JCI The Journal of Clinical Investigation

STUDIES IN METHIONINE METABOLISM. II. FASTING PLASMA METHIONINE LEVELS IN NORMAL AND HEPATOPATHIC INDIVIDUALS IN RESPONSE TO DAILY METHIONINE INGESTION

Laurance W. Kinsell, … , David P. McCallie, Jean R. Hess

J Clin Invest. 1949;28(6):1439-1450. https://doi.org/10.1172/JCI102209.

Research Article

Find the latest version:

https://jci.me/102209/pdf

STUDIES IN METHIONINE METABOLISM. II. FASTING PLASMA METHIONINE LEVELS IN NORMAL AND HEPATOPATHIC INDIVIDUALS IN RESPONSE TO DAILY METHIONINE INGESTION [1]

By LAURANCE W. KINSELL, HAROLD A. HARPER, GRACE K. GIESE, SHELDON MARGEN,[2] DAVID P. McCALLIE,[3] AND JEAN R. HESS

(From the Division of Medicine, University of California Medical School; Department of Biology, University of San Francisco; Metabolic Research Unit, University of California—U. S. Naval Hospital; and Department of Medicine, U. S. Naval Hospital; San Francisco and Oakland, Calif.)

(Received for publication March 12, 1949)

In earlier methionine studies (1), it was noted that the fasting plasma methionine [4] levels in some patients with acute and chronic liver disease, receiving DL-methionine, tended to be markedly increased. It was not too clear in all instances whether this elevation related to the methionine administration, to the disease process, or to a combination of the two. It was definite, however, that severe liver disease in itself could account for some elevation of the fasting plasma methionine level. Over an 11-month period we have attempted further to clarify this picture.

PROCEDURES

The actual quantitative assay procedures have been previously described (2, 3). It might be emphasized here that one concept held earlier has been modified in that the *normal* fasting plasma methionine level varies within a very narrow range, from 0.25 mgm. per 100 cc. to 0.48 mgm. per 100 cc., with a mean of 0.33 mgm. per 100 cc. The previous concept of a wider normal range related to a longer period intervening between the obtaining of the blood sample and the preparation of the protein-free filtrate. Considerable proteolysis apparently occurs in a matter of a few hours in some plasma specimens. All determinations here reported have been performed on protein-free filtrates prepared within three hours from the time of withdrawal of blood.

The first patients and controls in this series were given 3 gms. of DL-methionine three times daily by mouth as compressed 0.5 gm. tablets, over varying periods of time. Prior to, during, and following such periods of administration, fasting blood specimens were obtained for D- and L-methionine quantitation. In some of these individuals, urines collected over 24-hour and/or 72-hour periods were also assayed for D- and L-methionine.

When it became apparent that a striking difference existed in the methionine retention pattern between normal individuals and many patients with chronic liver damage, a standard program was set up consisting of the following procedures:

A. Studies prior to methionine administration:

1. Fasting blood specimens for plasma D- and L-methionine assay, Monday through Friday, during Week No. 1.
2. One or more three-day quantitative urine specimens during Week No. 1 for D- and L-methionine.
3. No medication of any sort during this period, except for routine "multi-vitamin" dietary supplementation in all patients,[5] and parenteral vitamin K in those individuals with significantly low prothrombin levels. Such medication was constant throughout the entire period of study.
4. A diet high in protein, moderate in fat, and adequate in calories, was administered throughout the study. The most severe cirrhotics received a semi-liquid, high-protein, low-salt intake.

B. Studies during methionine administration:

1. On Sunday of Week No. 2, 3 gms. of DL-methionine were administered at 1:00 p.m. and 8:00 p.m.
2. Thereafter, 3 gms. of methionine were administered at 9:00 a.m., 1:00 p.m. and 8:00 p.m., daily during Weeks No. 2 and No. 3, up to and including 9:00 a.m. on Monday of Week No. 4.
3. Daily fasting bloods were obtained Monday through Friday for D- and L-methionine during Weeks No. 2 and No. 3.
4. Three-day quantitative urine specimens were obtained for methionine assay throughout this period.

C. Studies following the cessation of methionine administration:

1. After the 9:00 a.m. dose on Monday of Week No. 4, as previously noted, methionine was discontinued.

[1] This work is supported by grants from the Research Division of the Bureau of Medicine and Surgery, U. S. Navy (BuMed No. 007046), and from the Office of Naval Research, under a contract between the latter and the University of California.

[2] Senior Research Fellow, U. S. Public Health Service, 1947–48; and Schering Research Fellow in Endocrinology, 1948–49.

[3] Lieutenant (j.g.) MC., USNR.

[4] Unless otherwise qualified, the word "methionine" will refer to L-methionine, *i.e.*, the natural isomer.

[5] "Hexavitamin Tablets" (Strong, Cobb & Co., Inc.), six tablets daily.

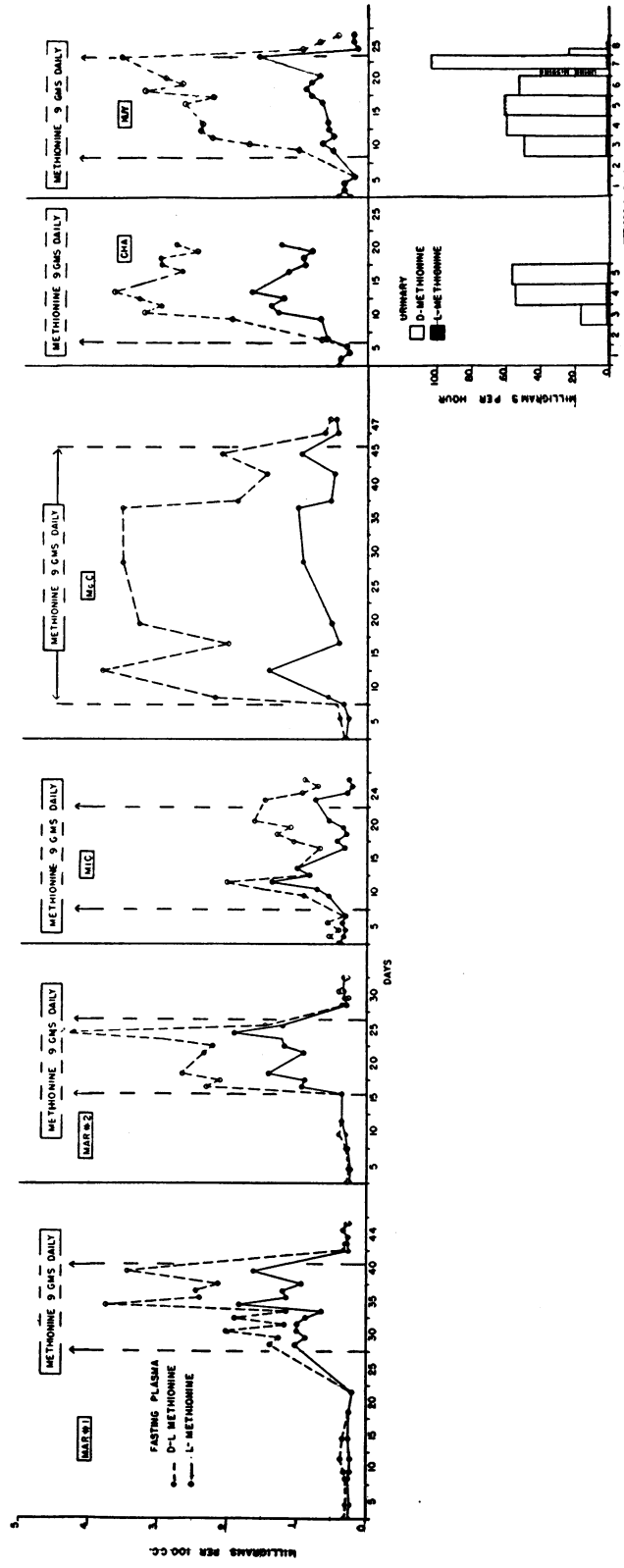

FIG. 1. NORMAL CONTROLS

It will be noted that 2 mgm. per 100 cc. is the upper limit of fasting plasma L-methionine, during methionine administration.

2. Daily fasting blood specimens, Monday through Friday of Week No. 4, were continued.

3. Twenty-four hour quantitative urine specimens were obtained and assayed for methionine during Week No. 4. (The change from a three-day to a one-day collection was made in order to determine more exactly the period of time necessary to eliminate all excess methionine from the tissues and blood.)

During this entire period of study, the patients were subjected to careful daily clinical evaluation by different members of the staff in order to detect any observable changes which might relate to the duration of methionine administration and the plasma level of methionine.

This procedure was found to be unsatisfactory for serial studies in patients with acute viral hepatitis. Such individuals have therefore received 9 gms. of methionine daily for two-day periods, at intervals during the course of their disease. The evaluation of the periods preceding and following methionine administration has been correspondingly shortened.

For purposes of description, comparison, and evaluation, the patients have been divided into arbitrary diagnostic categories (see below).

CHEMICAL EVALUATION

Normal Controls (Figure 1)

Six control studies have been obtained in five young adult males. In control subject MAR, two studies were conducted—one on an average diet (essentially the same type of diet as supplied to all other controls) and the second on an 800-calorie, relatively high-protein diet. This latter study was carried out because of a question as to whether the abnormal values noted in patients with liver damage might refer to the relatively poor dietary intake of one or two of the patients studied in the early phase of this investigation. As will be seen from the data which follow, there are no significant differences in methionine retention in this normal man while on the 800-calorie diet as compared to an average isocaloric diet.

Fasting Plasma Methionine Levels

L-methionine

1. *Prior to methionine administration:* All values were in the normal range previously noted.

2. *During methionine administration:* Elevation of the fasting level was noted in the first 24 hours in all subjects. In no normal man was a value noted in excess of 2 mgm. per 100 cc. at any time during the period of methionine administration.

3. *Following methionine administration:* A fall to normal levels was noted within 24–48 hours after the cessation of methionine administration in all normal control subjects.

D-methionine

1. *Prior to methionine ingestion:* Within the limits of the method, no D-methionine was found in any instance in the pre-treatment state.

2. *During methionine ingestion:* An elevation of the fasting plasma D-methionine was noted in all control subjects within the first 24 hours. Maximal values were 1.92, 2.54, 0.62, 2.39, 2.35 and 2.11 mgm. per 100 cc., respectively.

3. *Following methionine ingestion:* Except for the control subject MIC, all values returned to essentially zero within 24 hours after the cessation of the methionine.

Urinary D- and L-Methionine Excretion

Urinary excretion values were obtained in only two of the normal controls. Maximal excretion rates of 1.52 and 1.25 mgm. per hour for L-methionine and rates of 102.5 and 55.8 mgm. per hour for D-methionine were observed in these men. The behavior of the two isomers as "high and low (renal) threshold substances," respectively, has been emphasized in previous reports.

Patients with Acute Hepatitis (Figure 2)

Serial studies on two representative patients are shown using a short (two-day methionine administration) program for the reasons previously noted. The relationship between the status of the disease, as shown by the presence of icterus, and the degree of methionine retention is obvious. In these and other similar patients, the cephalin cholesterol flocculation and the methionine retention procedures were usually the last of a panel of liver function tests (which included the determination of bromsulfalein retention, free and combined bilirubin, and prothrombin time) to become normal.

Patients with Chronic Viral Hepatitis (Figure 3)

Studies in three patients are presented. All had histories compatible with an attack of acute viral hepatitis one and one-half or more years before this study was undertaken. All have been extensively studied from the standpoint of liver func-

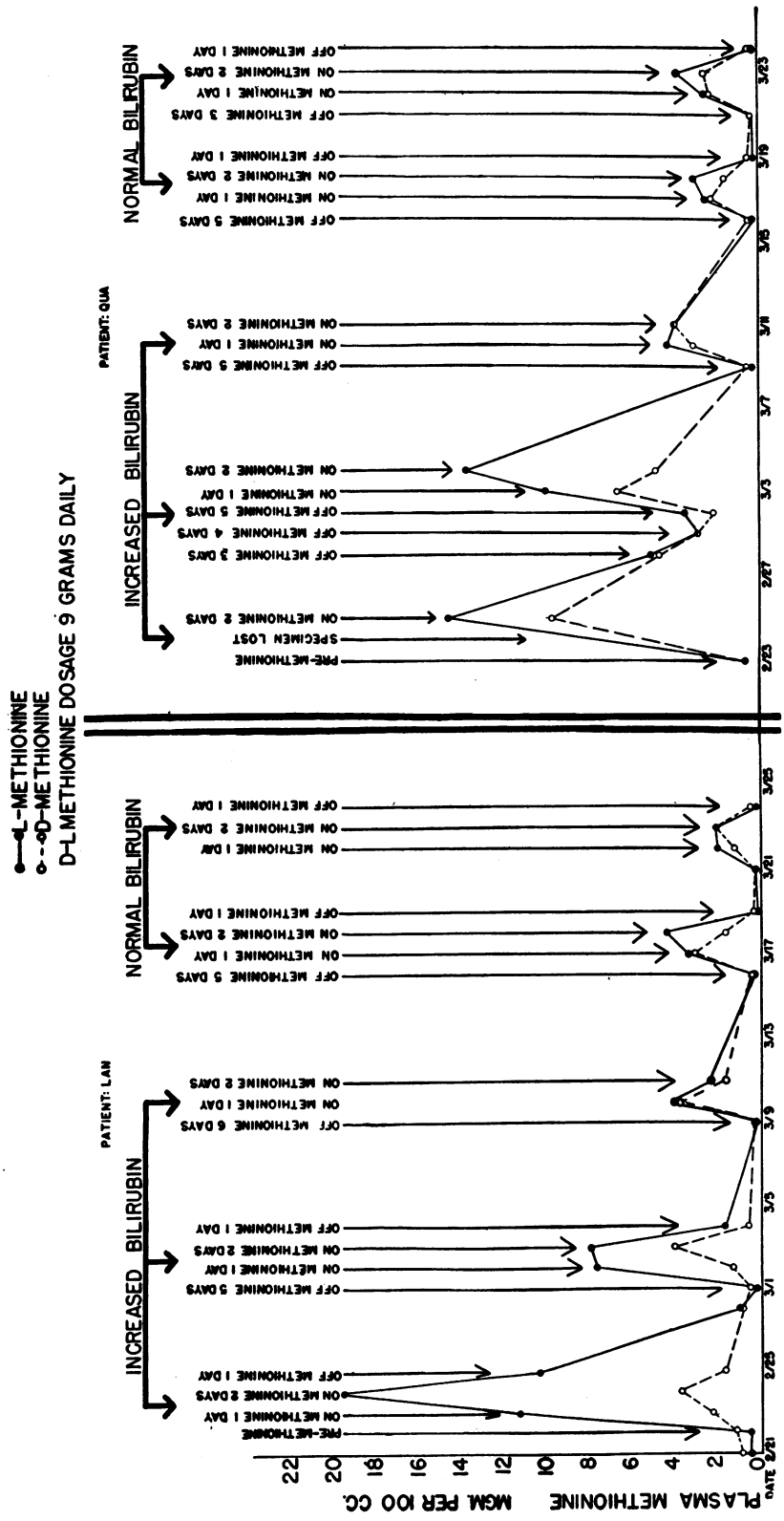

FIG. 2. ACUTE HEPATITIS

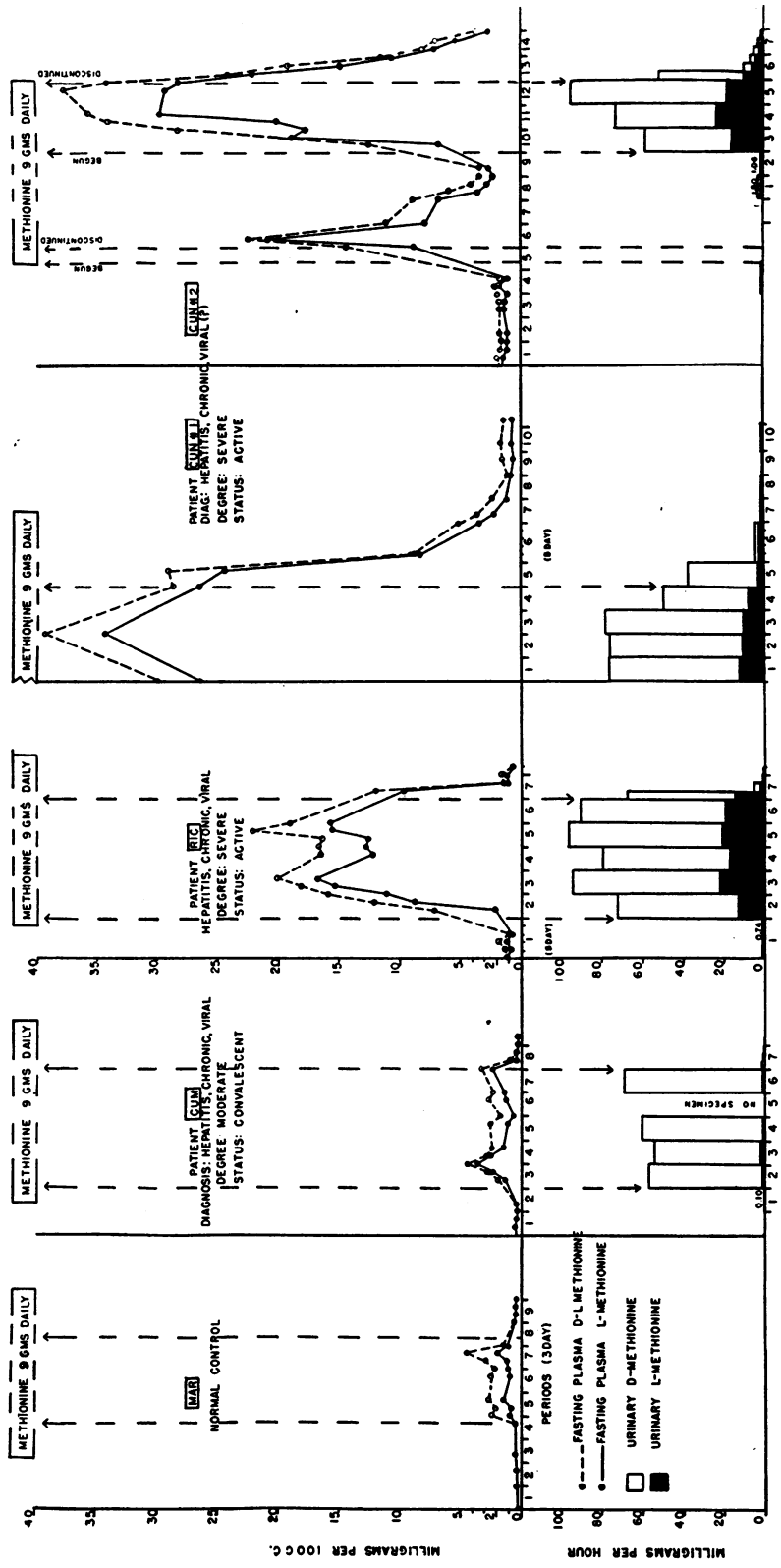

FIG. 3. CHRONIC HEPATITIS (VIRAL)

Plamsa L-methionine levels correlate well with the clinical and chemical evidence of hepatic insufficiency.

tion (all of the usual tests were abnormal); protein metabolism (balance studies); and liver histology (biopsy). The histological findings are those of localized areas of round cell infiltration, evidence of hepatocellular abnormality and moderate amounts of periportal fibrosis. The actual proof of viral infection is unfortunately not possible.

Patient CUM, aged 25—At the time of this study his disease process appeared to be progressively less active, as evidenced by diminution in hepatomegaly (to approximately 3 cm. below the rib margin, on deep inspiration), gradual disappearance of spider angiomata, and diminishing abnormality of liver function tests.

Patient RIC, aged 21, had had involvement of the liver for a period of one and one-half or more years, dating from an attack of acute hepatitis prior to his original admission to this Hospital. At the time this test was performed, he had major abnormality of all liver function tests including a retention of 14 per cent bromsulfalein (5 mgm. per kgm. × 45 min.). He also had evidence of portal hypertension including some splenomegaly, and had had bleeding from an esophageal varix about two months previously.

Patient CUN, aged 50, in the spring of 1946 noted gradual onset of painless jaundice. Six weeks later he was admitted to the Veterans Hospital in San Francisco at which time he had a major degree of ascites which required eight paracenteses. He was told at that time that he had viral hepatitis.

On March 1, 1948 he had a severe hemorrhage from esophageal varices. On admission to this Hospital ten days later, he was found to have considerable ascites, an enlarged, tender liver, moderate jaundice, and abnormality of all tests of liver function above noted. He was also found to have cardiac valvular disease probably referable to an old attack of rheumatic fever.

On a high-protein, adequate-calorie diet plus complete bed rest, he rapidly mobilized his ascitic fluid and showed considerable clinical improvement and some degree of improvement in liver function, although at no time did he have complete normality of any of the liver function tests used.

From March 18 to May 13, 1948, five fasting plasma methionine values were all significantly above the normal, the highest being 1.51 mgm. per 100 cc.

On July 9, 1948 he was placed on 9 gms. of DL-methionine daily. By July 27 he had become obviously toxic, the toxicity including some degree of disorientation and a penetrating odor which was not entirely that of methionine and which suggested the so-called "hepatic fetor." The same dose of methionine, that is, 9 gms. daily, was continued until the 2nd of August, 1948. From July 21 until August 2, he also received 9 gms. of choline chloride daily. The first methionine value shown in Study No. 1 in this patient was obtained on July 21, that is, 12 days after the institution of methionine in a dose of 9 gms. daily, and on the first day of choline chloride administration.

The second methionine study was carried on as part of a protein balance study and was instituted on October 11, 1948. His liver function tests at this time were still significantly abnormal although he no longer had any detectable ascites. The initial portion of the second study was interrupted because of the appearance of symptoms which were attributed to salt deprivation referable to a period of hot weather and low-sodium intake. When this condition was corrected the study was resumed with the results shown in Figure 3. During this second period of methionine administration, he again manifested the symptoms of major and progressive mental clouding coupled with a strong fetor which was similar to, but probably not identical with that which is commonly called fetor hepaticus.

Following the completion of this balance study, the patient was permitted to return home and was instructed to come in for a further evaluation after the Christmas holidays. Unhappily, he had a fatal hemorrhage from esophageal varices about one month after leaving this hospital and his local physician obtained no postmortem examination.

The deviations from normal of the fasting plasma L-methionine values in all three patients are quite definite, and in the last two are most striking. The degree of abnormality would appear to parallel the severity of the disease process. It is also apparent that increased urinary L-methionine appears in those individuals with very high plasma levels.

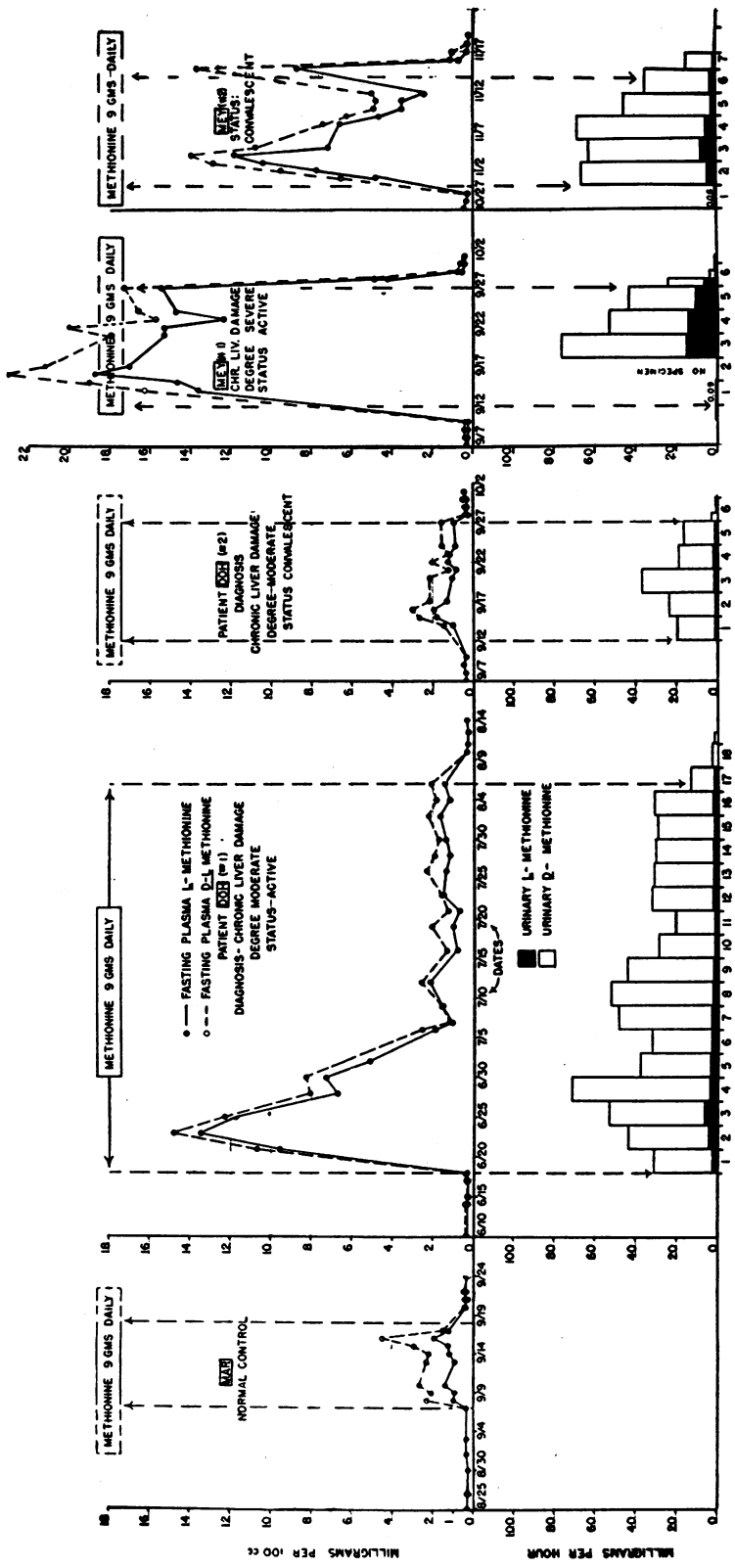

FIG. 4. CHRONIC NON-VIRAL HEPATITIS (CIRRHOSIS)

Methionine utilization improves as the liver becomes more competent.

Patients with Chronic, Non-Viral Liver Damage (Cirrhosis) (Figure 4)

Two successive studies on two patients are shown inasmuch as these men showed marked clinical and chemical improvement in the interval between these evaluations. Both men (DOH and MEY) had the common denominator of excessive and prolonged use and abuse of alcohol plus inadequate dietary intake during some of these periods of alcoholic indulgence. Patient DOH was 33 years of age and by virtue of his relative youth presumably had a more reversible degree of liver damage. Patient MEY was 54 years of age and when first admitted showed all the usual findings of the disease, including an enlarged, hard, irregular liver, multiple spider angiomata, clinical icterus, and general evidence of impaired nutrition. He had had several previous episodes of jaundice, as well as one episode of hematemesis, presumably from esophageal varices.

These patients received the usual therapy for this disease, that is to say, a high-protein, high-vitamin, adequate-calorie diet with supplemental choline chloride and with complete bed rest during the first several weeks of treatment. Routine liver function tests, which initially were abnormal in both men, became progressively more normal; and in Patient DOH, at the time of the second study shown in Figure 4, had all become normal except for slight elevation of the serum globulin and 3 + cephalin flocculation.

As in the patients with chronic viral liver damage, it appears that the degree of elevation of the fasting plasma L-methionine, under these conditions, bears some relation to the degree of hepatic damage.

Patients with Unusual Forms of Liver Pathology (Figure 5)

In Figure 5 are shown the findings on three patients. Patient DEP is a cirrhotic, 58 years of age, who has a long history of alcoholism, had antiluetic therapy with arsenicals in 1908–1910, and malaria in 1926. He entered this Hospital two months before the first methionine study with a red blood cell count of one million as the direct result of hemorrhage from esophageal varices. With adequate therapy he had rapid disappearance of ascites and hepatomegaly, and striking general clinical improvement. He has had at all times a moderate degree of proteinuria. At the time of his initial study, he was far along on the road to convalescence, having a barely palpable liver, no residual ascites, and clinically no complaints whatever. His other liver function tests at this time were normal. The methionine retention study showed a plasma L-methionine level which was within normal limits throughout. There was, however, a very marked elevation of the plasma D-methionine, a maximal level of 16.16 mgm. per 100 cc. being attained on the 11th day of methionine administration, together with most impressive elevation throughout. On the fourth day post-methionine, the plasma D-methionine was still elevated, the level being 0.95 mgm. per 100 cc. Evaluation of the urinary methionine levels showed a single peak excretion on the second three-day period during methionine administration, the level reaching 115.5 mgm. per hour, but at all other times the D-methionine excretion was so low as to approach the limits of accuracy of the procedure. From this observation we postulated that the mechanism of the entire picture related to some intrinsic renal defect which caused this man to reabsorb D-methionine at the same rate at which he normally would reabsorb L-methionine.

One month later the procedure was repeated in an identical fashion. The plasma D-methionine and L-methionine levels, as will be seen from Figure 5, were not significantly different from that noted in the first studies, but the urinary D-methionine values were of a high rather than low magnitude on this occasion. Urinary L-methionine was minimal throughout both studies. During these two periods of methionine administration he manifested symptoms and findings which may be referable to methionine toxicity; namely, a progressive anorexia coupled with malaise and with a strong fetor approximating that noted in the patients previously described. There was no major degree of disorientation, however, as was observed in Patient CUN (Figure 3).

Patient BIE, a 52-year-old man with a huge liver as part of his hemachromatosis and with marked abnormality of many liver function tests, proved to have a perfectly normal pattern of

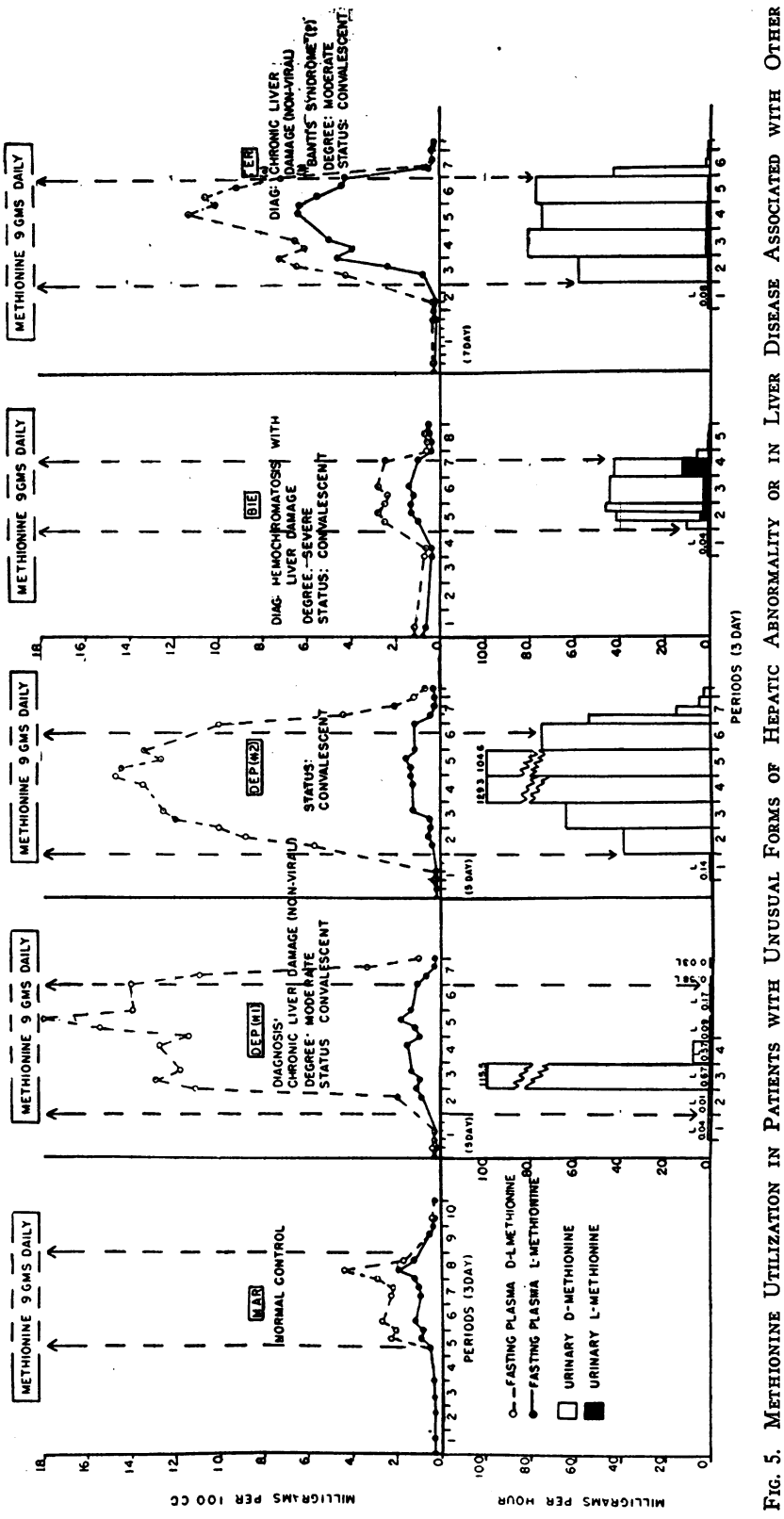

FIG. 5. METHIONINE UTILIZATION IN PATIENTS WITH UNUSUAL FORMS OF HEPATIC ABNORMALITY OR IN LIVER DISEASE ASSOCIATED WITH OTHER PATHOLOGY

methionine retention. He represents the only man so far with unquestionably active liver disease who has shown such a picture. It may be that hemachromatosis represents an extremely specific form of liver disease in which the utilization of methionine is not impaired. It should be noted, however, that he was undergoing rapid clinical improvement referable to a high-protein, low-sodium intake, and hence that widespread protein tissue formation might account for such a finding.

Patient FER, aged 50, has chronic liver damage, presumably resulting from prolonged advanced nu-

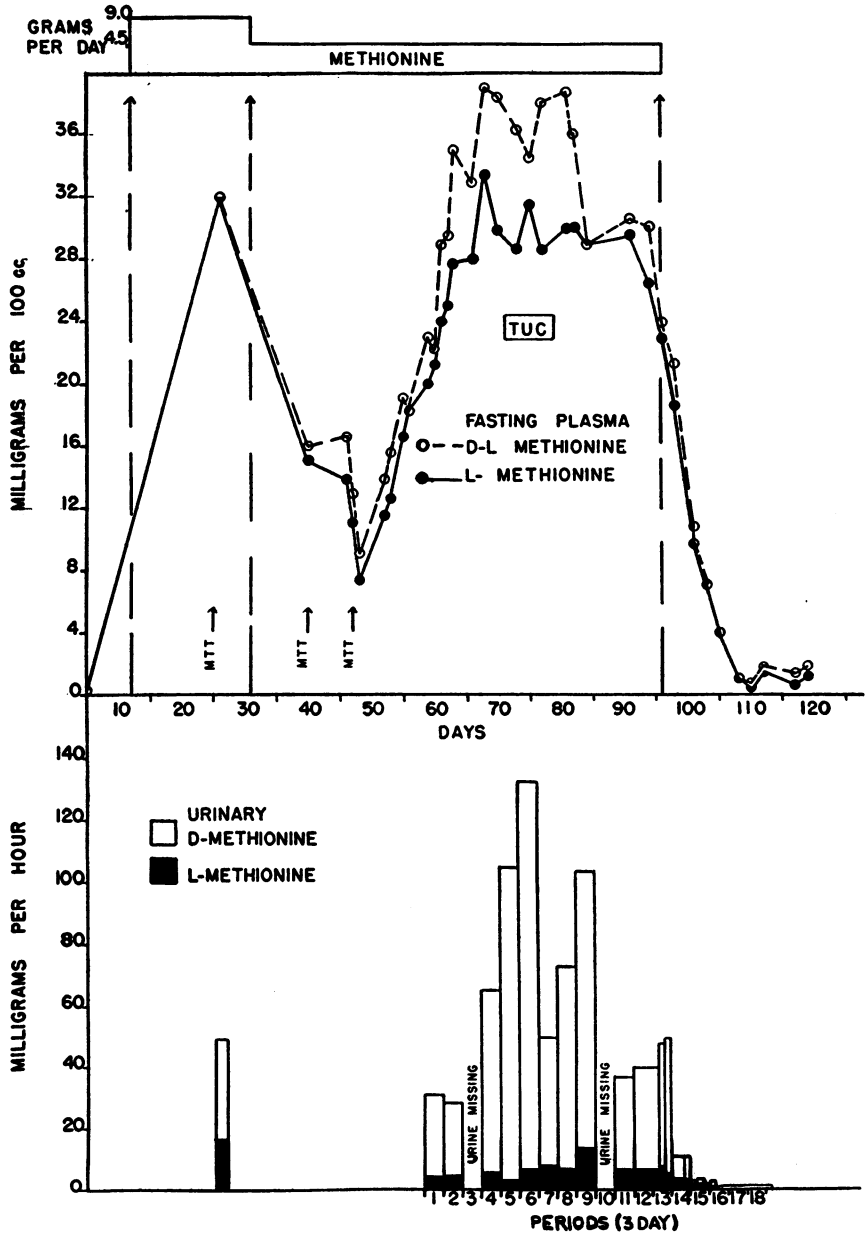

FIG. 6. PROLONGED METHIONINE STUDY IN PATIENT TUC (SEVERE HEPATIC INSUFFICIENCY)

Halving the dosage still resulted in marked elevation of the fasting plasma L-methionine. "MTT" refers to intravenous methionine studies (1).

tritional deficiency (he was a prisoner of war of the Japanese for four years). In addition to clinical and chemical evidence of moderate hepatic insufficiency, he has evidence of portal hypertension in terms of recurrent hemorrhages from esophageal varices and a considerable degree of splenomegaly. His plasma L-methionine pattern is similar to that seen in patients with moderate chronic liver damage.

One additional study (Figure 6) is included on a man with very severe cirrhosis, chiefly because of the length of the study and the fact that the dosage of methionine was diminished by one-half at one stage. It will be noted in this patient, TUC, that with reduction in dosage a temporary fall in the plasma methionine level occurred, but that with the continuance of the smaller dose a rise approaching the previous levels was obtained. The one high value on the dosage of 9 gms. of methionine was not entirely comparable with that observed in other individuals on the same dosage inasmuch as this fasting level was obtained as part of an intravenous methionine study and related to a discontinuance of orally administered methionine for a 48-hour period prior to the test. It is probable that had the fasting level been obtained without any such two-day interval, the value would have been higher than that noted in any other individual included in this report. The figure is presented chiefly to demonstrate that even moderate dosage of methionine in individuals with extreme degrees of hepatic insufficiency results in fasting blood levels which are quite unphysiologic.

DISCUSSION

From the preceding observations, it is apparent that many patients with liver damage are unable properly to utilize methionine, and that such impairment of methionine metabolism is no longer present when the liver damage disappears (in patients with acute hepatitis) or has become quiescent (in individuals with cirrhosis).

If one compares the abnormalities in plasma methionine in patients with liver disease with the blood sugar findings in diabetics, there appears to be some analogy. The individual with severe liver disease has a high fasting plasma methionine level without previous methionine administration. The abnormality in methionine metabolism in the patient with mild or moderate liver damage is only apparent when stress is applied. In our experience thus far, a spontaneously high fasting level is always correlated with a prolonged, difficult convalescence, whether the disease be viral hepatitis or "cirrhosis."

The possible mechanisms of impairment in methionine utilization provide material for speculation. Three metabolic pathways are normally open: (*1*) Incorporation of the methionine into protein tissue; (*2*) demethylation with resultant homocysteine formation; and (*3*) oxidation of the methionine molecule with resultant excretion of the oxidized sulfur as urinary inorganic sulfate. It appears probable that the block occurs in (*1*) or (*2*), inasmuch as the excretion of urinary sulfate is considerably increased in some of the patients with high plasma methionine levels. Simultaneous quantitation of plasma cysteine will help to rule in or out the presence of block in the methionine → homocysteine → cysteine sequence. Such studies are under way at the present time.

Also in the process of evaluation is the determination of methionine utilization in patients with non-hepatic disease. Should a high degree of specificity for liver disease be found, it is conceivable that the procedure may have some clinical applicability.

The appearance of a reproducible picture of toxicity in patients whose ability to utilize methionine is impaired, suggests the need for caution in the indiscriminate use of this material as a therapeutic agent in individuals with liver disease. It is probably best to regard dietary methionine as the logical source of the amino acid in such patients.

SUMMARY

Daily administration of 9 gms. of DL-methionine to patients with liver damage results in a significant elevation of the fasting plasma L-methionine, as compared to normal controls; such abnormal retention disappears when the hepatic status reverts to normal.

In some patients with severe liver damage, continued administration of methionine results in toxic manifestations.

ACKNOWLEDGMENTS

Acknowledgment is made to Wyeth, Inc., for supplies of DL-methionine, "Meonine," used in this study; to the Mead-Johnson Company, for supplies of "Amigen" and "Lonalac" (sodium-free, reconstituted milk); and to Merck & Co., Inc., for supplies of pure amino acids and vitamins.

Acknowledgment is made to Florence E. Olson, research dietician, for her assistance; and to M. F. Jack for editing and typing this paper.

BIBLIOGRAPHY

1. Kinsell, L. W., Harper, H. A., Barton, H. C., Hutchin, M. E., and Hess, J. R., Studies in methionine and sulfur metabolism. I. The fate of intravenously administered methionine, in normal individuals and in patients with liver damage. J. Clin. Invest., 1948, **27**, 677.
2. Harper, H. A., Kinsell, L. W., and Barton, H. C., Plasma L-methionine levels following intravenous administration in humans. Science, 1947, **106**, 319.
3. Kinsell, L. W., Harper, H. A., Barton, H. C., Michaels, G. D., and Weiss, H. A., Rate of disappearance from plasma of intravenously administered methionine in patients with liver damage. Science, 1947, **106**, 589.