THERAPEUTIC TRIAL OF METHIONINE IN INFECTIVE HEPATITIS*

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During the present war there has been a greatly increased incidence of liver disease due to infective hepatitis, arsenotherapy jaundice, and homologous-serum jaundice following inoculation with yellow-fever vaccine. The problem of treatment has produced a renewed interest in the relation of dietary factors to liver disease. Most animal experiments recorded in the literature have been concerned with the production of cirrhosis of the liver and its relation to the fat content of the diet or to liver metabolism of fat. More recently, however, certain protein constituents have been shown to have an important bearing on the production of acute liver damage. Davis and Whipple (1919) found that skimmed milk or casein protected the liver against the toxic action of chloroform. Subsequent work (Miller, Ross, and Whipple, 1940; Miller and Whipple, 1942) revealed that the sulphur-containing amino-acids, particularly methionine, were the specific protective factors. These experiments were performed in dogs fed on a very low protein diet. Messinger and Hawkins (1940) found that in dogs a high protein diet protected the liver against the toxic action of arsphenamine; and Goodell, Hanson, and Hawkins (1944), using a similar technique to that of Miller and his colleagues, showed that methionine exerted a specific protective action against mapharside. More recently it has been discovered that protein deprivation alone gives rise to acute liver damage, which can be prevented by the addition of sulphur-containing amino-acids to the diet. Daft, Sebrell, and Lillie (1942) reported that cystine and methionine prevented the hepatic haemorrhages and necrosis which occur in rats on a low-protein high-carbohydrate diet. Himsworth and Glynn (1944) demonstrated that this protective action was independent of other dietary factors.

From these experiments it is apparent that protein deprivation in animals produces or predisposes to acute hepatic necrosis and that the sulphur-containing amino-acids, particularly methionine, protect the liver from the effects of protein deficiency. It was on the basis of these experiments that the present therapeutic trial of methionine was undertaken. The object of the trial was to discover whether oral administration of the amino-acid had a beneficial effect on the course of the disease and, in particular, whether the period of incapacity could be shortened.

Material

The trial was carried out on 100 Service patients with infective hepatitis, admitted to hospital under the M.R.C. Jaundice Unit. Methionine was administered to alternate cases. All were males, except three in the treated group and two in the control group. The average duration of symptoms of all cases on admission was 8.3 days, the average interval between the appearance of dark urine and admission was 3.9 days, and the majority of the patients had been jaundiced one to two days.

Methods

dl-methionine was given in daily doses of 5 g. from the time of admission until the urine had been bile-free (using the foam test) for five days. This quantity of methionine is probably roughly equal to the average content of the normal

diet. The amino-acid was administered in morning and evening doses of 2.5 g. each. A fresh solution was made daily, kept in the refrigerator, and given ice-cold, flavoured with orangeade. Control subjects received orangeade alone. There was no restriction of diet except that greasy foods were omitted, and all patients received a supplementary pint of milk and one egg daily. It was impossible to give a fixed diet owing to variation in the appetite at different stages of the disease. Sample analyses showed that during the period of diminished appetite (i.e., during the first few days after admission) the amount of food actually consumed contained approximately 60 g. protein, 70 g. fat, and 240 g. carbohydrate, with a total calorie value of 1,900. During the later stages, after the appetite had returned to normal, the values were 120 g. protein, 130 g. fat, 450 g. carbohydrate; calorie value 3,500. The majority of patients were kept in hospital until they were fit to resume training at a convalescent depot, from which they returned for examination one month after discharge from hospital.

Comparison of the treated and control groups was based on clinical and biochemical criteria. The clinical criteria were: (1) duration of anorexia after admission; (2) duration of jaundice after admission; (3) duration of liver enlargement after admission; (4) duration of liver tenderness after admission; (5) period in hospital; (6) frequency of relapses or recrudescences. The majority of relapses or recrudescences were observed before discharge from hospital; we have included any deterioration of the patient's condition during recovery which led to a return of symptoms with increase in biliuria and rise in the serum bilirubin.

The biochemical criteria employed were: (1) duration of biliuria; (2) interval between admission and return of serum bilirubin to 2 mg. per 100 c.cm.; (3) maximum serum bilirubin; (4) hippuric acid synthesis after the disappearance of bile from the urine, as a test for recovery of liver function. The foam test for bilirubinuria was used, and serum bilirubin was estimated by the method of Malloy and Evelyn (1937). The intravenous modification (Quick, 1939) of the hippuric acid synthesis test was employed.

Comparability of Control and Treated Cases

The following criteria were used to determine whether the control and treated cases were comparable: (1) age; (2) interval between first symptom and admission; (3) interval between

TABLE I.—Comparability of Treated and Control Groups

	Treated	Controls	Difference	D./S.E.
Number of cases	50	50		
Age (mean in years)	26.7	27.2	0.5	0.42
Onset to admission (mean in days)	7.5	9.2	1.7	1.84
Dark urine to admission (mean in days)	4.4	5.7	1.3	2.14
Number of cases with rising serum bilirubin on admission	25	25		

appearance of dark urine and admission; (4) percentage of cases admitted with rising serum bilirubin.

The comparative figures for the two groups are given in Table I. The age distribution was very similar, with mean

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values of 27.2 (controls) and 26.7 years (treated cases). The mean duration of the disease before admission was a little shorter in the treated group (7.5 against 9.2 days), which might produce a slight bias in favour of the untreated cases in respect of criteria measured from the date of admission. There were 29 patients in the treated group with less than seven days' history on admission, compared with 23 in the control group: as will be seen later, comparison of these early cases does not give significantly different results from those obtained by comparison of all cases. Thus the groups may be regarded as comparable in spite of the shorter mean duration before admission in the treated cases. Furthermore, the duration of the disease before admission is largely determined by the length of the pre-icteric stage; this we have found to bear no relation to the severity or duration of the icteric stage, on which our comparison is mainly based. In both groups exactly half the cases showed a rising serum bilirubin after admission. This is not an entirely satisfactory criterion, since estimations were made on admission and every third day subsequently, so that if methionine had an immediate beneficial effect a rising serum bilirubin on admission might have been checked in some cases. The general results of the investigation indicate that such an occurrence is unlikely. On the several criteria employed, it may therefore be assumed that the control and treated groups are comparable.

Results

A comparison of the clinical and biochemical criteria in treated and untreated patients is made in Table II. Figures

TABLE II.—Comparison of Clinical and Biochemical Criteria of Severity and Duration in Treated and Control Groups

	All Cases				Early Cases			
	Treated	Controls	Difference	D/S.E.	Treated	Controls	Difference	D/S.E.
Number of cases	50	50			29	23		
Duration of anorexia (days)	8.3	8.7	+0.4	0.17	7.7	10.7	+3.0	0.79
Duration of jaundice (days)	16.0	18.6	+2.6	0.96	17.9	17.8	-0.1	0.02
Duration of liver enlargement (days)	20.8	23.9	+3.1	0.67	21.6	24.2	+2.6	0.40
Duration of liver tenderness (days)	21.5	22.8	+1.3	0.47	20.5	24.8	+4.3	0.67
Period in hospital (days)	32.0	36.4	+4.4	1.33	33.9	32.1	-1.8	0.35
Number of relapses	5	9	+4	1.14				
Duration of bilirubinuria (days)	13.2	16.7	+3.5	1.59	14.8	17.0	+2.2	0.55
Duration of hyperbilirubinaemia (days)	18.1	21.9	+3.8	1.20	21.1	20.2	-0.9	0.19
Maximum serum bilirubin (mg./100 c.cm.)	12.0	12.3	+0.3	0.20	12.0	12.2	+0.2	0.11
Final hippuric acid test	1.039	1.058	-0.019	0.36	1.007	1.125	-0.118	1.49

+ = Difference in favour of treated cases. - = Difference in favour of controls. Early cases were admitted within 7 days of onset. All figures except number of relapses are mean values. Time intervals are measured from admission — i.e., from the beginning of treatment. Duration of hyperbilirubinaemia is the interval between admission and return of serum bilirubin to 2 mg./100 c.cm. Hippuric acid test is expressed as grammes of sodium benzoate excreted in 1 hour.

are given for all cases, and for early cases admitted with less than seven days' history of symptoms. The majority of criteria show a slight bias in favour of the methionine-treated group, but the differences are not statistically significant.

The mean duration of jaundice after admission was 16.0 days in treated patients and 18.6 days in controls. This is not a significant difference (D./S.E.=0.96). In early cases the difference was negligible (17.9 against 17.8 days). Duration both of liver enlargement and of liver tenderness was again slightly longer in the control than in the treated series, and the early cases showed a similar but not significant difference. Duration of stay in hospital was 4.4 days shorter in the treated group (32.0 days) compared with the controls (36.4 days); in early cases the difference was reversed (33.9 days, treated; 32.1 days, controls). There were five relapses in the treated group, compared with nine in the control group, but this difference is less than twice the standard error (D./S.E.=1.14). Duration of anorexia was almost the same in the two groups (8.3 days, treated; 8.7 days, controls), although in early cases anorexia lasted 3 days longer in controls.

Comparison of the biochemical criteria showed similar small differences in favour of the treated group. The duration of bilirubinuria after admission was 13.2 days, treated, against 16.7 days, controls (D./S.E.=1.59); in the early cases the difference was somewhat smaller (14.8 against 17.0 days). The duration of hyperbilirubinaemia after admission showed a similar but not significant difference (18.1 days, treated; 21.9 days, controls; D./S.E.=1.2); this difference was reversed when early cases were compared (21.1, treated; 20.2 days, controls). The difference in duration of hyperbilirubinaemia was more pronounced in cases admitted with a falling serum bilirubin, which is partly explained by the fact that in these the mean admission serum bilirubin was higher in the control (7.65 mg. per 100 c.cm.) than in the treated group (5.9 mg. per 100 c.cm.), so that half the control cases started with a disadvantage, estimated at 2½ to 3 days, compared with the treated cases.

The mean maximum recorded serum bilirubin was almost identical in the two groups. The comparison of maximum serum bilirubin was confined to the 25 cases in each group admitted with rising serum bilirubin. If, instead of comparing the mean values, the cases with rising serum bilirubin on admission are subdivided into groups with maximum serum bilirubin of 0 to 5, 5 to 10, and over 10 mg. per 100 c.cm., the distribution of cases in these groups of differing severity is very similar in the treated and untreated series (Table III).

TABLE III.—Distribution of Cases admitted with Rising Serum Bilirubin, according to Maximum Observed Serum Bilirubin

Maximum Serum Bilirubin	Treated	Controls
0-5 mg./100 c.cm.	1	1
5-10 mg./100 c.cm.	10	7
Over 10 mg./100 c.cm.	14	17
Total	25	25

The hippuric acid test, performed at the end of treatment, showed no significantly different mean value in treated and control cases; in fact, the result was slightly favourable to the controls.

Discussion

One of the difficulties in interpreting the results of therapeutic trials in infective hepatitis is the choice of suitable criteria for comparison. In individual cases of the disease there often appears to be a very variable relationship between the clinical and biochemical findings. We have attempted to overcome this difficulty by comparing as many criteria as possible, so as to obtain a comprehensive picture of the severity and duration of the disease. In a study of over 200 cases we have found that in a large series there is in fact a fair correlation between the clinical and the biochemical criteria of severity used in this comparison.

Although the differences between treated and untreated cases do not appear to be statistically significant, the fact that all the criteria except hippuric acid synthesis show some bias in favour of the treated group suggests that possibly the methionine may not have been without benefit. Since, however, many of the criteria are interdependent their values should not be summated in assessing the effect of methionine treatment. Thus duration of jaundice and duration of biliuria are dependent on duration of hyperbilirubinaemia; and length of stay in hospital, except in a few cases, was decided by the time taken for the serum bilirubin to reach the 2-mg. level.

We conclude, therefore, that oral administration of methionine under the conditions of this trial had no significant effect on the severity or duration of the disease. It must be pointed out that infective hepatitis is rarely diagnosed until jaundice becomes manifest. Even if treatment is started on the first day of jaundice, symptoms may have been present for a week or more, and both symptoms and liver function tests are rapidly improving in the majority of cases. It is apparent that methionine administration does not prevent relapses. We observed five relapses during the course of methionine therapy, and there was no evidence that the degree of liver involvement during these relapses was less than in untreated subjects. Himsworth and Glynn (1944) have suggested that massive hepatic necrosis occurring in the course of infective hepatitis

may be due to nutritional factors. The rarity of this complication would make it extremely difficult to decide this point. It has been stated (Cohen, 1944) that methionine undoubtedly has beneficial effects in "subacute necrosis" of the liver. The proof of this would again demand a large and well-controlled series of cases. In our limited experience methionine has been ineffective in patients who had already developed symptoms of hepatic failure.

Summary

Five grammes of methionine has been administered daily by mouth to alternate cases in a series of 100 Service patients with infective hepatitis. A comparison of clinical and biochemical criteria has shown no significant effect on the severity or duration of the disease or on the incidence of relapses.

REFERENCES

- Cohen, H. (1944). *Practitioner*, **153**, 193.
Daft, F. S., Sebrell, W. H., and Lillie, R. D. (1942). *Proc. Soc. exp. Biol.*, N.Y., **50**, 1.
Davis, N. C., and Whipple, G. H. (1919). *Arch. intern. Med.*, **23**, 612.
Goodell, J. P. B., Hanson, P. C., and Hawkins, W. B. (1944). *J. exp. Med.*, **79**, 625.
Himsworth, H. P., and Glynn, L. E. (1944). *Lancet*, **1**, 457.
Malloy, H. T., and Evelyn, K. A. (1937). *J. biol. Chem.*, **119**, 481.
Messinger, W. J., and Hawkins, W. B. (1940). *Amer. J. med. Sci.*, **199**, 216.
Miller, L. L., Ross, J. F., and Whipple, G. H. (1940). *Ibid.*, **200**, 739.
— and Whipple, G. H. (1942). *J. exp. Med.*, **76**, 421.
Quick, A. J. (1939). *Amer. J. digest. Dis.*, **6**, 716.

TREATMENT OF INFECTIVE HEPATITIS WITH METHIONINE

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No therapeutic means have yet been found capable of producing a rapid alleviation of infective hepatitis. There would seem to be justification for seeking such a means among the constituents of proteins. Favourable effects of a diet of high protein and vitamin content have been claimed by some investigators in cases of liver disease (Pátek and Post, 1941). In animals it has been shown experimentally that the health of the liver and its capacity to resist the action of toxic agents are influenced by the protein content of the diet (Himsworth and Glynn, 1942). Diets low in protein may produce in animals a cirrhosis of the liver which in many features resembles that seen in man (György and Goldblatt, 1939; György, 1944; Lillie, Daft, and Sebrell, 1941). Of the amino-acids, methionine has proved most effective in preventing the experimentally induced deposition of fat in the livers of animals (for references, see McHenry and Patterson, 1944) and the cirrhosis associated with a low protein intake (György, 1944). From the work of Miller and Whipple (1942), who described its value in preventing necrosis of the liver following chloroform anaesthesia, methionine may have some value in protecting the liver against other toxic substances. In what way methionine acts in these conditions, whether by processes of trans-methylation or by a role in sulphur metabolism, is uncertain. Nevertheless, of all the amino-acids, methionine most justifies a trial of its effect upon liver disease in man. Peters *et al.* (1945) have given evidence of a slight beneficial effect of methionine and cysteine upon so-called post-arsphenamine jaundice. While that trial was in progress it seemed worth while to ascertain its usefulness in infective hepatitis.

Details of Trial

All patients suffering from infective hepatitis referred to the hospital between Oct., 1943, and March, 1944, were admitted to the same ward. The total number was 37, and their ages were between 17 and 45 years. Care was taken to exclude patients with liver disease from other causes; no patient who had received neoarsphenamine or who had been exposed to any toxic substance was included in the experimental group. All the patients were kept in bed. Every alternate patient was given methionine. They were not discharged until clinically well and their plasma bilirubin had fallen below 2 mg. per

100 c.cm.* One month after discharge they were seen again and their blood and urine examinations were repeated.

To minimize the chance variation of the methionine content of the meals, all patients, treated and untreated, were put on a diet of low-fat high-protein content, supplemented with extra vitamins. A record was kept of the amount eaten by every patient at each meal. It was estimated that the methionine content of the diet consumed daily was of the order of 2.5 g. To ensure an adequate supply of methionine twice this amount was given to the treated patients. Synthetic *dl*-methionine is not easily taken in solid form. It was therefore made up in the proportions of 10 g. to 283 ml. of water containing 2.8 ml. of concentrated hydrochloric acid; this solution contained 1 g. of methionine in 1 fl. oz. Throughout the day five 1-g. doses were given either in milk or after flavouring with fruit juice. Including that in the diet, the treated patients received daily nearly 7.5 g. of methionine. Some found the amino-acid nauseating, and in others it caused vomiting in the early days of the disease. There was only one instance of a patient's intolerance to the amino-acid. During the experiment one of us (G.H.) developed infective hepatitis. He was admitted to the ward and was given methionine. This induced persistent nausea. He became so intensely jaundiced and ill that the drug was stopped after 7 days. For this reason his case is not included in the series, but is described separately.

A detailed biochemical study was made on all patients. It included blood urea, plasma bilirubin, proteins, phosphatase determinations at 2-3-day intervals, daily urinary excretion of nitrogen, urea, creatinine, creatine, porphyrin, bilirubin, and urobilin, and faecal porphyrin and stercobilinogen studies. A detailed description of these findings will be published later. Only the more simple biochemical investigations, which were thought to reflect adequately the clinical course of the disease and provide criteria for comparing the treated and untreated groups, are being reported here.

Results

In a small series it might happen that all the treated cases were much milder or much more severe than the controls, or that the former were all admitted much earlier or later in the course of the disease than the others. Table I has been com-

TABLE I.—The Severity of the Bilirubinaemia in 18 Controls and 18 Treated Subjects, and the Number of Subjects in which the Bilirubinaemia Increased after Admission

	Number of Subjects	
	Treated	Controls
Males	8	11
Females	10	7
Highest level of plasma bilirubin:		
1. Over 10 mg./100 c.cm.	6	8
2. 10 to 6 mg./100 c.cm.	8	5
3. Under 6 mg./100 c.cm.	4	5
Rising plasma bilirubin	9	10

piled to show that in the present series there was no great bias in either group. The treated cases, omitting G. H., included 8 males and 10 females, and the controls 11 males and 7 females. If the severity of the disease be judged by the degree of bilirubinaemia it will be seen that there were 6 severe, 8 moderate, and 4 mild cases in the treated group, and 8 severe, 5 moderate, and 5 mild cases in the control group. This table also shows that in 9 of the treated cases and 10 of the controls the jaundice increased after admission, which suggests that the illness was at about the same stage in both groups when treatment was started. In further support of this is the fact that the length of time from the onset of the illness to the day of admission, which is given in Table II, is not significantly different in the two groups.

From a consideration of the detailed clinical, biochemical, and haematological studies made during the course of the illness and at the follow-up, it was decided that the duration of anorexia, raised plasma bilirubin, and biliuria provided adequate information for the assessment of the therapeutic value of methionine in infective hepatitis. All the patients

* Plasma bilirubin was estimated by the method of Thannhauser and Anderson (1921).