not develop significant clinical manifestations or other definite evidence of acute hepatitis as a result of either inoculation. This series of events suggest that his resistance to virus I. H., Pa. may have been due to immunity acquired as a result of the attack of “catarrhal jaundice” eighteen years earlier.

These cases show that two persons were not resistant to virus SH four and ten years after so-called “catarrhal jaundice.” They also show that two persons apparently were resistant to virus I. H., Pa. five and eighteen years after an attack of “catarrhal jaundice.” The observations suggest an antigenic similarity of the causative agent of so-called “catarrhal jaundice” and virus I. H., (Pa.). This is not unlikely as there is no available evidence of a difference in the two diseases. On the other hand, the findings suggest a difference in the antigenic properties of the causative agent of “catarrhal jaundice” and virus SH. In view of the evidence cited herein of an antigenic difference in viruses I. H., Pa. and virus SH, an antigenic difference between the virus of “catarrhal jaundice” and that of virus SH would be expected as the former probably is the same or closely related to virus I. H., (Pa.).

STUDIES RELATING TO POSSIBILITIES OF ACTIVE IMMUNIZATION AGAINST VIRUS I. H., PA.

In this study, advantage was taken of the apparent resistance of normal persons to parenterally injected virus I. H., Pa. and their marked susceptibility to this virus administered by the oral route. Nine men who failed to develop clinical or laboratory evi-

<table>
<thead>
<tr>
<th>Observation</th>
<th>Virus I. H., Pa.</th>
<th>Virus S. H.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Type of onset of hepatitis</td>
<td>Abrupt and usually with fever exceeding 100°F.</td>
<td>Comparatively insidious and usually afebrile or with fever not exceeding 100°F.</td>
</tr>
<tr>
<td>2. Interval from inoculation to onset of acute hepatitis</td>
<td>17 to 37 days</td>
<td>2 to 4½ months</td>
</tr>
<tr>
<td>3. Incidence of hepatitis in normal volunteers following oral inoculation</td>
<td>High</td>
<td>0</td>
</tr>
<tr>
<td>4. Incidence of hepatitis in normal volunteers following parenteral inoculation</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>5. Presence of agent in feces</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>6. Resistance to infection after previous infection with virus I. H., Pa.</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>7. Resistance to infection after previous infection with virus S. H.</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

* Summary of results with Viruses I. H., Pa. and SH indicating that they are not identical viruses and affording satisfactory evidence of the existence of at least two different strains or types of hepatitis viruses.
evidences of active infection following parenteral injection of virus IH (three with the Seitz filtrate of feces pool 1 FIH, three with serum pool 2 SIH, and three with serum pool 1 FIH) were inoculated orally with feces pool 1 FIH six to thirteen months after the previous parenteral injection. These men had no previous history of recognizable hepatitis. None of the nine men developed clinical or laboratory evidences of hepatitis during the six-month period of observation following their oral inoculation with feces pool 1 FIH. As hepatitis would be expected to occur in approximately 74 per cent of normal persons of this age group inoculated orally with this material, the failure of any of these nine men to develop evidences of active infection strongly suggests that they had been immunized by their previous parenteral inoculations which had produced no signs of active infection. Supporting this possibility is the evidence of acquired immunity provided by the negative results following challenge inoculations with orally administered virus I. H., Pa. (in feces pool 1 FIH) of the eight volunteers who previously had had very mild hepatitis without jaundice due to virus I. H., Pa.

Although these studies provide no information of the total duration of such acquired immunity, these observations suggesting that active immunization may be possible appear to be of considerable importance. They also provide evidence that the decreased susceptibility of the general population over thirty-five years of age to infectious hepatitis may be due, at least in part, to immunity acquired as a result of inapparent or subclinical infections. This is of interest in connection with the apparent presence of antibodies in human immune serum globulin prepared from large pools of normal human adult plasma which have been shown to be effective in protecting against virus I. H., Pa. and other strains of infectious hepatitis virus. 10, 11, 12

At the time of initiation of our studies on virus hepatitis in 1943, the authors were inclined to share the opinion of many that the etiological agents responsible for infectious hepatitis and homologous serum hepatitis probably were the same and that the differences noted in respect to incubation period, type of onset and frequency of secondary cases possibly were due to a difference in the route of entry. The results of studies reported herein have indicated, however, certain differences which appear to be incompatible with the concept of the existence of only one strain of hepatitis virus. The differences apparent from the results obtained to date in relation to the two viruses studied (virus SH and virus I. H., Pa.) are summarized in Table IV. These data, particularly the lack of cross immunity indicating an antigenic difference, justify the conclusion that virus SH and virus I. H., Pa. are not identical viruses and appear to establish the existence of at least two different strains or types of hepatitis virus.

Havens and his associates 9, 13 have studied a strain of infectious hepatitis virus obtained in Sicily. The behavior of this virus in volunteers apparently has been almost identical with that of virus I. H., Pa. with one possible exception. The reported data of Havens and Paul et al. indicate that the Sicilian strain has been almost as effective in inducing hepatitis in apparently normal persons when injected parenterally as when administered orally in contrast to the low incidence of hepatitis in normal persons injected parenterally with virus I. H., (Pa.). Whether or not this is indicative of a difference in the two viruses is uncertain. The preliminary studies, reported herein, of the immunological relationships of these two strains of infectious hepatitis virus suggest that they are antigenically similar. It is of particular interest that parenteral injection
of either of these viruses has been associated with the incubation period characteristic of infectious hepatitis (not over thirty-seven days in studies reported to date). This provides strong evidence that the two to four and one-half month interval between the parenteral injection of some other hepatitis viruses and the appearance of jaundice or definite hepatitis is not just the result of the parenteral route of entry.

Havens also has reported the finding of the Sicilian strain of infectious hepatitis in the feces of hepatitis patients who had acquired the disease as a result of parenteral injection of this agent. As previously stated, similar studies reported herein were inconclusive concerning the presence of virus I. H., Pa. in the feces of hepatitis patients who had acquired the disease following parenteral injection of this virus. However, the findings of Havens in this respect appear to be conclusive and constitute, as he points out, another apparent difference between infectious hepatitis and homologous serum hepatitis, the etiological agent not yet having been demonstrated in the feces of patients with the latter disease. Paul, Havens, Sabin and Philip also have studied a hepatitis virus obtained from the Middle East which apparently is similar to virus SH used in the present studies. This Middle East virus induced hepatitis in normal volunteers two to four and one-half months after parenteral injection. Volunteers recovered from hepatitis due to this virus again developed hepatitis twenty to twenty-five days after inoculation with the Sicilian strain of infectious hepatitis virus. Their findings with these two viruses thus have been similar to those observed in connection with viruses I. H., Pa. and virus SH. As the results obtained by other investigators in their studies of various hepatitis agents have been reviewed in our previous reports and in those of Havens, further consideration of these data in the present report appears to be superfluous.

There is urgent need, however, for a reconsideration of some of the reported experimental data in view of the newer knowledge concerning methods of transmission and the apparent existence of two, and possibly more than two, types of hepatitis viruses. There is a general tendency to diagnose all cases of hepatitis who have not had injections of blood, plasma, serum or biologicals containing blood products, as infectious hepatitis. Undoubtedly, some cases of hepatitis due to agents similar to virus I. H., Pa. have been called homologous serum hepatitis and some cases due to agents similar to virus SH have been called infectious hepatitis. This is particularly apt to occur because both the virus of serum hepatitis and that of infectious hepatitis can be transmitted by blood, plasma or serum. Thus, if the term homologous serum hepatitis is to be used for any type of virus hepatitis in which the agent has been transmitted by parenteral introduction of blood or blood products, the two types described in connection with parenteral injection of virus I. H., Pa. (or virus I. H., S., Havens) and virus SH must be recognized. Furthermore, it has been suggested that these viruses may be transmitted by improperly sterilized syringes and needles used only for withdrawal of blood or for parenteral injections of materials of any type. Such procedures, often performed on large groups of persons for prophylactic or diagnostic purposes, may be overlooked as sources of infection with the serum hepatitis virus and subsequent hepatitis developing in such persons thus regarded as a naturally acquired infectious hepatitis since no history of injection of a blood product may be obtained. Likewise a person may have been considered to have homologous serum hepatitis because of previous administration of a blood product which, however, may have con-
tained the virus of infectious hepatitis rather than that of serum hepatitis. These and other factors may account for some of the apparent inconsistencies in the behavior of various hepatitis viruses of supposedly similar or different origin reported by investigators in this field.

SUMMARY AND CONCLUSIONS

Some of the properties of hepatitis viruses obtained from three different immediate sources have been studied. Virus SH, present in a pool of plasma, induced hepatitis, after two to four and one-half months, in a high percentage (72 per cent) of normal volunteers inoculated parenterally but failed to induce the disease in any of ten normal volunteers who were inoculated orally. Feces obtained from volunteers with virus SH hepatitis failed to induce hepatitis when administered orally or parenterally (Seitz filtrate) to volunteers. In a preliminary study, nasopharyngeal washings and urine from volunteers with virus SH hepatitis also failed to induce the disease in volunteers to whom they were administered by the nasopharyngeal and oral routes but for reasons described, these studies do not warrant conclusions regarding the presence of the virus in these materials. Virus SH remained active after three and one-half years residence in frozen plasma. No consistent effect on the interval from inoculation with virus SH to the onset of hepatitis was noted with the different quantities injected parenterally. Compared to the type of onset of hepatitis due to virus I. H., Pa., the onset of hepatitis due to virus SH was relatively insidious and usually was not associated with elevations of temperature greater than 100°F. (oral). Following hepatitis due to virus SH, all volunteers tested were found to be resistant to reinfection with virus SH but were susceptible to infection with virus I. H., (Pa.).

Virus I. H., Pa., initially found in the feces of patients involved in an epidemic of hepatitis at a summer camp in Pennsylvania, induced active hepatitis, after seventeen to thirty-seven days, in a high percentage (73 per cent) of normal volunteers who were inoculated orally but in only one (11 per cent) of nine normal volunteers inoculated parenterally. In volunteers who had recovered from hepatitis due to virus SH, however, parenteral injection of virus I. H., Pa., in contrast to the results in normal persons, induced active hepatitis in three, (75 per cent) and possibly four, (100 per cent) of the four men so inoculated. Regardless of the route of inoculation (parenteral or oral), the interval from inoculation with virus I. H., Pa. to the onset of hepatitis did not exceed thirty-seven days and the onset was associated with elevation of temperature that exceeded 100°F. (oral) in all but one case. Virus I. H., Pa. was shown to be present in the blood and feces (including a Seitz filtrate obtained from a feces suspension) of patients with active hepatitis due to oral administration of this virus but apparently was not present, at least in sufficient quantities to infect volunteers, in the nasopharyngeal washing and urine pools tested. Virus I. H., Pa. apparently was not present, at least in quantities sufficient to infect volunteers, in single specimens of feces obtained from two persons three weeks after the disappearance of jaundice due to previous oral administration of this virus. Studies for the presence of virus I. H., Pa. in the feces of persons with hepatitis caused by parenteral inoculation with this virus were inconclusive. Two of the five volunteers inoculated orally had laboratory findings suggestive of mild hepatic disturbance but these findings alone were not considered sufficient to justify a definite diagnosis of hepatitis. The results with a relatively small range of orally administered doses of virus I. H., Pa. suggested that a decrease below a certain quantity (in terms of the amount of infective feces pool given) resulted in a prolongation
of the incubation period, although in no instance in the present studies was the incubation period longer than thirty-seven days. Following hepatitis due to virus I. H., Pa., all of the twelve volunteers tested were resistant to reinfection with this virus but two of four such volunteers were susceptible to parenterally injected virus SH as indicated by the occurrence of hepatitis with jaundice three months after inoculation. After recovery from infection with virus I. H., Pa., the five volunteers tested apparently were resistant to oral inoculation with virus I. H., S. (a strain of infectious hepatitis virus obtained in Sicily). Observations bearing on the relationship between the etiological agents responsible for so-called "catarrhal jaundice" and viruses SH and I. H., Pa. are briefly discussed. Studies suggesting that volunteers who failed to show signs of active infection following parenteral inoculation with virus I. H., Pa. were actively immunized by that inoculation are described. The significance of the results of some of the studies is briefly discussed, and possible explanations for apparent discrepancies in the results of different investigations are considered. It is concluded that the data presented constitute satisfactory evidence of the existence of at least two different strains or types of hepatitis viruses.

Acknowledgement. These investigations were made possible through the cooperation of the administrative staffs of Selective Service (Camp Operations Division), the National Service Board for Religious Objectors, the American Friends Service Committee, the New Jersey State Hospital (Trenton, New Jersey) and the Philadelphia State Hospital. We are particularly indebted to the members of the technical staffs of our laboratories in the Hospital of the University of Pennsylvania and in the New Jersey State Hospital, Trenton, N. J. The assistance of various members of the staffs of the Biochemical Laboratory of the Philadelphia General Hospital, the William Pepper Laboratory of Clinical Medicine of the Hospital of the University of Pennsylvania and the Laboratories of the New Jersey State Hospital in the conduct of certain phases of the studies is greatly appreciated. The advice and assistance of Dr. John G. Reinhold, Principal Biochemist of the Philadelphia General Hospital, who supervised the biochemical studies, were invaluable. Finally, the authors gratefully acknowledge the contribution made by the members of Civilian Public Service Unit No. 140, Philadelphia, Pennsylvania, who served as experimental subjects as well as assisting in the conduct of a large part of the technical work connected with the investigations.

REFERENCES


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