STUDIES ON HEPATIC DYSFUNCTION

I. CARBON TETRACHLORIDE POISONING TREATED WITH CASEIN DIGEST AND METHIONINE

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The interest of the case recorded below lies in the successful use of a method of treatment not hitherto applied to man. It was felt that the following considerations, which seemed to indicate a probably fatal outcome, justified its adoption: (1) The large quantity of carbon tetrachloride alleged to have been swallowed—30 to 40 ml. The maximum therapeutic dose is 4 ml., but fatalities have been reported with doses as low as 1.5 ml. (2) The probably complete absorption of the drug. Immediate symptoms indicated a rapid entry into the circulation. Vomiting was not induced until 45 minutes after ingestion. (3) The speed with which the liver enlarged. (4) The delay of 19 hours between the accident and the institution of treatment.

Case History

An Army Air Force sergeant-pilot (white) aged 23 ingested by mistake "two large swallows" of carbon tetrachloride at 4.15 p.m. on Oct. 2, 1943. The quantity was determined later to be between 30 and 40 ml. No food had been taken since breakfast at 7.15 a.m. The patient noted an immediate "hot burning sensation" in his "throat and stomach." He became rapidly "dizzy and light-headed," but was able to walk with assistance to the camp infirmary. At 4.35 p.m. he was given large quantities of salt and water by mouth to induce vomiting. This did not occur until 45 minutes after the accident, and was not repeated. The medical officer observed that at this time the patient was confused mentally, and subsequent questioning revealed that there had been a loss of memory of events between his arrival at the camp infirmary and his transfer to hospital at 6.30 p.m. On admission to hospital he was put to bed and given 1½ gr. of luminal to alleviate his apprehension and restlessness. He was still dizzy and nauseated throughout the night, but vomited only once. He refused food on the evening of admission and on the morning of the following day—Oct. 3.

At 11 a.m. on Oct. 3 he came under our observation. One of us (W.) examined the patient, who was restless and complained of dizziness. Jerking movements of the limbs and rolling of the head from side to side were observed. The pharynx was red and injected. Abdominal palpation revealed a tender liver with rounded edge. This edge extended 2 in. below the costal margin in the right anterior axillary line and 1½ in. below the costal margin in the left anterior axillary line. It was also palpated 1½ in. above the umbilicus. All these and subsequent measurements were taken during normal respiration, and were confirmed by three observers. No other abnormal signs or symptoms were noted.

At 12.30 p.m. the patient was given 2 g. of *dl*-methionine by mouth. This was retained. At 3.30 p.m. 1 ml. of a casein-digest-methionine solution was injected slowly into an antecubital vein. There was no immediate reaction. Five minutes later 5 ml. was injected, also without reaction. Continuous infusion of the solution by a Murphy drip apparatus was begun immediately. The rate of infusion was about 2 ml. a minute. At 6.30 p.m., when 436 ml. of

the solution had been infused, the patient complained of chilliness, intense headache and backache, and some aching of the limb muscles. The pulse was thin and the lips slightly cyanotic; the blood pressure was 130/20. Infusion was stopped, hot-water bottles and extra blankets were applied, and 5 gr. of an aspirin-codeine mixture was given. One hour later the colour had improved, the aching had been relieved, and the blood pressure was 120/88. The temperature was 100.8° and pulse 100.

At 6 p.m. it was noted that the liver was still tender and had enlarged considerably. The edge, which was rounded, had reached the level of the umbilicus on the middle line and was between 2½ and 3 in. below the costal margin in the anterior axillary line. At 7 p.m. the patient was given a small meal, which he ate and retained. Nausea, dizziness, headache, and muscle aching had disappeared before 11 p.m. The night was uneventful.

At 9 a.m. on Oct. 4 the patient was mentally alert and made no complaints. Abnormal muscle movements, dizziness, headache, and nausea were absent. The liver was no longer tender on palpation. The edge, which had now become sharp and firm, was half an inch below the costal margin in the right and left anterior axillary lines. He ate all the food placed before him during the day and said his appetite was good. He had a good night.

At 8 a.m. on Oct. 5 he complained that his dizziness and headache had returned. He felt tired and devoid of energy, and his appetite was poor. He was not nauseated and had no abdominal discomfort. The alertness noted on the 4th had been replaced by apathy and listlessness. Questions were answered slowly and with effort. Abdominal palpation revealed that the liver had again enlarged. The edge was rounded, soft, and tender. It was felt one inch below the costal margin in the right anterior axillary line. The spleen was palpable. At 9 a.m. 2 g. of methionine was given by mouth and another 2 g. at 7 p.m. All food presented was eaten. There was no elevation of temperature, pulse, or respiration rate. By 7 p.m. he stated that he felt better. The night was uneventful.

The following morning (Oct. 6) the patient was alert and made no complaints. He said he had a good appetite and expressed a desire to return to duty. Abdominal palpation showed that the liver had again retracted and was palpable about half an inch below the right costal margin on deep inspiration. The liver edge was firm, sharp, and not tender. The spleen could not be palpated.

The patient was retained for observation until Oct. 14, during which time he was symptom-free and received no medication. At no time during the period of observation was icterus noted. The liver was palpable only on deep inspiration during the whole of the period from Oct. 6. He was discharged as fit on Oct. 14 and returned to full duty with his squadron. Between that day and Nov. 2 he was on normal operational flights over enemy-occupied territory. On Nov. 2 he was readmitted for observation. He made no complaints, had an excellent appetite, and said he had been fit since his discharge on Oct. 14. Examination revealed no abnormal signs or symptoms. His liver edge could be palpated only on deep inspiration. It was not tender.

During the period of observation all food given was weighed and its nitrogen and sulphur content estimated from the tables of

McCance and Widdowson (1940). The daily diet contained 100 g. of protein, of which three-quarters was of animal origin. The calorie content of the diet varied between 2,800 and 3,000. The diet was low in fat.

Comment on Treatment

Miller and Whipple (1942) showed that fatal liver damage was produced by 30 minutes of chloroform anaesthesia administered to dogs which had been kept on protein-deficient diets for several weeks before. This damage was averted if within 3 to 4 hours after anaesthesia 3 g. of methionine in solution was injected into the circulation. The effect of carbon tetrachloride on the liver is comparable to that produced by chloroform. As our patient was a physically fit young airman whose diet was rich in protein it seemed possible that a massive dose of methionine intravenously might be effective despite the relatively long time which had elapsed since the accident. The small oral dose of methionine (2 g.) was administered pending the preparation of a solution for intravenous infusion.

The infusion was prepared by dissolving 20 g. of a dried papain-trypsin digest of casein in 600 ml. of distilled water. The solution was acidified, and to it was added 15 g. of *dl*-methionine. It was then boiled and filtered. The final pH was 7.6 and the solution was approximately isotonic. It was tested by injecting 20 ml. into the ear vein of a rabbit. No effect was observed within a period of two hours.

The original intention was to administer 15 g. of methionine. This dose was calculated on the basis of 0.25 g. per kg. of body weight, which was approximately the dosage used by Miller and Whipple. In fact, only 9.5 g. was infused, and 2 g. was given by mouth on Oct. 3. Casein digest was added to the solution because it was thought that amino-acids other than methionine might be of some value. As Miller and Whipple had secured good results in their experiments by a single massive dose of methionine it was decided to give no methionine during Oct. 4. The relapse on the 5th, which was treated by giving 4 g. of methionine by mouth, suggested either that our dosage of methionine on the 3rd was too small or that small doses of methionine should have been administered on two or three days after the original massive dose.

The febrile reaction observed in our patient was similar to those reported by other observers (Martin and Thompson, 1943) after infusion of amino-acids. Such reactions are due apparently to too rapid infusion of the amino-acid solutions. It was unfortunate, therefore, that in our patient the rate of infusion was rapid enough to bring on the reaction. A slower infusion might have resulted in the injection of all the solution without reaction, and possibly might have averted the relapse on the 5th.

Laboratory Investigations

Haematology.—Three blood counts were carried out. The results of these are given in Table I. The raised white count on the 3rd with 74% polymorphonuclear cells was followed three days later by a fall in the white count to 10,500 with a marked rise in the percentage of lymphocytes. There was no significant rise, however, in the total number of lymphocytes. The blood picture almost a month later showed that the lymphocytes had been little affected, and the rise in the white count must be attributed to a rise in the polymorphs. The red cell count calls for no special comment.

TABLE I

Date	R.B.C.	Hb %	W.B.C.	Poly-morphs (%)	Lymphocytes (%)	Mono-cytes (%)	Eosino-phils (%)
Oct. 3	5,030,000	100	16,600	74	24	1	1
" 6	5,480,000	—	10,500	53	44	3	—
Nov. 2	5,320,000	100	13,700	61	34	4	1

Blood Chemistry.—The various determinations are listed in Table II and commented on later.

Urine.—All urine passed after 12 noon on Oct. 3 was collected. Routine tests for albumin, sugar, bile, and urobilinogen were carried out twice daily. Except for the presence of a trace of sugar and a positive urobilinogen reaction in the sample collected between 7 p.m. on the 4th and 7 a.m. on the 5th no abnormality was noted. No acetone bodies were

found in this sample. A few red and white blood cells were seen in the sample collected between 3.30 p.m. on Oct. 3 and 7 a.m. on the 4th. No casts were seen in any specimen.

TABLE II

Date	Time	Serum Bilirubin (mg./100 ml.)	Blood N.P.N. (mg./100 ml.)	Blood Amino-N (mg./100 ml.)	Total Cholesterol (mg./100 ml.)	Total Protein (g./100 ml.)	A./G. Ratio
Oct. 3	12.30 p.m.	1.10	33	6.4	172	7.3	2.0
" 3	3.30 p.m.	1.30	34	8.7	165	7.5	2.2
" 3	6.30 p.m.	1.90	34	12.7	137	—	—
" 4	6.30 p.m.	0.80	30	5.6	136	7.3	1.9
" 6	8 a.m.	0.88	31	5.2	195	—	—
" 13	8 a.m.	1.00	34	4.9	212	7.4	1.8
Nov. 2	8 a.m.	1.10	39	4.8	197	—	—

The daily urinary output of nitrogen, sulphur, creatinine, and "total creatinine" was estimated. The values obtained are listed in Tables III-VI. The significance of these findings is discussed below.

TABLE III

Date	Urine Vol. (ml.)	Urinary Creatinine (g.)	Total Creatinine (g.)	Total Urinary Nitrogen (g.)
Oct. 3	1,588	1.06	1.12	7.77
" 4	2,730	1.97	2.06	20.64
" 5	1,810	1.55	1.57	12.10
" 6	2,420	1.86	2.08	14.64
" 7	2,210	2.06	2.14	14.78
" 8	2,120	2.01	2.26	14.76
" 9	1,860	2.08	2.10	13.18

The specimen listed for Oct. 3 was collected between 12 noon on that day and 7 a.m. on Oct. 4. All other specimens were from 7 a.m. to 7 a.m., and are listed under the date on which collection started.

TABLE IV.—Nitrogen Balance

Date	Input as Grammes N ₂			Urinary Output (g. N ₂)	Balance
	Food	Methionine and Digest	Total		
Oct. 3	4.8	2.1	6.9	7.8	-0.9
" 4	14.3	—	14.3	20.6	-6.3
" 5	16.4	0.4	16.8	12.1	+4.7
" 6	16.9	—	16.9	14.6	+2.3
" 7	15.7	—	15.7	14.8	+0.9
" 8	16.3	—	16.3	14.8	+1.5
" 9	17.1	—	17.1	13.2	+3.9
	101.5	2.5	104.0	97.9	+6.1

The period listed as Oct. 3 covers the hours between 12 noon on the 3rd and 7 a.m. on the 4th. All other periods are between 7 a.m. and 7 a.m., and are listed as belonging to the day on which collection of urine began.

TABLE V.—Sulphur Balance

Date	Input as Grammes S ₂			Urinary Output (g. S ₂)	Balance
	Food	Methionine and Digest	Total		
Oct. 3	0.33	2.57	2.90	1.00	+1.90
" 4	0.97	—	0.97	2.65	-1.68
" 5	1.09	0.86	1.95	1.01	+0.94
" 6	1.07	—	1.07	1.08	-0.01
" 7	1.01	—	1.01	0.99	+0.02
" 8	1.01	—	1.01	0.97	+0.04
" 9	1.03	—	1.03	0.73	+0.30
	6.51	3.43	9.94	8.43	+1.51

TABLE VI.—Sulphur Partition in Urine

Date	Total S ₂ (g.)	Total Sulphate S ₂ (g.)	Neutral S ₂ (g.)	% Neutral S ₂
Oct. 3	1.00	0.67	0.33	33.0
" 4	2.65	1.30	1.35	51.0
" 5	1.01	0.77	0.24	23.8
" 6	1.08	0.98	0.10	9.3
" 7	0.99	0.91	0.08	8.1
" 8	0.97	0.89	0.08	8.3
" 9	0.73	0.67	0.06	8.2

Methods.—Serum bilirubin estimations were carried out by Gibson and Goodrich's (1934) modification of van den Bergh's method. Non-protein nitrogen was determined in a Folin-Wu

filtrate of whole blood, as was the amino-nitrogen. The former determination was done by the method of Folin and Wu (1919), and the latter by van Slyke's method (1929). Blood cholesterol levels were determined by Leiboff's method (1924), the blood proteins by Andersch and Gibson's (1933) modification of Wu and Ling's method. Urinary nitrogen was determined by the Kjeldahl method, and the creatinine and "total creatinine" by the colorimetric method of Folin (1914). The total sulphates (inorganic plus ethereal sulphates) were estimated by the method of Folin (1905). Some difficulty was experienced in obtaining consistent results in total sulphur estimations until a method after Pirie (1932) was used. Estimations on each individual sample were repeated until a constant value was obtained. To check these results a pooled sample made from proportional parts of each daily sample was prepared and the sulphur content estimated. The final value obtained from the pooled sample was within 2% of the sum of the values from the individual samples.

Comment on Laboratory Investigations

The first abnormality revealed by the routine investigations was the rise in the serum bilirubin level between 12.30 p.m. and 6.30 p.m. on Oct. 3—i.e., during the period when the liver was enlarging rapidly. Apart from the rise in blood amino-nitrogen at the end of the period of infusion and the transient appearance of urobilinogen on the day when relapse occurred, the routine investigations showed no other significant abnormality. It is of interest to note that the serum bilirubin on Nov. 2 had remained at the same level as on the day before discharge (Oct. 13).

In addition to these routine tests an attempt was made to follow the mode of action of methionine by estimating the nitrogen and sulphur balances of the patient and by determining the partition of the urinary sulphur between the oxidized (total sulphate) and unoxidized (neutral) fractions. It would appear from the balance figures, that there was a nitrogen retention of 6 g. over the whole period. This may well be accounted for by nitrogen loss in the faeces. It should be noted, however, that on the 3rd and 4th there was a total negative balance of 7 g., which was almost eliminated by the sum of the positive balances of the 5th and 6th. While it is difficult to believe that the negative balance represents the rapid destruction of cellular elements followed by an equally rapid replacement, it may well be that on the 3rd and 4th the processes of protein synthesis in the liver were impaired, but returned to normal on the 5th and 6th and continued so during the rest of the period of observation.

The positive sulphur balance is, however, proportionately much greater. Even when a 5% error in estimated intake, a faecal loss of 10%, and an error of 2% in the total urinary sulphur estimations are deducted from the positive balance, there is still a small amount of sulphur unaccounted for. Since it is unlikely that all these factors operate at a maximum value and in the same direction the retention of sulphur is probably significant.

That the liver damage was in the nature of a metabolic derangement rather than an actual destruction of liver substance is indicated by the partition of the urinary sulphur. We have not yet followed the metabolism of large quantities of methionine given intravenously to normal individuals. Medes (1937), however, has shown that of 3.2 g. of *dl*-methionine given orally to normal individuals 95% is oxidized to sulphate and excreted in the urine within 24 hours, causing no appreciable rise in the unoxidized sulphur fraction. As it has also been shown by Martin and Thompson (1943) that amino-acids injected at the rate of 10 g. an hour do not spill over into the urine, it is safe to assume that a normal individual receiving the same treatment as our patient would have excreted in the urine the major portion of the methionine sulphur as oxidized (total sulphate) sulphur within 24 hours. The rate of injection of the solution used in our patient was almost identical with that quoted by Martin and Thompson. Our patient, however, excreted an excessive amount of unoxidized sulphur during the first two days, a quantity which was roughly equivalent to 65% of the amount of sulphur given therapeutically as methionine. While there is a possibility that some of the unoxidized sulphur excreted on the first day might be

due to spill-over into the urine, this cannot be true of that excreted on the second day. Moreover, a similar phenomenon was noted on the day of relapse, when the methionine was given orally. Thereafter the neutral sulphur fraction was within the usually accepted normal limits.

These considerations lead us to believe that the intimate cause of the liver disturbance induced by carbon tetrachloride is an abnormal metabolism of methionine and related compounds, and, moreover, that in our patient it was specifically the administration of methionine which prevented further liver damage. We hope to be able to determine the degree of permanent liver damage, if any, and the patient's response to large doses of methionine when he is readmitted to hospital for investigation at the end of six months.

Summary

The history of a case of acute carbon tetrachloride poisoning has been presented.

The patient was treated successfully by *dl*-methionine administered partly by infusion and partly by mouth.

An attempt has been made to follow the metabolism of the methionine administered.

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A CLINICAL EVALUATION OF SOME TESTS OF LIVER FUNCTION

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Before biochemical tests are accepted for the diagnosis or assessment of disease of the liver in man they should conform to certain conditions. First, it is essential to know the extent of the normal variations. To do this the tests must be applied to a large number of healthy men and women of different ages. Secondly, the tests should be applied in proved disease of the liver to show their sensitivity. To be satisfactory the results should differ materially from those found in the normal series. Detailed analysis might also reveal points by which one could distinguish between the various forms of liver disease and judge the severity of the liver damage. Thirdly, the tests should be applied in other diseases to show how specific they are.

Only when tests have been considered from these stand-points can they justifiably be applied to unselected clinical material. Although obvious, these criteria have often been ignored in the past, and even now are not fully appreciated. The number of observations on control cases is often small, and the worth of the tests in the diagnosis of disease of the liver is often open to doubt. Applied to cases in which the diagnosis is uncertain, they have given abnormal findings which have led to the assumption that the patient is suffering from disease of the liver. Then, by a familiar logical fallacy, it