Experiments with volunteers have established the viral etiology of human hepatitis. Several species of animals are susceptible to hepatitis caused by viruses which appear to differ from each other and from the virus or viruses infecting man. Horses (1–4) may develop hepatitis following the inoculation of homologous serum. Swine (5, 6), dogs (7, 8), and birds (9) are all susceptible to epizootics of virus hepatitis. A form of hepatitis in old mice, which appears to be non-transmissible and produces no gross signs of disease was described by Olitsky and Casals (10) and others (11–13). This hepatitis is characterized by the occurrence of cytoplasmic inclusion bodies. Gledhill and Andrewes (14–16) have described hepatitis in mice due to a mouse hepatitis virus which occurred in their stock mouse colony and was transmissible to suckling mice proving fatal to them. Nelson (17–20) has described a somewhat similar acute disease in mice, with an associated leukemia and Japanese workers have encountered a similar disease (21–24). Each of these animal diseases appears to be due to a different virus but none to the agent causing hepatitis in man. Many unsuccessful attempts to adapt the human virus to an animal host have been published (25–27).

In a preliminary report (28) we have described the occurrence of hepatitis in mice following the serial intraperitoneal passage of extracts of mouse liver, spleen, and kidney. The mice had received urethane or an adjuvant monocrotaline in their drinking water and were injected with an extract of the liver of a patient (C.B.) who died of hepatitis. The nature of the disease that followed, its agent, and its possible relation to other types of hepatitis in mice and in human beings are discussed in this and the accompanying paper.

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† Fellow of the National Foundation of Infantile Paralysis, Inc.

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AN INFECTIOUS HEPATITIS OF UNDETERMINED ORIGIN. I

Materials and Methods

Materials from Human Beings.—Liver (C.B.): The patient C.B. was a 28 year old pathologist who cut his finger on two occasions, 6 weeks apart, during the autopsies of patients with diffuse liver necrosis. 19 days after the second autopsy he developed myalgia, fever, and the symptoms of an upper respiratory infection. 5 days later he became jaundiced and had nausea, vomiting, abdominal pain, weakness, and mental confusion. He died on the 27th day following his exposure and at autopsy was found to have acute diffuse necrosis of the liver. Slices of the liver were stored in sterile Petri dishes in a dry ice chest for about 18 months before starting the present experiments.

Liver (A.U.): The patient A.U. was a 37 year old alcoholic who died of cirrhosis of the liver. There was a history of previous jaundice occurring 1 year prior to death. The liver was stored in a dry ice chest for 6 months before utilization.

Liver (A.E.): The patient A.E. was a 4 year old girl who died on the day following an operation for congenital heart disease. She had received 10 transfusions during the 8 months before death, six of them during the last 24 hours. The liver was obtained immediately after death and was stored in the dry ice chest for 3 months before use.

Serum (S.H.—Great Lakes): This was the pooled serum obtained from 8 patients 3 weeks after their inoculation with icterogenic serum. It had been placed in a sealed pyrex ampoule and stored at −12 ° for approximately 4 years before use in the present experiments. It was thought to be icterogenic in man because 8 similar pools proved to be so on inoculation into human beings.

Serum (Akiba): This was pooled serum obtained through the courtesy of Dr. Joseph E. Stokes, Jr. It had been collected in the pre-icteric and early icteric periods of 2 volunteers who had been inoculated with the Akiba strain of hepatitis (IH) virus and subsequently developed hepatitis. This pool was known to be infectious for human beings (29). It had been stored frozen for about 9 months before mouse inoculation.

Sera (EL), (LZ), (JJ), (MH): These sera were obtained from 4 healthy human beings and were stored in the dry ice chest for several days to 2 months before use. None of the donors had a history of, or subsequently developed, clinical hepatitis and they had not received any inoculations for over 6 months.

Strains of Mice.—1. O’Grady Strain of Webster Swiss mice. These had been pen-bred by a commercial breeder, Mrs. Flora O’Grady. They were approximately 3 weeks old on arrival and averaged 8 to 10 gm. in weight. As routine they were inoculated 2 to 7 days after arrival. Unless otherwise stated the O’Grady strain of mice was used in all the experiments described further on.

2. Strain A mice were obtained from our own breeding colony started with mice originally donated by Dr. C. L. Larsen of the National Cancer Institute.

3. CVII strain mice were supplied by Dr. Frederik Bang, descendents of animals originally obtained from the Jackson Memorial Laboratory in Bar Harbor.

4. Bar Harbor Swiss mice were purchased from the Jackson Memorial Laboratory.

5. C57 Brown mice were purchased from the Jackson Memorial Laboratory.

6. BSVS (Bacteria-susceptible, virus-susceptible), a strain of mice inbred by Dr. Webster of The Rockefeller Institute for Medical Research, and obtained through the courtesy of Dr. Howard Schneider.

Housing and Feeding of Mice.—The mice were kept on pine shavings in 12 X 9 X 9 inch metal boxes or glass jars 7 X 7 1/2 inches, with wire mesh tops. Regularly, they were fed pellets of Purina dog chow and given water to drink ad libitum. In some experiments they were fed modifications (30) of the diets of Fenton and Cowgill containing 4 per cent or 10 per cent protein; in other experiments a 30 per cent protein diet free from pyridoxine was used.

Urethane.—In some experiments ethyl urethane (Merck & Co., Inc.) was originally em-
ployed as an adjuvant, in attempting to adapt human hepatitis virus to mice, because of its demonstrated enhancing effect on the severity of PVM infection in mice (31). A 0.1 per cent solution was made in tap water and stored in stoppered dark bottles at room temperature. In the experiments described it was given the mice as drink for 1 week before inoculation and throughout the subsequent experimental period.

Monocrotaline.—Monocrotaline, an hepatotoxic alkaloid from plants of the genus *Crotonaria*, was obtained through the courtesy of Dr. Roger Adams and Dr. K. K. Chen (32). It was used as an adjuvant because ingestion of plants of the related genus *Senecio* has apparently enhanced indigenous hepatitis in horses (1). It was dissolved in distilled water and stored in the refrigerator at 4°C. In the experiments described 200 mg./kg. was injected once intraperitoneally 2 to 7 days before the virus inoculation.

Preparation and Storage of Mouse Organ Extracts.—As a routine procedure the liver, spleen, and kidneys of the mice were ground in a mortar with a pestle and alundum, the resulting brei was thoroughly mixed in 0.86 per cent saline solution to a concentration of 10 to 20 per cent by weight; and after centrifugation at 1500 r.p.m. for about 10 minutes the supernatant was decanted into lusteroid tubes with metal screw caps. Ordinarily these organ extracts were either used at once or frozen quickly in an alcohol-dry ice mixture and stored in a dry ice chest.

EXPERIMENTAL

Initial Attempts to Adapt Human Hepatitis Virus to Mice.—Certain clinical observations have led to the concept that the susceptibility of human beings to hepatitis may be influenced by non-specific environmental factors (33–36) or by drugs (37, 38). Moreover, dietary deficiencies may under certain circumstances result in increased susceptibility of the host to hepatitis (6, 39) and other viral infections (40). Urethane was used as an adjuvant in the experiments to be reported because previous experiments had shown that this substance increases the susceptibility of mice to infection with pneumonia virus of mice (PVM) and results in an increased titer of this virus in the lungs of the infected mice (31).

Two groups of 12 mice each of the O'Grady strain were inoculated intraperitoneally with human materials thought to contain hepatitis virus. Each mouse in one group received 0.5 cc. of a 5 per cent saline suspension of human liver (C.B.). In the other group each received 0.5 cc. of pooled human serum (G.L.) which was thought to be icterogenic in man. Two other groups of 12 mice each served as controls and were not inoculated. The two inoculated and one uninoculated groups were given 0.1 per cent urethane in their drinking water for 1 week before inoculation and throughout the whole experimental period. The remaining control group had water only. Three mice from each group were sacrificed and examined for evidence of disease at the end of 1 week, three more at the end of 2 weeks, and three at 4 weeks, and three at 4 months. In the serial passages, saline extracts of the pooled livers, spleens, and kidneys from the mice sacrificed at 1, 2, and 4 weeks respectively were injected immediately into the peritoneal cavities of normal 10 gm. mice. Ten serial passages made in this fashion. Separate sterile syringes and needles were used for inoculating each group. None of the mice observed for 4 months developed obvious signs and at autopsy they appeared normal.

A mouse in the 4th monthly passage of the line originally injected with C.B. liver extract, which received water containing urethane and was killed after 4
weeks, was found to have developed hepatitis and ascites (Text-fig. 1). Two other mice in this group had died of unknown causes. Two of the three mice injected with an extract of the organs of this mouse had hepatitis and ascites when sacrificed 1 month later. At that time the routine use of urethane was discontinued; yet hepatitis and ascites occurred in thirteen subsequent serial monthly passages (five of these are shown in Text-fig. 1).

It can be seen in Text-fig. 1 that one mouse with ascites was observed in the 6th and 8th passages respectively of the water and urethane control series. Obviously an infectious agent of some sort was responsible for the disease. Since ascites and hepatitis are its most striking features, we have decided to call the etiological agent ascites hepatitis agent.

_Fig. 1. Attempts to isolate hepatitis virus._

It can be seen in Text-fig. 1 that one mouse with ascites was observed in the 6th and 8th passages respectively of the water and urethane control series. Obviously an infectious agent of some sort was responsible for the disease. Since ascites and hepatitis are its most striking features, we have decided to call the etiological agent ascites hepatitis agent.

The _Signs of Infection with Ascites Hepatitis Agent (AHA) of Mice._

2 to 3 weeks after inoculation with an extract containing the agent the mice become thirsty, slowly develop abdominal enlargement, and gain weight. The fur becomes ruffled and the
respirations labored. After 1 to 4 weeks of illness (average 2 weeks), a rapid diuresis occurs and thereafter they nearly always appear completely normal, live the usual life span, and reproduce normally. In only 3 mice out of about 10,000 inoculated was jaundice ever noted.

The weight curve of a mouse infected with the ascites hepatitis agent (AHA) is shown in Text-fig. 2 and photographs of this mouse at the peak of its ascites and after recovery are shown in Figs. 1 and 2.

The period before illness was noted varied between 15 and 35 days with an average of 21 days. In separate experiments the incidence of ascites ranged from none to 100 per cent. The inoculation of fresh organ extracts intraperitoneally into 130 mice weighing 10 gm. of the O'Grady strain in 6 separate experiments resulted in ascites in 46 per cent of the mice while 85 per cent had in the gross pathological livers. In certain large individual experiments ascites would occur in 93 to 100 per cent of the mice for unknown reasons. As described below its incidence may be governed to some extent by the route of inoculation, size and virulence of the inoculum, the type and length of its storage, the time at which the animals were killed, the strain and age of mice employed and perhaps other factors.

Pathology of the Disease.—Since so few mice infected with AHA died, the evolution of the infection was studied by sacrificing groups of mice at various intervals after inoculation. The gross pathological changes observed are recorded in Table I. The most prominent gross pathological features of the disease other than ascites were hepatomegaly and splenomegaly, (Fig. 3). 80 per cent of animals developing the disease had splenomegaly 1 week after inoculation, but the livers were of normal size and appearance. After 2 weeks about 70 per cent also had hepatomegaly but no ascites. At that time the livers of infected mice were 2 to 3 times normal size, dark red, friable, and sometimes mottled, as shown in Fig. 4. At the end of 3 weeks the livers still had the same appearance but about 28 per cent of the mice now had some light yellow
serous ascitic fluid. By the end of the 4th or 5th week 50 to 60 per cent of the mice had ascites and about 90 per cent had hepatosplenomegaly. The ascitic fluid varied in amount from about 0.5 to 30 cc. At 5 to 6 weeks the disease regressed, and 2 months or more after inoculation only 10 to 20 per cent had hepatosplenomegaly, and ascites was present in only 2 per cent. Loose flecks of fibrin were occasionally seen in the peritoneal cavity. The leukocytes in the tail blood of infected mice ranged from 7,000 to 30,000 per c.mm. but were usually less than 12,000.

The ascitic fluid was usually thin, clear, and light yellow, but in an occasional mouse it was hemorrhagic. It clotted on standing and contained about 3.4 gm. of protein per 100 cc. of which 2.5 gm. was albumin. It contained less than 1000 cells per c.mm. which were usually mononuclear with occasional large endothelial cells.

Tissues from normal mice, from mice given urethane, and from a number of infected mice with the typical pathological changes were fixed in formalin for pathological study. Subsequent microscopic examinations were done ordinarily only when there was doubt about the diagnosis.

Sections of the livers of infected mice showed two different pathological pictures. Those from the large, dark red, thick, friable, and sometimes mottled livers revealed dilated sinusoids, diffuse and focal infiltration with mononuclear cells, and perivascular collections of similar cells. Both the portal and central vessels were surrounded. In addition frequent multinucleated giant cells were seen. These findings are illustrated in Figs. 5 to 7. In the presence of ascites the livers were usually less enlarged and paler with slightly pitted surfaces. Mononuclear cell infiltration, as described above, was seen in microscopic sections. Moreover, in some livers, masses of eosinophilic material, presumably necrotic cells, were observed. These areas usually occurred without apparent relation to the hepatic architecture, as shown in Fig. 8, and rarely this process involved the whole central portion of the lobules (Fig. 9).

Biopsy of the anterior gastric lobe of the liver under ether anesthesia was done on several

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### TABLE I

The Incidence of Splenomegaly, Hepatomegaly, and Ascites in Mice in Relation to Time after Inoculation

<table>
<thead>
<tr>
<th>Time of sacrifice after inoculation</th>
<th>Pathological changes</th>
<th>Ascites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Splenomegaly</td>
<td>Hepatomegaly</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>Per cent</td>
</tr>
<tr>
<td>1 week</td>
<td>5/6*</td>
<td>83</td>
</tr>
<tr>
<td>2 weeks</td>
<td>32/37</td>
<td>87</td>
</tr>
<tr>
<td>3 weeks</td>
<td>26/29</td>
<td>90</td>
</tr>
<tr>
<td>4 weeks</td>
<td>24/25</td>
<td>96</td>
</tr>
<tr>
<td>5 weeks</td>
<td>4/8</td>
<td>50</td>
</tr>
<tr>
<td>6-8 weeks</td>
<td>17/24</td>
<td>71</td>
</tr>
<tr>
<td>9-23 weeks</td>
<td>6/45</td>
<td>13</td>
</tr>
<tr>
<td>24-32 weeks</td>
<td>7/41</td>
<td>17</td>
</tr>
</tbody>
</table>

* Numerator, number of mice with characteristic; denominator, number of mice examined.
occasions after ascites had developed. Livers with the typical pathological infiltrations described above when examined in this way at 30 days after inoculation usually showed a normal histological picture when the mice were sacrificed 2 months later. Only one mouse had perihepatic adhesions and a tremendous mononuclear cell infiltration of the liver and kidneys when sacrificed 6 months after infection.

The livers of the three jaundiced mice already mentioned deviated from the typical pathological picture above only in showing more extensive areas of necrosis. Extracts of these livers were inoculated separately into normal mice and resulted in the usual disease without jaundice. Moreover, the infectious titer of these livers was no higher than that of other preparations as described further on.

The brains, hearts, and lungs of mice infected with AHA appeared normal. Histological sections of enlarged spleens showed engorgement of the sinusoids with blood, giant cell formation, and increase in number of lymphocytic cells. Occasional areas of cellular infiltration were seen in the pancreas and the kidney.

Transmission of AHA Infection in Mice.—As described above, saline extracts of the combined liver, spleen, and kidneys obtained 1 month after inoculation from a mouse infected with AHA usually resulted in the disease when injected intraperitoneally into mice of the O'Grady strain. Other materials, routes of inoculation, and strains and ages of mice were tested. It was found that extracts of the liver, spleen, or kidneys separately were infectious. Moreover, blood or ascitic fluid from infected mice also transmitted the disease when inoculated intraperitoneally. The inoculation of infectious extracts or ascitic fluid intravenously, subcutaneously, into the footpad, or intracerebrally also resulted in infection. Ascites was, however, somewhat less common following infection by these routes. Serial intracerebral passage of brain extracts from mice inoculated intracerebrally failed to produce any signs or pathological evidence of meningitis, encephalitis, or other neurological disease but resulted in hepatitis with minimal ascites. All evidence of disease disappeared after four serial passages by the intracerebral route. Unfiltered urine and the fluid obtained by filtration from suspensions of feces through a Seitz pad did not result in obvious infection after intraperitoneal inoculation. Moreover, materials that were infectious intraperitoneally failed to produce any demonstrable disease when inoculated intranasally or fed to susceptible mice.

Some experiments have been done to determine the optimal time to harvest the agent from infected mice. Twenty-three groups of 3 to 6 mice were inoculated intraperitoneally with fluids containing AHA and were sacrificed at various intervals after inoculation. The infectivity of their pooled liver-spleen-kidney extracts was tested by inoculating normal mice. The results are shown in Table II. It will be seen that in neither of two experiments was agent-demonstrable 1 week after inoculation. However, the samples collected after all other intervals up to 6.5 months, the last samples tested, were infective. Evidently the infection was chronic despite the fact that most mice appear to be well 6 to 8 weeks after inoculation. Moreover, this finding raises interesting questions concerning immunity, which will be considered subsequently.
None of the great number of uninoculated young mice which have been sacrificed for various reasons over a 5 year period have shown any ascites or evidence of infection with AHA. Normal uninoculated young mice were placed in the same jars with infected mice and observed for several months for evidence of infection. None showed signs of illness due to AHA and on sacrifice all appeared normal. Nevertheless, as already pointed out, an occasional mouse in the control passage series had ascites and hepatitis at the time of sacrifice 4 weeks after inoculation. Twenty "normal" mice, over a year old, were obtained from our breeder, Mrs. O'Grady, and when sacrificed, one was found to have ascites and hepatomegaly. This supported the impression that the disease was indigenous, and was disseminated in this strain of mice, despite the fact that no natural means of transmission was demonstrated experimentally.

Most of the tests were made with O'Grady mice but several other strains were tested for susceptibility to infection with AHA and found to be as susceptible as the O'Grady strain. These were Strain A, CVIII, Bar Harbor Swiss, and C57 Brown. Insufficient numbers have been tested to be certain that some may not be more susceptible. The disease produced in the BSVS strain of Webster (bacteria-susceptible, virus-susceptible), did not seem to be more severe or fatal than in the others tested.

O'Grady strain mice and strain A mice of various ages were tested for susceptibility. It can be stated that suckling mice 2 to 3 days old and mice up to 1 year in age were susceptible. In very young mice the incubation period seemed to be about 1 week shorter, but the mortality no greater than in the 10 gm. mice usually employed.

### TABLE II

<table>
<thead>
<tr>
<th>Time after inoculation when virus pool was harvested</th>
<th>No. of Experiments</th>
<th>Total No. of mice</th>
<th>Ascites</th>
<th>Hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>Per cent</td>
</tr>
<tr>
<td>1 wk.</td>
<td>2</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 wks.</td>
<td>3</td>
<td>17</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td>3 wks.</td>
<td>5</td>
<td>27</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>4 wks.</td>
<td>4</td>
<td>21</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>5–6 wks.</td>
<td>4</td>
<td>14</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>7–8 wks.</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>2 mos.</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>4 mos.</td>
<td>1</td>
<td>6</td>
<td>5</td>
<td>83</td>
</tr>
<tr>
<td>6½ mos.</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
</tbody>
</table>

Total: 23 117
Tests for Immunity to AHA Infection.—No resistance to infection with AHA could be demonstrated. Mice which had apparently completely recovered from the disease were susceptible on challenge at a later date. This is at variance with the experience of Morris as cited in reference 27. Uninoculated normal mice kept in the same cages with actively infected mice did not become immune as a result of this exposure, but were susceptible to infection when inoculated 2 months later. Mice given five weekly intraperitoneal injections of AHA and then sacrificed at the end of another month showed evidence of mild hepatitis but no ascites at the time of sacrifice 9 weeks after the first inoculation. The litters of mice convalescent from AHA infection were susceptible at the time of weaning. Moreover, the sera from mice convalescent from the disease failed to neutralize AHA, as reported in the accompanying paper.

The Susceptibility of Other Species to AHA Infection.—Attempts were made to infect other species with AHA.

Four rabbits were inoculated intravenously with 1 ml. of 2 separate infectious AHA preparations. Two rabbits were immunized with 18 ml. divided into nine intravenous doses of increasing size. Two rabbits were immunized by a subcutaneous inoculation of a mixture of virus, fat, and mineral oil. None developed obvious disease and when sacrificed 2 to 10 months later the livers of all appeared normal. Serum from two of these rabbits obtained about 1 month after inoculation was injected intraperitoneally into mice and failed to induce infection.

Four guinea pigs were inoculated intraperitoneally with 0.5 ml. of two separate pools of AHA. None of these animals developed fever or other evidence of infection, and microscopic sections of the livers of two when sacrificed 1 month later, were normal. Organ extracts and/or blood obtained from all these animals obtained about 1 month after inoculation produced no disease when tested in mice.

Four monkeys were inoculated intraperitoneally with 2 ml. of a suspension of AHA and two with 2 ml. of an extract of the human liver (C.B.) obtained in the usual way. Half of each group were given 0.1 per cent urethane to drink and in each the serum bilirubin, thymol turbidity reaction, and bromosulfalein retention at 25 minutes following the injection of 10 mg. of dye per kg. were measured at weekly intervals for 6 weeks. None of these monkeys developed evidence of infection or abnormal liver function, as measured by these tests. Four serial intraperitoneal blind passages in monkeys of 10 per cent extracts of monkey liver obtained by biopsy 1 month after inoculation gave negative results.

Seven serial amniotic passages and 6 yolk sac passages in embryonated hens’ eggs were carried out. No obvious disease of the embryos resulted, and mice inoculated with amniotic fluid, or yolk sac extracts, or extracts of the embryos themselves showed no evidence of disease.

One ml. of a pool of infectious ascitic fluid was inoculated intraperitoneally into three mature hamsters as shown in Table III. Likewise, 0.5 ml. of pooled mouse liver-spleen-kidney extract which contained AHA was inoculated into half-grown hamsters of the same kind. They were sacrificed and examined 1 month later. None of the 3 older hamsters but 2 of the 3 young ones showed slight evidence of disease on gross examination. There was slight mottling.
and pitting of the liver surfaces and a definite though minimal ascites. Sections of the livers from some of the animals showed small focal and periportal areas of mononuclear cell infiltration. Two serial intraperitoneal passages into young hamsters of extracts of the pooled livers, spleens, and kidneys did not

### TABLE III

*The Results of Inoculating Hamsters with AHA from Mice*

<table>
<thead>
<tr>
<th>AHA Source</th>
<th>Hamster</th>
<th>Passage to Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse material</td>
<td>No.</td>
<td>Age</td>
</tr>
</tbody>
</table>
|--------------|-----|-----|----------------------|----------------------------------|-----------------|-------|-----------|----------------
| **A. Ascitic fluid** | 1 | Old | 0 | ±* | LSK | 3 | 4 | 12 |
| | 2 | Old | 0 | ± | LSK | 0 | 1 | 9 |
| | 3 | Old | 0 | + | Sera | 0.1 ml. | 4- | 0 |
| | 4 | Young | ±† | 0 | LSK | 4- | 4- | 0.1 ml. | 12 gm. |
| | 5 | Young | ± | ± | LSK | 4- | 4- | 0.2 ml. | 12 gm. |
| | 6 | Young | ± | - | LSK | 4- | 4- | - | - |
| | 7 | Young | ± | + | LSK | 4- | 4- | - | - |
| | 8 | Young | ± | ± | LSK | 4- | 4- | - | - |
| | 9 | Young | ± | - | LSK | 4- | 4- | - | - |
| **B. LSK§** | 10 | Young | ± | - | LSK | 0 | 2 | 6 |
| | 11 | Young | ± | - | LSK | 0 | 0 | 6 |
| | 12 | Young | 0 | - | LSK | 0 | 0 | 6 |
| | 13 | Young | ± | 0 | LSK | 0 | 0 | 6 |
| | 14 | Young | 0 | 0 | LSK | 0 | 0 | 6 |
| | 15 | Young | 0 | 0 | LSK | 0 | 0 | 6 |

When not otherwise indicated the passage material was a 10 per cent saline extract of pooled livers, spleens, and kidneys.

* ±, periportal and focal infiltrations of mononuclear cells.
† ±, slight mottling and pitting of the liver surface and slight ascites.
0, no obvious disease.
§ LSK, 10 per cent saline extract of pooled livers, spleens, and kidneys.

result in any gross lesions. The inoculation of mice with the pooled sera or organ extracts obtained from hamsters 1 month after inoculation caused hepatomegaly in seven of twenty-seven mice but ascites in only three. However, the inoculation of mice with material from the second passage in hamsters resulted in no detectable disease.

It is concluded that hamsters are somewhat susceptible to experimental
JANET JORDAN AND GEORGE S. MIRICK

infection with AHA and may transmit the disease it induces. Further experience may show them to be a satisfactory experimental animal for studying this infection.

The results of inoculating AHA into human volunteers will be described in detail in a subsequent paper.

Tests for the Influence of Certain Adjuvants and Diets on AHA Infection.—Urethane in large doses may produce focal liver necrosis in rats (41), and its prolonged administration may occasionally result in anasarca in mice (42). In the doses we have employed there is some suppression of lymphoid tissues (31) but no gross or microscopic pathological changes in the liver, and no ascites have been noted in uninoculated mice given 0.1 per cent urethane. The routine use of this adjuvant was discontinued when it became apparent, after the fourth serial passage of the disease in the first series that it was not essential to the development of the disease. Its possible effect in facilitating or enhancing the disease was studied in several later experiments and it was utilized in repeated attempts to isolate the agent, as described in the following section. Infected mice given urethane solution to drink were usually a little smaller than controls and their spleens were not so large. The character and degree of liver involvement in the 2 groups seemed equal. Although the agent did not infect urethanized mice in higher titer than it did controls, ascites tended to be more common and abundant in infected mice given urethane.

Monocrotaline could not be effectively used in attempts to adapt human hepatitis virus to mice, because the serial intraperitoneal passage of mouse organ extracts in O'Grady strain mice given monocrotaline invariably resulted in a fatal generalized infection with a Gram-positive micrococcus, Gaffky tetragenus. In several individual titrations of AHA in mice given monocrotaline and in normal mice no difference in the severity of infection was noted.

Because of the apparent enhancing effect of protein-deficient diets on the hepatitis in rats described by MacCallum and Miles (39) and the hepatitis in pigs described by Andersen and Tulinius (6), low protein diets identical with those employed by Mirick and Leftwich (30) were fed to mice from the time of inoculation with AHA. No detectable difference in the severity of infection was noted when mice were fed 4 per cent protein or 10 per cent protein, as compared with controls given 30 per cent protein in their diet. Moreover, a pyridoxine-free diet, which under certain conditions increased the severity of PVM infection in this strain of mice (30), did not seem to influence their susceptibility to infection with AHA.

Attempts to Procure AHA from Human Beings.—The circumstances of the initial demonstration of AHA suggested that it might have come from the liver of C.B. Attempts to establish the disease due to AHA by the inoculation
of mice with extracts of other livers or with sera from human beings are summarized in Text-fig. 3. It will be seen that two additional series (II and III) inoculated with the extract of C.B.'s liver became positive as concerns hepatitis and ascites on the first or second passage, and this whether the mice were given urethane or not. A mouse series inoculated originally with pooled human sera containing the Akiba strain of human hepatitis virus likewise showed hepatitis in the 4th passage. In this instance ascites developed only in the mice given urethane. The disease seemed identical with that described above but was lost on serial passage. In the second passage of a series inoculated with an extract of another human liver (A.E.) five out of twelve mice developed ascites, but 5 passages in a series inoculated with an extract of a liver (A.U.) from a patient with cirrhosis remained negative.

The results in other series of O'Grady strain mice inoculated primarily with serum from normal human beings or only with normal mouse organ extracts are summarized in Text-fig. 4. It will be seen that evidence of liver
infection and ascites occurred in each instance in the 2nd or 3rd passage. To date two passage series, using C57 brown mice inoculated primarily with liver-spleen-kidney extract from normal mice of that strain, have remained negative through the 4th serial monthly passage. The significance of these observations will be considered in the discussion.

**Fig. 4. Attempts to isolate hepatitis virus.**

**RECAPITULATION AND DISCUSSION**

As described above, a mild transmissible disease in mice characterized particularly by hepatosplenomegaly and ascites resulted from the serial intraperitoneal passage of saline extracts of organs or ascitic fluid from the sick mice. Its transmission in series by a variety of routes of inoculation indicates that it is due to an infectious agent. Of the other species tested, only hamsters seemed susceptible. Evidence is presented in the accompanying paper that the agent of this disease has not been previously described. The disease was first observed in the course of experiments designed to adapt human hepatitis virus to mice.

No transmissible disease appeared in the first control series but the observation of occasional positive mice suggested early that the agent might be carried by normal mice of the strain under study or alternatively that the disease was contagious and was transferred inadvertently from the positive mice of the C.B. series. The first suggestion was substantiated by the occurrence of a similar pathological process in the second series of control mice inoculated with organ suspensions from normal mice.

The infection was usually benign with apparently complete recovery by most mice. However, the agent was harbored for months afterward by mice which had been infected; yet during this period we were unable to demonstrate resistance to reinfection.
The failure of some mice to develop overt disease following inoculation is unexplained. The presence of acquired immunity from infection by other routes than those we have employed experimentally is one possibility. Interference by some other agent has been considered, and also the possibility that the disease may represent a complex infection.

Since most mice of all ages and of all strains tested seem to be susceptible to AHA, it is difficult to understand why this disease has not been encountered previously, notably in the course of the many previous attempts to adapt the virus of human hepatitis to mice (25, 26). The most obvious explanation is that the serial passage of mouse organ extracts at intervals of a month or more was rarely tried (43). It may be that the disease is rare in mice and occurred only by unusual chance in the O'Grady strain we employed. Lackey, Eichman, and Havens (44) have reported the occurrence of a similar disease in the O'Grady strain of mice, serially inoculated by methods as described in our preliminary note (20) and in CFW albino mice obtained from Carworth Farms but not in an inbred strain (dba). However the disease proved transmissible in our laboratory to 4 inbred strains of mice, dba (subline 1), C3H (Jax), C57 (brown dc.), A albino C (C) all obtained from Jackson Memorial Laboratory, Bar Harbor.

SUMMARY

Serial intraperitoneal passage in mice of a saline extract of the pooled livers, spleens, and kidneys of such animals has led to the demonstration after three or more passages of a transmissible agent causing hepatitis. The mice developed an illness after 3 to 4 weeks characterized by hepatosplenomegaly, and serous ascites. Spontaneous diuresis and recovery usually occurred during the subsequent 2 to 4 weeks.

Histological studies of the livers showed diffuse mononuclear infiltrations, focal accumulations of mononuclear cells, perivascular mononuclear cuffing, dilated sinusoids, and occasionally focal areas of necrosis.

Mice which have recovered from the disease showed no noteworthy resistance to it, and their sera failed to protect against the infectious agent. Attempts to infect rabbits, guinea pigs, monkeys, and embryonated hens’ eggs yielded negative results, but young hamsters developed the disease in mild form.

We wish to acknowledge the valuable technical assistance of Mrs. Marie Maselbas and Mr. John Lockner.

BIBLIOGRAPHY

29. Stokes, J. E., Jr., personal communication.
EXPLANATION OF PLATES

PLATE 72

FIG. 1. Photographs of a mouse of the 8th serial passage 33 days after intraperitoneal injection of liver extract containing AHA. A portion of the liver with which it was inoculated is shown in Fig. 4. No urethane was given. \( \times \frac{3}{4} \).

FIG. 2. Photograph of the same mouse 69 days after inoculation. \( \times \frac{3}{4} \).

FIG. 3. Photograph above (Fig. 3 a) of a mouse of the 7th passage which had been infected with AHA ascitic fluid. No urethane had been given to the animal and no ascites was present but the animal had become somewhat jaundiced. Compare its enlarged liver and spleen with those of a normal mouse below (Fig. 3 b). \( \times \frac{3}{4} \).

FIG. 4. AHA—Liver (left (Fig. 4 a)) of a mouse of the 7th passage which died 35 days after injection with an extract of pooled livers, spleens, and kidneys. Compare with normal liver on the right (Fig. 4 b). No urethane was given. \( \times \frac{3}{4} \).

FIG. 5. Photomicrograph of the liver from a mouse sacrificed 25 days after inoculation with AHA in the 6th passage. 8 cc. of ascitic fluid was present. Note dilatation of liver sinusoids, diffuse infiltration, and occasional giant cells. Hematoxylin and eosin. \( \times 150 \).
(Jordan and Mirick: An infectious hepatitis of undetermined origin. I)
PLATE 73

FIG. 6. Photomicrograph of the liver from a mouse sacrificed 22 days after inoculation with AHA in the 6th passage. Note perivascular cellular infiltration. Hematoxylin and eosin. × 150.

FIG. 7. Photomicrograph of section shown in Fig. 6 to show mononuclear character of the infiltrating cells. Hematoxylin and eosin. × 150.
(Jordan and Mirick: An infectious hepatitis of undetermined origin. I)
PLATE 74

Fig. 8. Photomicrograph of liver section from AHA infected mouse shown in Plate 3. Note focal areas of acidophilic material. No amyloid was found in this tissue after special staining. Hematoxylin and eosin. × 150.

Fig. 9. Photomicrograph of liver section from AHA-infected mouse in the 10th passage which died 25 days after inoculation. No urethane was given. Note areas of necrotic liver cells which seem to surround the central veins. Hematoxylin and eosin. × 150.
(Jordan and Mirick: An infectious hepatitis of undetermined origin. I)