

STUDIES ON THE AGENT OF INFECTIOUS HEPATITIS*

III. THE EFFECT OF SKIN TESTS FOR INFECTIOUS HEPATITIS ON THE INCIDENCE OF THE DISEASE IN A CLOSED INSTITUTION

By MILES E. DRAKE, M.D., CHARLES WARD, M.D., JOSEPH STOKES, JR., M.D.,
WERNER HENLE, M.D., GEORGE C. MEDAIRY, M.D., FRANÇOISE
MANGOLD, M.D., AND GERTRUDE HENLE, M.D.

(From The Children's Hospital of Philadelphia, the Division of Virology, Department of Public Health and Preventive Medicine and the Department of Pediatrics, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, and the Rosewood State Training School, Owing Mill, Maryland)

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It has been shown that the virus of infectious hepatitis (IH) can be propagated in chick embryo tissue culture and in the embryonated hen's egg (1). Upon oral administration, the cultivated virus induced hepatitis without jaundice in the majority of volunteers. This illness resembled in every respect hepatitis without jaundice observed frequently after infection of volunteers with virus contained in serum taken from patients during the acute stage of the natural disease (2). Furthermore, individuals convalescent from infection with the cultured virus were found to be immune when exposed to natural IH virus (1).

It was reported early in 1950 that amniotic fluids derived from infected chick embryos, after irradiation with ultraviolet light, could be used as a skin test antigen (3). Individuals known to have suffered an attack of infectious hepatitis gave positive dermal reactions in more than 90 per cent of the cases, whereas the incidence of positive responses in subjects without known history or convalescent from serum hepatitis was strikingly less, in adults 30 to 40 per cent, in children of the order of 10 per cent. More extensive experience gained during recent epidemics and in random groups of individuals has amply confirmed the original report.

In the course of these studies it became evident that the skin test antigen is labile and deteriorates rapidly upon storage at 4°C. Furthermore, some antigens prepared from a given seed (stored at -70°C.) proved to be worthless, whereas others derived from the same subculture were satisfactory as skin test antigens. These problems are under intensive investigation at present.

In spite of these difficulties, certain observations have been made which

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warrant publication at this time. According to all indications, a positive skin test would seem to imply past apparent or inapparent infection with IH virus and, therefore, probable immunity of an individual. The negative skin reactions, correspondingly, would seem to indicate no past contact with IH virus, and thus, susceptibility. Attempts to put this interpretation to test led to unexpected results. It was hoped that the skin test would permit (*a*) the proper selection of volunteers for IH studies and (*b*) a prediction of the approximate number of cases to be expected during an outbreak. However, experiments in volunteers who had been skin-tested 6 to 10 weeks prior to being put at risk by feeding of icterogenic IH serum, suggested that the skin test had altered somewhat the attack rate. When the opportunity arose to study the meaning of positive and negative reactions during an epidemic at Rosewood State Training School, evidence of a protective effect again became apparent. The latter experience is the subject of this report.

Methods and Materials

Skin Test Antigen.—The amniotic fluid of the 6th to 8th amniotic passage (after 10 prior tissue culture transfers) of the Akiba strain of IH virus was used and irradiated by ultraviolet light as described (3). The control material was prepared in the same manner from chick embryos (of the same batches as used for preparation of antigens) injected with normal instead of infected amniotic fluid. The antigens and the control material were injected in 0.1 ml. amounts intradermally into the midflexor region of the right and left forearms, respectively. The results were read 24 to 36 hours later. The criterion for a positive test was arbitrarily selected as an area of erythema of an average diameter of 10 mm. or greater and at least one plus induration, with minimal reactions at the control site. In the few cases in which both the specific and control antigens gave similar reactions the test was considered negative for the purposes of this study.

Laboratory Studies.—The criterion for this study was hepatitis with jaundice. The liver function tests used have been described previously (2). They were performed on most of the sick patients but not on individuals who were apparently well. They included daily urine examinations, serum bilirubin determination, and flocculation tests as requested by the attending physician. Infectious mononucleosis and leptospirosis were eliminated by appropriate tests.

EXPERIMENTAL

Early in 1950 an epidemic of infectious hepatitis began in the Rosewood State Training School. The institution had a total population of about 1300 which was distributed among 7 boys' and 5 girls' cottages. There had been an epidemic of infectious hepatitis in 1944 in the girls' cottages and an occasional case in the boys' cottages giving a total of 45 cases. From 1945 to November, 1948 a total of 10 cases of jaundice was recorded, 8 in 1945, and 2 in 1948. However, in the fall of 1949 an employee developed hepatitis with jaundice and up to May of 1950, 31 individuals had developed the disease.

In May, 1950, 363 persons were skin-tested. Since the amount of skin test material available was insufficient it was decided to test about one-quarter of

each cottage with a single exception. In this cottage which had contributed most of the cases during the previous 6 months all but 4 boys were tested. Two of the cottages housing the youngest age group (4 to 14 years) were selected for additional studies: a boys' cottage (Keating) with a population of 85 and a girls' cottage (Holland) with a population of 87. One boy had developed jaundice early in May and was excluded from this study. None of the others gave a history of jaundice. Skin test antigen was given intradermally to 22 of the boys and 21 of the girls. At the same time, an additional 34 boys and 18 girls in these cottages received intramuscularly 0.01 ml. gamma globulin per pound of body weight. The results obtained with the gamma globulin were part of a separate study (4). These children are not included in the calculations below. Thus there remained in these 2 cottages, 29 boys and 48 girls as controls, none of whom had a record of past jaundice. The children for the skin test, gamma globulin, and control groups were selected at random by choosing, as

TABLE I
Results of Skin Tests in May, 1950

| Group | Total No. | Skin test | | | |
|--------------------------------------|------------|------------|-----------|------------|-----------|
| | | Positive | | Negative | |
| | | No. | Per cent | No. | Per cent |
| Jaundice prior to test..... | 43 | 41 | 95.3 | 2 | 4.7 |
| No known jaundice prior to test..... | 320 | 144 | 45 | 176 | 55 |
| Total..... | 363 | 185 | 51 | 178 | 49 |

far as possible, at least one name for each available letter of the alphabet for each group. They remained in intimate contact for over a year, except when hospitalized for hepatitis with jaundice. The patients were admitted to the institutional hospital under strict isolation. No children admitted to the institution after April 20, 1950, are included in the evaluation of the control group.

Later reference to the records revealed that of the total of 363 individuals who were skin-tested in May, 1950, 43 had a record of hepatitis with jaundice between 1944 and 1950 and in 320 no history was available. The history of the subjects was unknown to the persons applying the skin tests until after the tests had been read and recorded. It was found that 41 of the 43 individuals with a history of hepatitis showed positive reactions to the IH antigen. The 2 cases which gave a negative test had developed jaundice during the outbreak in 1944. Of the 320 persons with unknown histories prior to 1944, 144 gave positive and 176 negative skin reactions. These data are recorded in Table I. The incidence of positive reactions among the individuals without known history of jaundice was somewhat higher than reported previously (3) but is in keeping

with experience gained more recently in institutions where IH had been endemic (5).

The epidemic of infectious hepatitis continued and during the period from May, 1950 to April, 1951, a total of 117 cases of jaundice was recorded. Most of these cases occurred among the controls. Table II shows the incidence of hepatitis with jaundice in the skin-tested and non-tested individuals in Keating

TABLE II
The Incidence of Hepatitis with Jaundice in the Skin-Tested and Non-Skin-Tested Groups

| Cottage | Group | No. in group | Jaundice | |
|---------------------|-------------------------|--------------|----------|----------|
| | | | No. | Per cent |
| Keating | Skin test positive..... | 2 | 0 | 0 |
| | Skin test negative..... | 20 | 1 | 5 |
| | Total skin-tested..... | 22 | 1 | 4.5 |
| | Not skin-tested..... | 29 | 22 | 75.8 |
| Holland | Skin test positive..... | 2 | 0 | 0 |
| | Skin test negative..... | 19 | 2 | 10.5 |
| | Total skin-tested..... | 21 | 2 | 9.5 |
| | Not skin-tested..... | 48 | 22 | 45.8 |
| Keating and Holland | Skin test positive..... | 4 | 0 | 0 |
| | Skin test negative..... | 39 | 3 | 7.7 |
| | Total skin-tested..... | 43 | 3 | 7.0 |
| | Not skin-tested..... | 77 | 44 | 57.1 |
| Total institution | Skin test positive..... | 144 | 1 | 0.7 |
| | Skin test negative..... | 176 | 4 | 2.2 |
| | Total skin-tested* | 320 | 5 | 1.6 |
| | Not skin-tested* | 825 | 112 | 13.6 |

* Excluding those with a past history of jaundice.

and Holland cottages as well as in the whole institution. It is seen that the 4 children in Keating and Holland with positive skin tests remained well; 3 cases of hepatitis with jaundice occurred among the 39 children with negative skin tests. In contrast, among the 77 non-skin-tested children 44 cases of hepatitis with jaundice were observed. Thus, the incidence of jaundice among the skin-tested and control groups was 7 and 57 per cent, respectively. Considering the institution as a whole, there were 5 cases among the 320 skin-tested individuals without a record of jaundice (1 among the 144 with positive skin reactions, 4

among the 176 negative reactors) and 112 cases of hepatitis in the 825 non-tested subjects with an unknown history, or an attack rate of 1.6 and 13.6 per cent, respectively. These differences in incidence are highly significant, the χ^2 for Keating and Holland Cottages on the one hand, and the total institution, on the other, being 29 and 40 respectively. Thus it appears that the skin test had conferred a marked protection.

The date of admission to the institution could conceivably be an important factor in the apparent immunity in the skin-tested children as compared to the high incidence of hepatitis in the non-skin-tested group, especially if the latter consisted predominantly of relatively recent admissions. However, this was not

TABLE III

Year of Admission to Institution in Relation to Incidence of Jaundice during Experimental Period in Keating and Holland Cottages

| Year admitted | Total No. | Non-skin-tested | | Skin-tested | |
|---------------|-----------|-----------------|-------------------|-------------|-------------------|
| | | No. | Cases of jaundice | No. | Cases of jaundice |
| 1944 | 16 | 10 | 8 | 6 | 0 |
| 1945 | 14 | 7 | 5 | 7 | 0 |
| 1946 | 9 | 3 | 2 | 6 | 2 |
| 1947 | 20 | 14 | 12 | 6 | 0 |
| 1948 | 22 | 14 | 7 | 8 | 0 |
| 1949 | 23 | 16 | 7 | 7 | 1 |
| 1950* | 16 | 13 | 3 | 3 | 0 |
| Total | 120 | 77 | 44 | 43 | 3 |
| 1951† | 8 | 8 | 0 | 0 | 0 |

* Up to April 20, 1950.

† After April 20, 1950.

the case as seen in Table III, which lists the number of admissions per year to Keating and Holland and the incidence of jaundice in the current outbreak. It is apparent that the distribution of the children by this criterion in the 2 groups was fairly equitable. Furthermore, it became apparent that the incidence of jaundice in the non-skin-tested group was, if anything, higher in those who had been present for a number of years than in the more recent admissions. In those admitted in 1947 or earlier the incidence was 79 per cent, whereas in the later admissions it was only 35 per cent. In fact, on additional analysis of the most recently admitted children, there appeared to be a lag of at least 9 months between admission and onset of jaundice and no cases were observed among the 8 children added to the cottages after May, 1950. This observation appears to be in keeping with the experience of the Army in Europe and Korea where 6 to

10 months elapsed before replacements began to develop hepatitis with jaundice (6).

The cases of jaundice in the non-skin-tested groups of Keating and Holland cottages occurred over a period of 8 to 11 months as seen in Table IV, with a maximum of 8 in a given month and a minimum of none, the average being 2 to 3 cases per cottage per month. The 3 cases in the skin-tested groups occurred 2 and 8 months after injection of the antigen, *i. e.*, 2 in July and 1 in January.

TABLE IV
The Monthly Number of Cases of Jaundice Occurring in Skin-Tested and Non-Skin-Tested Groups in Keating and Holland Cottages over a Period of One Year

| Cottage | Group | No. in group | No. of cases of jaundice occurring during | | | | | | | | | | | |
|-----------------|-----------------|--------------|---|------|------|-------|------|------|------|------|------|------|------|-----|
| | | | 1950 | | | | | | | | 1951 | | | |
| | | | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May |
| Keating (boys) | Not skin-tested | 29 | 3 | 5 | 2 | 2 | 1 | 2 | 2 | 5 | 0 | 0 | 0 | 0 |
| | Skin-tested | 22 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Holland (girls) | Not skin-tested | 48 | 0 | 4 | 0 | 2 | 0 | 0 | 8 | 3 | 3 | 1 | 1 | 0 |
| | Skin-tested | 21 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

TABLE V
Result of the Retesting of Originally Skin Test-Negative Children in Keating and Holland Cottages One Year after First Test

| Cottage | No. tested | Skin test | | | |
|---------------|------------|-----------|----------|----------|----------|
| | | Positive | | Negative | |
| | | No. | Per cent | No. | Per cent |
| Keating | 20 | 13 | 65 | 7 | 35 |
| Holland | 15 | 8 | 53.3 | 7 | 46.7 |
| Total..... | 35 | 21 | 60.0 | 14 | 40.0 |

In view of this result, it became important to retest some of the previously negative individuals at the end of the epidemic to determine if any changes in dermal sensitivity had occurred. Consequently, 35 of the children in Keating and Holland who had shown originally a negative skin test were skin-tested again after one year. The results are shown in Table V. It was noted that at this date 60 per cent gave a positive skin reaction. Repeated skin tests in non-exposed individuals in other institutions thus far have failed to indicate that a negative skin test may be converted into a positive test by intradermal injection of antigens. It is possible therefore that these children sustained in-

apparent or low grade infections in the interval between the 2 skin tests. As a matter of fact, the incidence of reversions from negative to positive skin test in the skin-tested group is not unlike the incidence of hepatitis with jaundice in the non-skin-tested children (Table II). In Keating cottage, 75.8 per cent of the non-skin-tested group developed hepatitis with jaundice and 65 per cent of the skin test negative boys became positive. The corresponding figures for Holland cottage were 57.1 per cent and 53.3 per cent respectively. However, this similarity may be misleading since no skin tests were applied at the end of one year to the non-skin-tested children to avoid changes in their susceptibility at this time.

DISCUSSION

These studies failed in their original purpose to clarify the meaning of a positive or negative skin test for infectious hepatitis in terms of immunity or susceptibility. Only 5 cases of jaundice were recorded among the 320 tested individuals with as far as could be ascertained negative histories of hepatitis. Four of these cases had previously shown a negative skin reaction, one had been positive. These facts indicate that, as in the case of the skin test for mumps (7, 8), a positive reaction is not always an absolute measure of immunity. In the light of the unexpected observations reported here, indicating a change in susceptibility as a result of skin testing, it will be difficult to obtain the desired answer.

The difference in the incidence of hepatitis with jaundice among the skin-tested and non-skin-tested individuals was striking throughout the institution, *i. e.*, for every case of jaundice in the skin-tested group, 8 occurred among the controls. Since hepatitis had occurred in the institution in the past, the possibility of inadvertent selection in the group had to be considered. However, the skin-tested children were selected at random; furthermore, analyses of the dates of admission of the children in the 2 groups in Keating and Holland cottages revealed that the average length of stay in the institution was quite similar in both groups. Thus it appears likely that the skin test had altered the susceptibility.

In considering this possibility, two explanations may be entertained. Immunization by inactivated viruses might induce some temporary immunity but, in the cases in which this is known to occur, large quantities of the inactive agent are required. In the absence of convenient laboratory tests it is impossible at present to estimate the concentration of IH virus in the amniotic fluid. Since volunteers are not readily available, it has not been possible as yet to titrate infected amniotic fluid in man. It may be surmised, however, that the amount of virus is rather small since no evidence of complement fixation activity has been obtained with amniotic fluid preparations. In as yet unpublished experiments, it was found that injection into chick embryos of infected amniotic

fluid diluted a 100-fold still can produce a skin test antigen after the usual incubation period, but not a 1000-fold dilution. Furthermore, a satisfactory IH skin test antigen can be diluted at most 10-fold, and as a rule not more than 2-fold, and still induce skin reactions. On the other hand, although mumps skin test antigen frequently could be diluted 100-fold and still be effective in the majority of cases, yet it failed to induce significant immunity (9).

Although the amount of antigen present in the IH skin test material is probably too small to produce solid immunity, it might be sufficient to produce partial immunity and reduce the severity of the disease. Since jaundice was the criterion of infection and no laboratory tests were done on apparently well individuals, this viewpoint must remain a hypothesis for the present. However, at the end of the epidemic at Rosewood State Training School, many of the individuals who were originally negative to the skin test had developed dermal sensitivity, a result which conceivably may be ascribed to an inapparent or low grade infection during the epidemic period. As pointed out, no evidence has been obtained as yet to indicate that the skin test *per se* induces dermal sensitization (5). According to this interpretation the skin test would effect partial immunization reinforced by exposure to active natural virus.

As the second possible explanation of the results, it must be considered that the virus in the skin test material was not completely inactivated and that the remaining active virus multiplied sufficiently to produce active immunity. The exposure to ultraviolet light was five times that sufficient to inactivate influenza virus (3), but it is possible that the IH virus is more resistant to irradiation. This cannot be readily demonstrated because of the lack of a susceptible laboratory animal; volunteers will be required in the future for testing the infectivity of the skin test material by the oral route. It should be pointed out, however, that intradermal application of the skin test materials has produced in no instance to date any disease recognizable by clinical or laboratory observations, within the usual incubation period of 14 to 40 days. Furthermore, Neefe, Stokes, and Gellis (10) have shown that the subcutaneous injection of icterogenic IH serum (Akiba strain) rarely induced disease, whereas the same material fed to volunteers produced hepatitis with jaundice. Havens (11), on the other hand, with another strain of IH virus, showed that parenteral injection was as effective as feeding in inducing hepatitis with jaundice in human volunteers. This difference may be due to strain differences or more likely, to a difference in quantity of virus, because cross-immunity was demonstrated between the Akiba strain and the strain used by Havens (12).

If the second explanation should be correct, one may expect that the immunizing effect of the skin test material will be variable. Some antigen may become completely inactivated by irradiation, and thus might not produce immunity, whereas those only partially inactivated may induce active immunity. The latter type may conceivably lead to dermal sensitization.

Although it is impossible at the present time to determine which is the correct explanation, the observations recorded above suggest certain experimental approaches. Further exploration of the problem in the laboratory as well as in volunteers appears to be justified.

SUMMARY

At the onset of an epidemic in a closed institution about one-fourth of the inmates were skin-tested. During the year following the skin test, 5 cases of hepatitis with jaundice were recorded among 320 skin-tested individuals with unknown histories, one in the 144 skin test-positive, and 4 in the 176 skin test-negative subjects. In contrast 112 cases occurred among the 825 non-skin-tested individuals. Thus, the incidence of jaundice in the skin-tested group was 1.6 per cent as against 13.6 per cent in the non-skin-tested individuals. Possible explanations for this observation have been discussed.

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