CLINICAL CONFERENCE
Clinical and Experimental Studies of Infectious Hepatitis

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DR. KRUGMAN: Since 1953 approximately 400 cases of infectious hepatitis with jaundice have been observed at the Willowbrook State School on Staten Island. The studies to be described were carried out in collaboration with Dr. Robert Ward and Dr. Joan Giles of our staff, Dr. A. Milton Jacobs of Willowbrook State School and Dr. Oscar Bodansky of Sloan-Kettering Institute.* I should like to present a progress report of our investigations which have been concerned with the prevention and natural history of infectious hepatitis at Willowbrook. (A report of these studies has recently appeared in the New England Journal of Medicine (248:407, 1958) to which the reader may refer for further details.)

It had been previously reported by Stokes and associates that the administration of gamma-globulin was followed by not only a lower incidence of hepatitis but also a prolongation of the protective effect. Stokes postulated that “passive-active” immunity was responsible for this phenomenon. The epidemic of hepatitis at Willowbrook provided us with an opportunity to test this hypothesis.

Effect of Gamma-globulin on the Frequency of Infectious Hepatitis. Figure 1 illustrates the course of the outbreak at Willowbrook beginning in January, 1955. As can be seen, hepatitis continued to occur at a rate of about two to three cases per week. The cases, predominantly in children, occurred in 18 buildings in the institution. In June of 1956 gamma-globulin, 0.01 ml/lb, was administered to approximately a third of the inmates of each building. The control and inoculated groups were comparable as to age and time of admission to Willowbrook.

The results of this first trial of gamma-globulin are shown in Table I. The rate of cases of hepatitis per 1,000 in the gamma-globulin group was 7.4 as compared with 19.7 in the control group. The reduced frequency of hepatitis, although significant, was not as striking as that reported by Stokes.

We wondered if the dose of 0.01 ml/lb was perhaps inadequate. Figure 2 shows the effect of a larger dose of gamma-globulin (0.06 ml/lb). It is apparent that this increased dose was followed by a striking reduction in the incidence of infectious hepatitis. During a 52-week period following administration of gamma-globulin the hepatitis rate per 1,000 was 2.5 in the gamma-globulin group as compared with 21.4 per 1,000 in the control group. Only three cases of hepatitis occurred in the group of patients receiving gamma-globulin: one in the fifth week, one in the twelfth week and one in the fiftieth week.

These findings confirmed Stokes’ observations of prolonged protective effect following the administration of gamma-globulin.

Attempts to Induce Passive-Active Immunity. Although the incidence of hepatitis at Willowbrook was high, the disease was fortunately very mild, even in adults. These circumstances provided an unusual opportunity for artificial induction of “passive-active” immunity by feeding virus to patients shortly after administration of gamma-globulin.

We gave serious consideration to a number of conditions before undertaking this study. Our decision to carry out this important investi-

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gation was based on the following factors: 1) Infectious hepatitis, a mild disease in children, was particularly benign in Willowbrook; 2) it was inevitable that most of the newly admitted, retarded children would acquire the disease; 3) only the Willowbrook strain of virus would be employed in these studies; and 4) facilities were available to provide isolation quarters and special medical and nursing care. In view of the above factors the investigation was begun with small groups of newly admitted patients whose parents gave consent. This study was approved by the New York State Department of Mental Hygiene and was sponsored by the Armed Forces Epidemiological Board, Office of the Surgeon General, U.S. Army. Our first task was to prepare a suspension of the Willowbrook strain of hepatitis.

**Fig. 1.** Occurrence of cases of hepatitis at Willowbrook before and after the first trial of gamma-globulin (each square represents one case of hepatitis with jaundice). (From Ward, Krugman, Giles, Jacobs and Bodansky: New England J. Med., 258:407, 1958.)

**Fig. 2.** Occurrence of cases of hepatitis at Willowbrook for both the first and the second trials of gamma-globulin (each square represents one case of hepatitis with jaundice). (Modified from Ward, Krugman, Giles, Jacobs and Bodansky: New England J. Med., 258:407, 1958.)
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FIG. 3. Preparation of hepatitis virus suspension
(Willowbrook stool pool No. 1).

Tissue culture monkeys were newly admitted and had no contact with other patients in the institution.

Neither the 0.00001 nor a 0.001 gm dosage produced hepatitis. However, when the dose was increased, cases of hepatitis were produced as follows: with 0.1 gm, hepatitis occurred in two of eight subjects after an incubation period of 39 and 71 days; with 1 gm, 5 of 11 developed hepatitis after incubation periods ranging between 39 and 61 days; and with larger doses the number of cases of hepatitis increased. The 50% infectivity dose* of this virus pool was approximately 1 to 2 gm.

Attempt to Induce Passive-Active Immunity Artificially. Having determined the 50% infectivity dose of virus, we were now ready to attempt to induce passive-active immunity by administration of gamma-globulin followed by feeding of virus. Gamma-globulin, 0.06 ml/lb, was given to 11 of 16 subjects in a group. Within 30 minutes, all 16 were fed the equivalent of 0.00001 gm of material. The subjects in this phase of the study

* The concentration of virus estimated to produce hepatitis in 50% of the subjects tested.

<p>| TABLE II |
| --- | --- | --- | --- |
| Titratian of Virus After Oral Administration* | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th>(gm)</th>
<th>Patients Fed Virus</th>
<th>Jaundiced Patients</th>
<th>Incubation Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00001</td>
<td>8</td>
<td>0</td>
<td>(days)</td>
</tr>
<tr>
<td>2</td>
<td>0.001</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>8</td>
<td>2</td>
<td>39, 71 (?)</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>11</td>
<td>5</td>
<td>39, 39, 43, 49, 61</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>5</td>
<td>4</td>
<td>39, 39, 46, 60</td>
</tr>
<tr>
<td>6</td>
<td>4.0</td>
<td>13</td>
<td>1†</td>
<td>44, 44, 44, 46, 47, 54</td>
</tr>
<tr>
<td>7</td>
<td>0.0</td>
<td>5</td>
<td>5</td>
<td>54, 54, 58, 58, 62, 67</td>
</tr>
</tbody>
</table>

* Virus contained in Willowbrook pool No. 1 (heated); 50% infectivity dose estimated to be equivalent of 1 or 2 gm of stool.


virus, free of other agents or microorganisms. The preparation of the virus suspension would then be titrated to determine its infectivity, and finally it would be administered along with gamma-globulin in an attempt to induce passive-active immunity.

Preparation of Virus Suspension. Figure 3 illustrates how the virus pool was prepared. Stools were collected from patients in the institution within the first 8 days of the onset of jaundice. This material was first subjected to centrifugation at 2,000 rev/min for 20 minutes; the resulting supernate was centrifuged at 8,500 rev/min for 1 hour. It was then heated at 56°C for 30 minutes, treated with antimicrobial agents, and centrifuged again at 8,500 rev/min. The final supernate was cultured and safety-tested in baby mice, in tissue cultures and in monkeys. All of these safety tests were negative, thereby indicating the absence of poliomyelitis virus or other agents detectable by these means.

Titration of Virus Pool. The virus pool was now deemed safe to feed. As can be seen in Table II the feeding was begun with a small dose. Eight presumably susceptible children received the equivalent of 0.00001 gm of material. The subjects in this phase of the study

TABLE I

FIRST TRIAL OF GAMMA-GLOBULIN*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Inmates</th>
<th>Hepatitis with Jaundice</th>
<th>No. of Cases</th>
<th>Rate/1,000†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2,988</td>
<td>50</td>
<td>19.7</td>
<td></td>
</tr>
<tr>
<td>Gamma-globulin*</td>
<td>1,204</td>
<td>9</td>
<td>7.4</td>
<td></td>
</tr>
</tbody>
</table>

* Dose, 0.01 ml/lb.
† Over 7-month period after gamma-globulin was administered.

TABLE III
RESULTS OF TRIAL (UPPER SECTION)* AND TITRATION (LOWER SECTION)†

<table>
<thead>
<tr>
<th>Dose (gm)</th>
<th>Patients Fed Virus</th>
<th>Jaundiced Patients</th>
<th>Incubation Periods (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1**</td>
<td>10</td>
<td>3</td>
<td>39, 41, 46</td>
</tr>
<tr>
<td>0 (6 controls)</td>
<td>10</td>
<td>3</td>
<td>39, 41, 46</td>
</tr>
<tr>
<td>4.0‡</td>
<td>13</td>
<td>10</td>
<td>42, 42, 44, 46, 47, 54, 54, 54, 58</td>
</tr>
<tr>
<td>2.0</td>
<td>5</td>
<td>3</td>
<td>39, 39, 46</td>
</tr>
<tr>
<td>1.0</td>
<td>11</td>
<td>4</td>
<td>39, 39, 43, 49</td>
</tr>
<tr>
<td>0.1</td>
<td>8</td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>0.001</td>
<td>8</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>0.00001</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Of 10 patients fed virus, jaundice developed 39, 41, and 46 days later in 3; in 2 at 60 and 71 days. Of six control subjects not fed virus, but intimately exposed to others, hepatitis with jaundice developed after 60, 63, 67 and 71 days respectively in four.
† Interrupted vertical line arbitrarily separates incubation periods at 60 days; longer ones probably represent secondary infections.
** Willowbrook stool pool No. 1 (unheated).
‡ Willowbrook stool pool No. 1 (heated).

Fig. 4. Detection of hepatitis virus in stool during the incubation period. Each solid oval represents one case of hepatitis with jaundice. The number adjacent to the solid oval indicates the incubation period. Four who were not fed virus, but who were in intimate contact with the others, developed hepatitis after 60, 63, 67 and 71 days (see Table III). Stools collected 25 days after virus feeding and 2 or 3 weeks before onset of jaundice were pooled. Stools collected 11 days after feeding were also pooled. Suspensions were prepared and safety-tested. The 11-day material was fed to eight patients with negative results. The 25-day suspension was fed to 13 patients, as shown on the top line; hepatitis with jaundice developed in three. This establishes the presence of virus in the feces during the incubation period. (Modified from Ward, Krugman, Giles, Jacobs and Bodansky: New England J. Med., 259:407, 1958.)
lent of 2 gm of the virus pool. Four of five controls given only virus manifested hepatitis. Of the 11 subjects who had received the combination of gamma-globulin and virus, only one developed mild hepatitis without jaundice.

About 2 months after this phase of the study was begun, the subjects had to leave the isolation unit. They were transferred to various buildings in the institution where hepatitis was endemic. Therefore, it became impossible to evaluate them for a future challenge of immunity. Consequently, this phase of the study had to be deferred.

**Incubation Period of Infectious Hepatitis at Willowbrook.** The unusually long incubation periods (Table II) intrigued us. We wondered if the incubation periods in excess of 60 days could possibly be explained by secondary infection. That is, hepatitis may not have resulted from the original feeding, but rather from contact with the patients within their unit who acquired the disease after the usually-observed incubation period of 40 days.

Table III illustrates our attempt to confirm this hypothesis. The results of the trial are shown in the top section of the table. Sixteen subjects were admitted to the isolation unit but only 10 of this group were fed virus. As can be seen, of the 10 who were fed virus, 3 came down with hepatitis 39, 41 and 46 days later. Four of the six control patients, who were intimately exposed to the three initial cases, acquired hepatitis following incubation periods ranging between 60 and 71 days.

These findings indicated that the long incubation periods were, indeed, spurious, and suggested that hepatitis virus was probably excreted in the stools several weeks before the onset of jaundice.

**Test for Virus Excreted During Incubation Period.** Figure 4 depicts the results of attempts to detect hepatitis virus in the stools during the incubation period. Each solid oval represents one case of hepatitis with jaundice. The number above or below the solid oval indicates the incubation period. As already mentioned, three patients fed virus manifested hepatitis after 39, 41 and 46 days (upper half of Fig. 4). Four of the control patients manifested their disease between 60 and 71 days (lower half of Fig. 4).

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**Fig. 5.** Course of hepatitis after feeding Willowbrook pool No. 1 (unheated). Mild jaundice was first noted after 49 days. However, liver function tests became abnormal after 45 days. The illness was extremely mild with transient icterus and hepatomegaly. Gastrointestinal symptoms included: A—anorexia and V—vomiting. (From Ward, Krugman, Giles, Jacobs and Bodansky: New England J. Med., 255:407, 1958.)
Fig. 6. Course of hepatitis after feeding Willowbrook pool No. 1 (unheated). Jaundice was detected after 41 days. Hepatomegaly and abnormal liver function tests were observed after 39 days. The illness was mild and the patient gained 3 lb during its course. Gastrointestinal symptoms included: A—anorexia and V—vomiting. (From Ward, Krugman, Giles, Jacobs and Bodansky: New England J. Med., 258:407, 1958.)

Fig. 7. Schematic diagram of clinical course of infectious hepatitis; correlation of liver function tests with clinical manifestations. (From Krugman, S., and Ward, R.: Infectious Diseases of Children. St. Louis, Mosby, 1958.)
Stools had been collected periodically during the incubation period. Stools which were collected 25 days after feeding of virus (2 or 3 weeks before jaundice appeared) were thawed, pooled, prepared as described earlier and safety-tested. Stools obtained on the eleventh day (4 or 5 weeks before jaundice appeared) were processed in the same manner.

As shown in the upper part of Figure 4, hepatitis was produced by preparations from stools excreted on the twenty-fifth day of the incubation period, but 11-day material was not infectious. The result of this trial established the excretion of virus in the feces during the incubation period as long as 2 or 3 weeks before onset of jaundice.

This interesting finding suggests that infectious hepatitis has an "alimentary tract" phase during the incubation period. The multiplication and excretion of virus in the absence of clinical and laboratory findings undoubtedly has a considerable bearing on the infectious period of the disease.

Correlation of Clinical Findings and Liver Function Tests, Including Serum Enzymes. During the course of these studies, 41 of 95 patients fed the Willowbrook strain of infectious-hepatitis virus manifested clinical or laboratory evidence of infectious hepatitis. These circumstances provided us with a unique opportunity to follow the course of hepatitis from its earliest inception.

Figures 5, 6 and 7 depict a correlation of the following liver function tests with the development of fever, hepatomegaly and jaundice: bilirubin in the serum; thymol turbidity; cephalin flocculation; and the serum enzymes, phosphohexose isomerase and glutamic oxaloacetic transaminase.

Generally, the illness was very mild and most patients gained weight during its course. A typical case of infectious hepatitis is schematically illustrated in Figure 7. The increase in transaminase in the serum preceded the appearance of other evidence of abnormal liver function by approximately 5 days and persisted for approximately 3 weeks. The jaundice was transient, and within a period of 1 or 2 weeks most of the symptoms had subsided. As already indicated, our studies are still in progress.

Chairman Holt: Would anybody like to ask Dr. Krugman any questions?

Dr. Muncie, Rockville, Illinois: Was the erythrocyte sedimentation rate done in relation to the testing?

Dr. Krugman: No.

Dr. Serkin, Nashville, Tenn.: In these extremely mild cases did you have unusual gastrointestinal symptoms and lassitude?

Dr. Krugman: In the majority of instances the diagnosis would have been missed if the patients had not been followed carefully with serial liver-function tests and daily physical examinations. The symptoms, if present, consisted of low-grade fever, mild anorexia and vomiting. A transitory enlargement of the liver was frequently seen. Jaundice, if present, was also transient. Hepatitis without jaundice was a frequent finding.

Infectious hepatitis is primarily a disease of childhood, the highest incidence occurring in this age group. In children the disease is most likely to be either inapparent or mild. In some instances immunity to this disease has been acquired following a mild attack, which has been erroneously labelled as either "grippe" or "intestinal flu."