

# Studies on the Active Immunization Against Epidemic Hepatitis

## II. The Role of the Relative Amounts of Virus and Gamma Globulin in the Inocula; the Effect of Ethanol Treatment on the Virus

By

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In our previous studies [1] it has been observed that in the acute phase the blood of epidemic hepatitis patients contains highly resistant virus. Thus the possibility to carry out active immunization experiments without having isolated the agent appeared to be possible. Part of the experiments on this problem has revealed that gamma globulin, when prepared from virus containing sera, contained a certain amount of live virus. Thus the treatment with ethanol at the concentration and duration applied in the manufacturing of gamma globulin may only result in an attenuation and not in the inactivation of the virus. Gamma globulin from normal sera has a protective effect against inoculated epidemic hepatitis virus. The incubation period of the ethanol treated, inoculated epidemic hepatitis virus is remarkably increased, thus on the basis of the incubation periods it is not always possible to distinguish between hepatitis caused by inoculation of IH or SH virus.

The effectiveness of an artificial infection with IH virus appears to de-

pend on the absolute amount and virulence of the virus inoculated, on the ratio of virus and antibody introduced, on the possible effects of ethanol treatment during preparation, and, last but not least, on the sensitivity of the organism. In order to develop an adequate method of active immunization, the importance of the said factors should be examined.

In our previous study the serum samples of 10 adult hepatitis patients were pooled, treated with 21 per cent ethanol for 40 hours, and then lyophilized. This preparation doubtlessly contained virus. Nevertheless, the long incubation period (45—80 days) observed in the inoculated persons (idiotic children) did not permit the definite exclusion of the possibility that only SH virus had been present in the mixture. Therefore in the present experiments the serum mixture used as an inoculum was prepared by pooling the sera of 20 adult and 60 children patients with acute hepatitis, thus from a total of 80 persons. The sera were treated with ethanol, and lyophilized. The studies of TAMÁSI [2]

and others have shown that SH virus is rare in children, the majority of hepatitis cases in young age being caused by the IH virus. Thus the presence of IH virus in the above inoculum was assured, except if it had been destroyed by treatment with 21 per cent ethanol for 60 hours.

In the previous study the mentioned group had been inoculated intramuscularly with a mixture of 1.0 ml 10 per cent serum protein solution of hepatitis patients (ethanol treatment: 21 per cent for 40 hours) and 9.0 ml of 10 per cent normal gamma globulin. In the present experiments the aim was to ascertain whether a decrease in the amount of the gamma globulin added had any effect on morbidity and whether a moderate increase in the duration of ethanol treatment would suffice to compensate for the decrease in the amount of gamma globulin.

#### METHODS

The serum for group I was treated for 60 hours with 21 per cent ethanol, lyophilized and a protein solution of 10 per cent was prepared of it. The serum for groups II, III and IV was treated with ethanol of various concentration and for various times, lyophilized, and a 10 per cent protein solution was prepared from it.

The experiments were performed on idiotic children ranging in age from 3 to 14 years.

#### RESULTS

The results are summarized in Table I.

In group I, 10 children received a mixture of 1.0 ml hepatitis serum and

6.0 ml normal gamma globulin intramuscularly. Out of the 10 children 3 showed the signs of icteric hepatitis on the 52nd, 53rd, and 76th day, respectively, following the inoculation, while 1 developed anicteric hepatitis on the 75th day. Thus, decreasing the amount of gamma globulin resulted in an increase of positive "takes" as compared to the corresponding data in our previous study, where two children had contracted hepatitis. This increase probably resulted from the amount of gamma globulin being less and this fact could not be influenced by the longer duration of ethanol treatment.

It had to be decided, therefore, whether the ethanol treatment had in fact any effect on the virus. To study this, the mixture of hepatitis sera used in group I was additionally treated with 40 per cent ethanol for 14 days at  $-5^{\circ}\text{C}$  and then lyophilized. This material served for the preparation of 10 per cent solutions of hepatitis sera for group II.

The inocula used for group II was composed of 6.0 ml normal gamma globulin and 1.0 ml of the pooled hepatitis sera. Among the 10 children inoculated, one developed icteric hepatitis after 64 days of incubation. Thus apparently the virus was attenuated by treatment with 40 per cent ethanol for 14 days. Nevertheless some virus was still active after this treatment.

As in the above experiment high amounts of gamma globulin and hepatitis serum were applied, it was difficult to provide for sufficient material

TABLE 1

Group I 60 hours 21 per cent ethanol (10 children)					Group II 14 days 40 per cent ethanol (10 children)				
Date	10 per cent hepatitis serum ml	Normal gamma globulin ml	Incidence and nature of disease	Incubation time (days)	Date	10 per cent hepatitis serum ml	Normal gamma globulin ml	Incidence and nature of disease	Incubation time (days)
12.3 1959	1	6	3 ict 1 anict	52, 53, 76 76	19.8 1959	1	6	1 ict	64
1.7 1959	1	3	—	—	—	—	—	—	—
23.9 1959	1	—	—	—	—	—	—	—	—

  

Group III 40 hours 21 per cent ethanol (12 children)					Group IV 40 hours 21 per cent ethanol (36 children)				
Date	10 per cent hepatitis serum ml	Normal gamma globulin ml	Incidence and nature of disease	Incubation time (days)	Date	10 per cent hepatitis serum ml	Normal gamma globulin ml	Incidence and nature of disease	Incubation time (days)
23.9 1959	0.1	0.9	2 ict	60, 71	26.6 1959	0.05	0.95	1 anict 2 ict	57 76, 81
—	—	—	—	—	23.9 1959	0.05	0.45	—	—
—	—	—	—	—	23.12 1959	0.05	—	2 ict	50, 102

for a more extensive experiment. We had therefore to examine the effect on the incidence of "takes" of a lower dose of inoculum.

For this experiment each of 12 children in group III received intramuscularly a mixture of 0.1 ml of 10 per cent hepatitis serum treated with 21 per cent ethanol for 40 hours and 0.9 ml normal gamma globulin. Out of 12 children in group III one exhibited signs of icteric hepatitis on the 60th, and a second child on the 71st day of incubation. It seemed that this lower-

ing of the inoculum dose had no effect on the incidence of "takes".

Next, the effects of a further decrease in the amount of hepatitis serum in the inoculum were studied. In this experiment 0.95 ml of normal gamma globulin was mixed with 0.05 ml of the hepatitis serum used in group III. As in this case a remarkably lower incidence was expected, the number of test persons was increased from 10—12 to 36.

In group IV, out of a total of 36 children 2 developed icteric hepatitis

on the 76th and 81st day, respectively, and one child fell ill with anicteric hepatitis on the 76th day. These results favoured the supposition that on lowering the amount of hepatitis serum in the inoculum there will be a decrease in the incidence of disease among inoculated persons. The same 36 children received three months later a mixture of 0.05 ml hepatitis serum and 0.45 ml of normal gamma globulin. None of them developed hepatitis. After an additional period of three months, the 36 children were inoculated with 0.05 ml hepatitis serum without any gamma globulin. To our surprise one of them developed icteric hepatitis on the 50th and another child on the 102nd day of incubation. Thus a total of 5 children became ill in this experiment. This fact showed that a further lowering of the amount of serum in the inoculum seemed to be necessary to avoid the disease among vaccinees.

All children with hepatitis were hospitalized. The disease was mild in every case and complete recovery ensued. No disease occurred among the contacts.

#### DISCUSSION

From the results the following conclusions have been drawn.

1. On inoculating large doses (1.0 ml) of hepatitis serum mixed with decreased amounts of antibody (group I), the incidence of "takes" increased. A moderate increase of ethanol treatment of the virus did not compensate

the use of smaller amounts of antibody.

2. On intensive, prolonged ethanol treatment the pathogenicity of the virus appeared to decrease. The invasiveness of the virus was weakened by that treatment, which was, however, insufficient to inactivate the agent completely.

3. A tenfold decrease in the volume of the inoculum at an unchanged hepatitis serum—gamma globulin ratio did not cause any change in the incidence of the disease.

4. The incubation period was long in every group. The inoculum used in groups I and II was prepared from the pooled serum samples from 20 adults and 60 children with acute hepatitis, and of a certain amount of normal gamma globulin. It seemed improbable that the mixture should have contained only SH virus. It seemed also improbable that the virus of IH had been destroyed by 21 per cent ethanol in 40 to 60 hours, while the SH virus was still active after an additional treatment for 14 days with 40 per cent ethanol. The occurrence of such a difference in ethanol sensitivity between the two agents is highly improbable.

It is thought that the prolonged incubation was the result of not only the gamma globulin given simultaneously but also of the effect of the ethanol on the virus.

5. The results obtained in group IV seem to be very instructive. Out of the 36 children 3 contracted the disease after the first inoculation, thus the mixture used had not been neu-

tralized. After the second inoculation no disease ensued. On the third inoculation, when hepatitis serum was used alone, 2 additional children from this group proved to be still sensitive. The incubation was 50 days in one, and 102 days in the other person. This difference could only be explained by the different immune-biological status of these two children.

6. In group IV, 3 children contracted the disease in spite of having received a gamma globulin containing inoculum. Two children became ill only when they had received hepatitis serum alone as the third injection. It seems that these two children were protected against the first injection by the simultaneously administered gamma globulin. It is known that because of failing cross-immunity normal gamma globulin does not protect against SH virus infection. Had only

SH virus been present in the mixture of hepatitis sera, all the five children should have become ill following the first injection.

7. Among the 36 children 31 did not develop disease even after having been inoculated with hepatitis virus containing serum. It is quite improbable that all the 31 had been immune against IH virus. It seems much more likely that the first two injections had induced immunity in the majority of the children. Two subjects had, however, failed to develop adequate immunity and fell ill. The reason for this failure must have been in the actual status of these children. A similar observation was made in our previous study.

8. It cannot be expected that two injections, even if they contain live virus, will be sufficient to immunize every vaccinated person.

#### SUMMARY

Human inoculations have been made with ethanol treated lyophilized hepatitis sera combined with gamma globulin. The results allowed the following conclusions.

1. On decreasing the amount of gamma globulin in the inoculated mixture of hepatitis sera and gamma globulin, an increase of morbidity was observed.

2. The invasiveness of the virus present in hepatitis sera was lowered by intensive ethanol treatment. The virus was not completely destroyed

after 14 days' treatment with 40 per cent ethanol.

3. Lowering the total amount of the inoculum did not influence the incidence of the disease following inoculation, provided the ratio of hepatitis serum—gamma globulin had not been altered significantly.

4. A certain protection against the hepatitogenic action of the virus was afforded by the administration of gamma globulin.

5. Two injections with hepatitogenic material failed to immunize all the persons inoculated.

## REFERENCES

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