Investigations into the Immunoprophylaxis of Viral Hepatitis

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Based on trials in mentally retarded children, the effect of alcohol on hepatitis virus A is discussed. The virus while kept in alcohol gradually loses its infectivity. The loss is in direct relation to the alcohol concentration and the period of treatment. First the oral infectivity is lost and this is followed by the loss of the parenteral infectivity. As a result of alcohol treatment, virus A becomes similar to virus B. In virus carriers a similar transformation of virus A into virus B occurs. There might exist a transient form (virus AB), which has retained its oral infectivity, but the incubation period of the illness is prolonged. It is suggested that virus B is a derivative of virus A. The intestinal wall as a barrier has a significant role in the prevention of hepatitis. The control of posttransfusion hepatitis of virus B origin is more difficult than that of infectious hepatitis. However, considering that virus B is a derivative of virus A, a successful control of infectious hepatitis may indirectly reduce the incidence of the posttransfusion cases, which may be caused by any of the viruses A, AB and B. The possibilities of active immunization against viral hepatitis are discussed.

Viral hepatitis is one of the most important problems of medicine. In Hungary, 16 000 cases are reported yearly, and a similar number of cases is thought to remain unregistered.

Stokes et al. [11] were the first to stop hospital epidemics by administering gamma globulin. The dose of 16% gamma globulin was initially 0.12 ml/kg, subsequently this dose was gradually reduced to 0.02 ml/kg. The protective effect of such low doses of gamma globulin proved to be strikingly lasting. Stokes et al. assumed that during the period of passive immunity children in institutions may be infected subclinically and acquire active immunity.

We are sceptical about such an explanation. It has been shown with the prevention of measles that the passive immunity induced by gamma globulin is of short duration while hepatitis spreads in children communities slowly. Thus, assumed that commercial gamma globulin, having been prepared from blood samples collected from thousands of donors may contain small amounts of hepatitis virus, thus may induce an active immunity without causing hepatitis. Hepatitis is not induced as

(i) the amount of virus is not sufficient; *(ii)* its hepatitogenic capacity is reduced by the alcohol treatment; *(iii)* the specific antibody in the gamma globulin may also reduce the viral effect (neutralization?).

(1) To check the assumption that gamma globulin may contain hepatitis virus, we prepared gamma globulin from the pooled plasma of female patients with acute hepatitis. The donors had received no injection in the six preceding months. It was beyond doubt that the plasma pool must have contained large amounts of hepatitis virus. Then from the pool, gamma globulin was prepared by Cohn's fractionation technique including exposure to 21% alcohol for 40 hours at −5°C. With the preparation thus obtained ten mentally and somatically retarded children were inoculated. Of these, two became ill with hepatitis on the 56th and 76th day, respectively. It was therefore concluded that the gamma globulin prepared from highly infectious plasma had in fact contained hepatitis virus. The inoculated children remained in the community without isolation until developing illness — in spite of this, no secondary cases occurred [3].

(2) To check the effect of alcohol treatment on the virus, the pooled serum obtained from 10 patients with acute hepatitis was exposed to 21% alcohol for 40 hours or 60 hours, or to 40% for 14 days. The lowest number of those contracting the disease occurred in the group having received the last preparation, though

even in this, the virus was not killed completely. The incubation period ranged between 52 and 102 days, and the course of the illnesses was mild. No contact case occurred [6].

(3) That gamma globulin acts against the hepatitis virus needs no confirmation.

We believe that the arguments in favour of our three suppositions are acceptable.

To obtain further evidence for the role of the quantitative factors, we reduced the dose of the gamma globulin preparation pretreated in 40% alcohol for 14 days. The dose was gradually reduced from 0.1 g to 0.01 g protein [7]. This latter dose caused no hepatitis. Unfortunately, because of technical errors we could not prove whether the preparation actually contained hepatitis virus.

For further experiments blood was collected from 50 children suffering from acute hepatitis. The pooled serum was kept with 40% alcohol at −5°C for 14 days or, in another experiment, for 12 days. Children were inoculated with each preparation. Neither of the children became ill. Since it would be unjustified to suppose that this serum pool had contained no hepatitis virus, we suppose that the resistance to alcohol of different virus strains may be variable [4].

As regards immunoprophylaxis, we recommended as early as 1961, to administer two doses of gamma globulin at an interval of four months. Our experiments in a small number of children supported this view. Subsequently, ČERVENKA [1] carried

out a similar trial in Slovakia. He immunized school-children with 16% gamma globulin preparations, administering 0.02 ml/kg, intramuscularly. Group I including 5347 children was inoculated in June, group II including 5355 children in September, group III including 4606 children one in June and again in September, and group IV including 8267 children served as uninoculated control. In the autumn of the same year four cases of hepatitis occurred in group I, five cases in group II, no case in group III and 70 cases occurred in the control group.

The question may arise whether the passive immunity due to the gamma globulin still persisted in October or November, *i.e.*, four or five months after the immunization. Moreover, it is of interest that in group II three of the five children became ill as early as in the month of the immunization, *i.e.* in the period of incubation. It might be supposed that in group III the second injection acted as a booster dose.

It is well-known that gamma globulin prevention of infectious hepatitis is successful in children living in communities, but its beneficial effect in the family is doubtful. Patients shed the virus as early as 1 or 2 weeks before the onset of jaundice, and the contacts are usually inoculated several days thereafter, *i.e.*, in an advanced state of the supposed incubation period.

Consequently, gamma globulin should be administered before the infection. We disbelieve that gamma globulin prevention in the family is reasonable except for cases when the patient remains with the family. In such cases, however, large doses should be given.

We were somewhat surprised by the lack of virus dissemination in the environment. To obtain more information, we initiated further experiments. For this purpose gamma globulin was prepared from pooled sera of six sportsmen. None of these had been given any injection within half a year before their arrival at a resort place on 6 February, 1966. Soon after their arrival one of the waitresses in the hotel's restaurant became ill with hepatitis. On 15 and 16 March, 6 of the sportsmen were admitted to our hospital with acute hepatitis. The incubation periods were not longer than 36 days. No doubt that these were cases of infectious hepatitis.

Blood was taken from the patients on the day of their admission. The pooled serum was kept in 21% alcohol for 70 hours at $-5°C$ and then lyophilized. The lyophilized specimen was halved, and from one half a 1% solution was made. With this solution 15 children were inoculated intramuscularly, with 4.0 ml ($= 0.04$ g protein) each.

Two of the children became ill. One had anicteric hepatitis with an incubation period of 90 days, the other developed jaundice and in this case the incubation period lasted 99 days. There is no doubt that the lyophilized serum contained virus.

After careful preliminary experi-

TABLE I

Experiments with an infectious hepatitis serum pool pretreated with 21% alcohol for 70 hours

Experiment	No. of inocula-ted subjects	Date of inoculation	The preparation			Occurrence of		Incu-bation period days
						hepatitis		
			Protein concentration	Dose	Route of inoculation	icteric	anic-teric	
No. I.	15	15 Sep, 1966	1%	4 ml	intra-muscular	1	1	99 90
No. II.								
a) first inoculation	23	1 Sep, 1966	Lyophilized serum	0.1 g	oral	—	—	—
b) second inoculation	19	1 Apr., 1967	1%	4 ml	intramuscu-lar	1	1	88 88

ments [4] 23 children were given of the lyophilized serum, orally. The individual dose was 0.1 g, which corresponded to 1.5 ml serum.

The children remained in their environment, and their serum bilirubin, SGPT, SGOT, thymol turbidity and gold sol values were followed. Within the following 100 days no case of hepatitis occurred either in the inoculated children or in their environment.

Nineteen of the 23 children fed with the serum containing virus were subsequently given of the same lyophilized serum, intramuscularly. Two children became ill, the one with icteric, the other with anicteric, hepatitis. The incubation period lasted 88 days in both cases. Obviously, active virus was present in the preparation even after the oral experiment.

We attribute the prolongation of the incubation period and the loss of oral infectivity to the alcohol treat-

ment. Supposedly, as a result of this treatment, the virus became similar to hepatitis virus B. The lack of contact cases is consistent with this assumption. The change of the virus was both quantitative and qualitative.

Furthermore, the defence of the organism against this virus was successful when the virus was administered orally, whereas it was unsuccessful after parenteral administration. In the latter case a barrier, the intestinal wall, was lacking.

Similar experiments were published by KRUGMAN et al. [8]. These authors obtained blood from 27 children with acute hepatitis three to seven days before the onset of jaundice. The pooled serum was fed to 11 newly admitted mentally retarded children, in a dose of 1.0 ml. Feeding of the serum was followed by hepatitis in ten of the 11 subjects. The incubation period was in the range of 40 days. Jaundice was observed in

six of the ten subjects with hepatitis. Six months later 10 children who had participated in the previous trial were given of the same pool a dose of 0.25 ml, intramuscularly. Hepatitis was observed in four of the inoculated children, and two of these developed jaundice. The occurrence of hepatitis in four children represented a second attack. In this trial the incubation period ranged between 66 and 82 days. Thus, the first attack had left behind no protection against the intramuscular infection with the same pool of serum. Nevertheless, in our opinion this observation does not indicate that these children had no intestinal immunity.

From a child who had developed two attacks, a serum specimen was obtained prior to each attack. With the pool obtained before the second attack (MS-2), children were infected intramuscularly and serum from one of the infected children (second-passage virus) was fed to six further children. Hepatitis with a prolonged incubation period was observed in five of the orally infected children. Consequently, the length of the incubation period was independent of the route of infection.

KRUGMAN et al. [8] suggested that the original serum pool contained two different viruses: a short-incubation virus (MS-1) and a long-incubation virus (MS-2); during the attack following parenteral infection MS-2 virus appeared in the serum of the same patient whose first attack had been due to the MS-1 virus. In the experiment with the second-passage MS-2 virus the children were infected with this virus only.

We prefer another explanation. The virus, which had been exposed to immunobiological effects in the first infected subject might have undergone changes which supposedly shifted its properties toward the properties of hepatitis virus B; the incubation period became longer, but the virus did not lose its oral infectivity. This is a change similar to that observed with our alcohol-treated virus. We therefore suppose that there exists a single basic hepatitis virus, and this is virus A. Virus B is its derivative.

SUGGESTIONS

(i) Gamma globulin prepared by Cohn's alcoholic method from sera obtained from patients with acute hepatitis can cause hepatitis if given intramuscularly.

(ii) The pathogenicity of the residual virus is low even after a short treatment with alcohol and inversely related to the alcohol concentration and to the duration of treatment. There may be some variability in the alcohol tolerance of different strains of virus.

(iii) ČERVENKA's experiments support our view that even commercial gamma globulin preparations may exert an active immunizing effect. Mostly those children remained unprotected who had received the gamma globulin in the incubation period. Consequently, gamma glo-

bulin should be administered prior to exposure.

(iv) Oral infection is not followed by massive immunity against parenteral infection.

(v) If a subject orally infected with virus A develops hepatitis and is excreting the virus, the virus in his faeces may infect contact subjects [9]. Our alcohol-treated virus A, on the other hand, had lost its oral pathogenicity as shown by the lack of dissemination in the environment. However, when administered parenterally, it caused hepatitis with a long incubation period.

(vi) The intestinal wall is of great importance in the immunity against infectious hepatitis. A protection satisfactory against oral infection may be unsatisfactory against parenteral infection.

(vii) We have shown that posttransfusion hepatitis can be prevented by administering giant doses of gamma globulin [2, 10]. This is only of theoretical importance. It is difficult to prevent posttransfusion hepatitis, as in this case the virus escapes an important barrier, the

intestinal wall. Besides, according to theoretical consideration, commercial gamma globulin must be richer in anti-A than in anti-B antibodies.

(viii) We suppose that there is only one basic hepatitis virus; this is virus A, and virus B is its derivative, continuously arising from the former. This is the most plausible explanation why virus B did not die out long ago, when there were hepatitis epidemics, but injections had not yet been given by the physicians.

(ix) The transformation of virus A into virus B appears to be gradual; first the incubation becomes prolonged, and this is followed by the loss of oral infectivity. The virus with a prolonged incubation period and retained oral infectivity might be called virus AB.

(x) Posttransfusion hepatitis may be caused by virus A, the tentative virus AB, or virus B. In the first case (which may occur after the transfusion of blood obtained from donors who were in the incubation period of infectious hepatitis or had subclinical infection) the incubation period is short and the faecal communicabi-

TABLE II

Characteristics of viruses A, AB and B

	Virus A	Virus AB	Virus B
Transmissibility of virus in blood by injection	+	+	+
orally	+	+	—
Incubation period of secondary cases	35 days	90 days	90 days
Transmissibility of virus in faeces by injection	+	+	?
orally	+	+	—
Incubation period of secondary cases	35 days	90 days	

B = blood , F = faeces , o+ = infective by the oral route ,
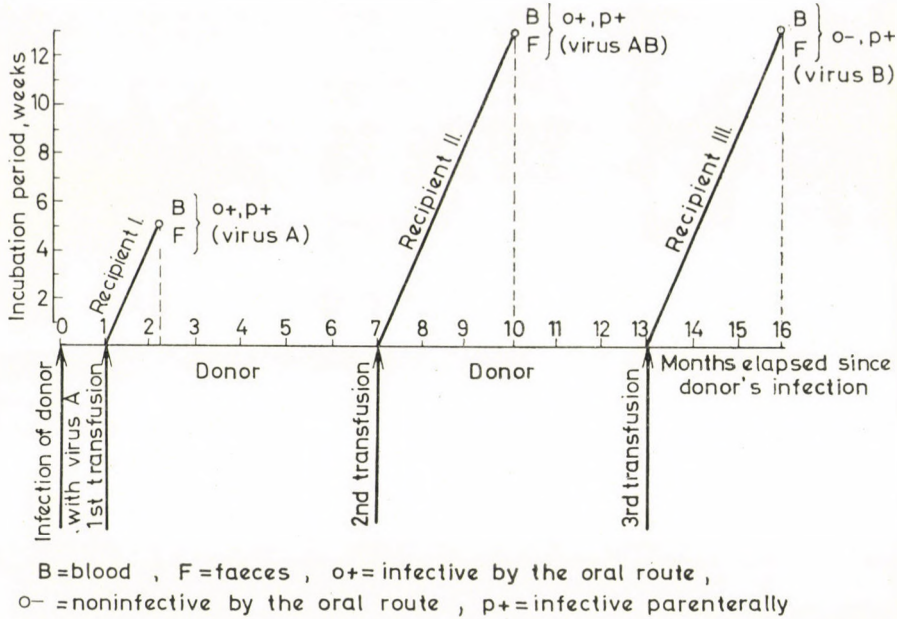o− = noninfective by the oral route , p+ = infective parenterally

FIG. 1. N. J.. a fictive blood donor. was infected orally with virus A on January 1, 1967. He developed an inapparent hepatitis with viraemia. Blood obtained from N. J. on January 30 was transfused to K. I.. who subsequently developed virus-A hepatitis with an incubation period of 35 days. His blood and faeces became infective and the virus was transmissible both orally and parenterally (virus A). Another blood specimen from N. J., obtained later in July, was transfused to K. P.. who developed hepatitis with an incubation period of 90 days. The blood and faeces of K. P. were infective and the virus was transmissible parenterally, to some extent also orally. The incubation periods of the secondary cases were prolonged, irrespective of the route of infection. This is characteristic of virus-AB infection. A third specimen. obtained from N. J. in January. 1968 was transfused to B. P., who subsequently developed hepatitis with an incubation period of 90 days. The virus which appeared in his blood. and to some extent in his faeces, did not spread by the oral route but was transmissible parenterally. In this case the causative agent was virus B

lity is of high degree; in the second case the incubation period is prolonged, but some faecal communicability is still retained; in the third case the incubation period is prolonged and faecal communicability has been lost.

(*xi*) If virus B is indeed a derivative of virus A, the control of infectious hepatitis must be indirectly effective against posttransfusion hepatitis.

(*xii*) The natural virus B and our alcohol-treated virus are similar in the prolonged incubation period and in the lack of oral infectivity. However, the natural virus B is highly virulent, whereas our virus shows low virulence (low morbidity, mild cases) when injected parenterally.

POSSIBLE WAYS OF THE CONTROL OF HEPATITIS

(i) It would be justified to carry out trials in schools, kindergartens, and in hospital staff. Subjects should be injected with gamma globulin three times: the first injection might be administered in June, or September when the epidemic curve begins to rise, the second injection 4 months after the first and the third one after 8 further months.

(ii) The second schedule is based on our own experiments. Serum containing hepatitis A virus should be treated with 21% alcohol for 40—48 hours at −5°C and lyophilized. In such preparations the virus is still living, but has lost its oral pathogenicity. Subjects to be immunized should be given 0.05—0.1 g of the lyophilized material, orally, alone or combined with gamma globulin injection.

The protection might be checked by epidemiological methods or by feeding native virus to experimental subjects several months after oral administration of the alcohol-treated virus. Such a challenge carries some risk but, as shown by our oral experiments adequate care can reduce the risk to very low levels.

It is questionable whether humoral immunity develops after feeding the alcohol-treated virus. However, a local intestinal immunity preventing the implantation of wild hepatitis virus would be a significant progress toward the control of hepatitis.

In preparing serum for immunization purposes, overtreatment with alcohol should be avoided.

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