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STUDIES ON HEPATIC DYSFUNCTION

II. THE VALUE OF SULPHUR-CONTAINING AMINO-ACIDS AND CASEIN DIGEST IN THE PREVENTION OF POST-ARSPHENAMINE JAUNDICE

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Miller and Whipple (1942) observed that in protein-depleted dogs chloroform anaesthesia was followed by fatal liver damage. This damage could be averted completely by the administration of methionine before anaesthesia. These observations suggested to us that the liver damage which occurs during an attack of post-arsphenamine jaundice might be averted by some similar treatment.

We have pointed out before (Beattie and Marshall, 1944) that in a clinic under the supervision of one of us the incidence of post-arsphenamine jaundice during the years 1942 and 1943 was over 50% of all cases treated entirely at the clinic. The incidence did not vary from month to month. Moreover, the time of greatest incidence of the disease lay between the 13th and 19th weeks after starting arsenical therapy. In view of these observations it seemed worth while to attempt a series of experiments to determine if methionine, or any of the other sulphur-containing amino-acids, or casein digests rich in methionine, would exert some demonstrable protective effect against post-arsphenamine jaundice during the time when the disease was most frequently developed. Apart from the scientific interest of such a study, a positive result would have some immediate practical value. It is important in antisyphilitic therapy with the arsphenamines to ensure an uninterrupted series of arsenical injections at weekly intervals over a period of at least six months except for a four-weeks rest period beginning at the end of the 10th week of treatment. As an attack of post-arsphenamine jaundice necessitates the withholding of arsenic during the period of icterus and for some weeks or months thereafter, the chances of a cure of the syphilitic infection are considerably diminished. Any treatment which would tend to reduce the incidence of post-arsphenamine jaundice, postpone it to late in the course of treatment, or, alternatively, so to reduce its severity as to enable the patient to continue to receive arsenical treatment, would be of value.

The Investigation

The experiments covered the whole of the year 1943 and the first three months of 1944. During this 15 months the post-arsphenamine jaundice occurred in the control groups in 22 out of 36 cases (61%). The time of greatest incidence of the disease in these groups was between the 14th and 19th weeks of antisyphilitic treatment, when 18 cases occurred—i.e., 82% of all cases. The control and treated groups consisted of patients who received all their antisyphilitic treatment at one clinic (M.I.H.). The experiments were designed so that protective treatment would begin just before the time of greatest incidence of the disease—i.e., at the 14th week. The numbers in each group were determined by the patients available, the quantity of the various preparations and amino-acids at our disposal, and the inevitable loss of men from the groups owing to transfer outside the area served by the clinic. It was not possible to control the diet, as all patients were serving with their units and attended the clinic only as out-patients. It was noted that there was no significant difference in the incidence of jaundice among syphilitics from different units.

The routine antisyphilitic treatment used on all the patients in the control and treated groups consisted of 4 courses, each of 10 weeks, with a rest period of 4 weeks between courses. The weekly treatment consisted of an intravenous injection of 0.6 g. neoarsphenamine and an intramuscular injection of 0.2 g. bismuth in watery suspension. The first week of antisyphilitic treatment (A.S.T.) was reckoned to be the 7 days immediately following the first injection treatment. The weeks of treatment were reckoned consecutively from the first, and included the rest periods. Thus the 15th week of treatment was the week following the first injection of the second course.

Alternate patients arriving at the 14th week of treatment were chosen as controls and given no protective treatment. The remainder were allocated to one or other experimental group. In this way a continuous series of control and experimental groups was maintained for 15 months.

At the start of the investigation we had available a small supply of a dried casein digest prepared by spray-drying a papain-trypsin digest of pure casein. Owing to its unpalatable taste the powder was administered in capsules each containing 0.5 g. of digest. It was found that the practical upper limit of dosage was 20 capsules (10 g. digest) each day. These were given in 4 doses each of 5 capsules at 4-hourly intervals. This experiment was Series I.

To increase the amount of sulphur-containing amino-acids in the daily doses of digest we reinforced the dried casein digest with cystine. As it is well known that cystine can act as a "sparer" of methionine provided the minimal daily requirements of methionine are present in the diet, we thought that the reinforced digest might be more effective than the simple digest powder. To each 97.5 g. of digest 2.5 g. of cystine was added. Assuming that each molecule of cystine in the mixture would "spare" two molecules of methionine, the "maximum effective" methionine content of a daily dose of reinforced digest of 10 g. would be approximately:

Casein digest (9.75 g.)	=	312 mg. methionine
Cystine (0.25 g.)	=	155 mg. methionine
"Maximum effective" methionine content ..		467 mg. per day

It is probable that such an estimate exceeds the actual effective methionine content, but we had no means of assessing the latter. This experiment was Series II. It was necessary to determine if cystine alone was of value, and four different experiments with different dosages and covering differing periods of administration were carried out. These form Series III. Finally, when pure methionine became available this substance

was given in doses of 0.6 g. per day. This formed the Series IV experiment.

The scheme of each of the experiments is detailed below:—

Series I: 10 g. casein digest daily during the 14th and 15th weeks of A.S.T.

Series II: 10 g. reinforced casein digest daily from the 14th to 19th weeks (inclusive) of A.S.T.

Series III A: 0.5 g. cystine from the 14th to 16th weeks (inclusive of A.S.T.

B: 1.2 g. cystine daily during the 14th to 16th weeks (inclusive) of A.S.T.

C: 0.6 g. cystine daily during the 14th to 19th weeks (inclusive) of A.S.T.

D: 1.2 g. cystine daily during the 14th week, followed by a single dose of 2.4 g. cystine at the time of arsenical injection on the 15th to 19th weeks of A.S.T.

Series IV: 0.6 g. methionine daily during the 14th to 19th weeks (inclusive) of A.S.T.

During arsenical treatment it had been observed that when some or many of the pre-icteric signs and symptoms of post-arsphenamine jaundice are present an injection of neoarsphenamine (0.6 g.) almost invariably was followed by icterus within 7 days. When the injection is withheld most patients (80% or more) developed icterus within the same time. Biopsy findings made it clear that liver damage is usually well marked before icterus appears. For these reasons we considered that post-arsphenamine jaundice had occurred when some or all of the characteristic prodromata were present. In some patients, however, the prodromata were absent and icterus was the first sign of the disease. To avoid using the term "post-arsphenamine jaundice" when no icterus was present, we propose to use the term "liver damage" to include both icteric and non-icteric cases of post-arsphenamine jaundice. The general results are summarized in Table I.

TABLE I

Series	Total Cases	Appearance of Liver Damage (Week of A.S.T.)					Total Jaundice Cases
		14–15	16–19	20–24	25–28	29–38	
I	8	2	4	–	–	–	6
II	11	–	5	1	2	1	9
III A	6	1	3	–	–	–	4
III B	6	–	6	–	–	–	6
III C	6	1	3	–	–	1	5
III D	6	–	–	1	–	1	2
IV	10	–	1	3	1	–	5
Totals treated ..	53	4	22	5	3	3	37
Control ..	36	8	10	4	–	–	22

Explanation of Graphs.—The detailed results are expressed graphically in Figs. 1–6. The vertical bars at weekly intervals indicate the injection of neoarsphenamine; the height of the bar is proportional to the dose of arsenical given. The horizontal shaded bar shows the duration of the protective treatment, and increases in its width are proportional to the daily dose given when this was increased. The narrow horizontal black bar indicates the appearance of typical prodromal signs and symptoms. Icterus is indicated by a doubling of the width of the bar. Duration of the illness is shown by the length of the bar. The broken line in Fig. 3 indicates treatment with reinforced casein digest.

The effect of the various treatments in deferring liver damage is summarized in Table II.

TABLE II

Series No.	Total Cases	Duration of Treatment (Weeks)	Additional Sulphur in mg./day	Cases of Liver Damage	
				During Treatment	During 21 Days following Treatment
III D ..	6	6	640*	0	0
III B ..	6	3	320	0	6
III C ..	6	6	160	4	0
II ..	11	6	143	6	0
III A ..	6	3	133	1	3
IV ..	10	6	122	1	1
I ..	8	3	78	5	1

* Given as a single dose on day of injection of neoarsphenamine.

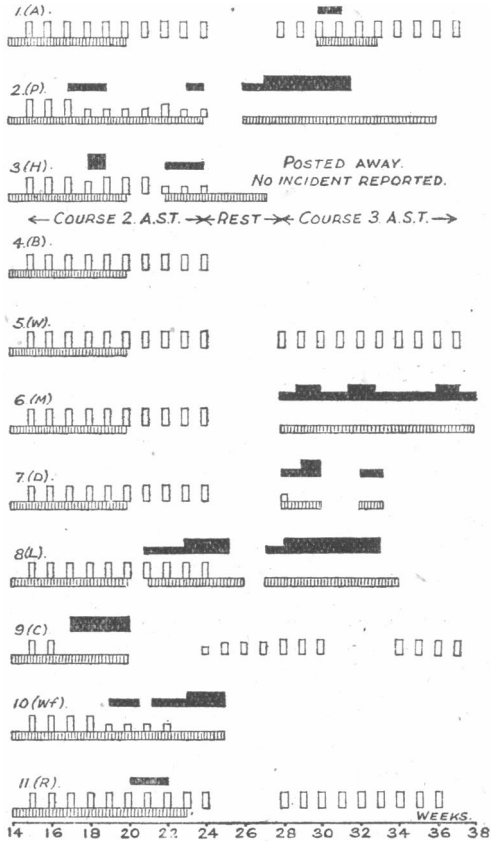

FIG. 1.—Series II.

Comment

In assaying the value of the "protective" treatments we felt we had to provide answers to the following questions:

(*a*) Is the "protective" treatment of value in reducing the incidence of liver damage during the 15th to 24th weeks of anti-syphilitic treatment (A.S.T.)?

(*b*) Is there any evidence that the "protective" treatment shifts the incidence of liver damage towards the end of the second course of A.S.T.—i.e., towards the 24th week?

(*c*) Does the "protective" treatment prevent the appearance of icterus when prodromal signs and symptoms have appeared and arsenical administration is continued?

(*d*) Is the "protective" treatment to prevent the development of icterus effective only when arsenical injections are suspended or the dose is reduced?

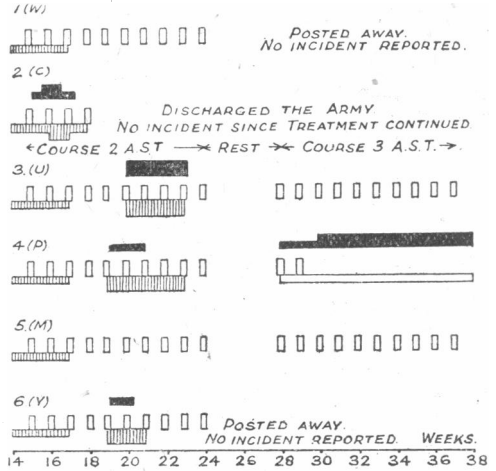

FIG. 2.—Series III A.

The immediately effective treatments are apparently those given in Series III B and D, when no sign of liver damage appeared during the period of treatment. In Series III B, however, all 6 patients in this group had evidence of liver

damage within 21 days after the cessation of preventive treatment, while no such cases appeared in Series III D. The next most effective treatments seemed to be those in Series III A and Series IV, in which only one case in each appeared during treatment. Within 21 days three cases of liver damage occurred in Series III A and one in Series IV.

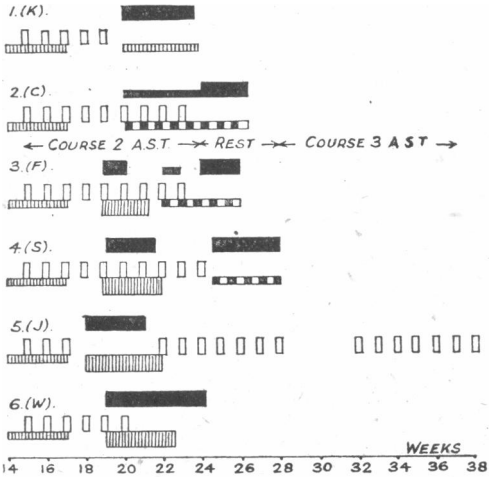

FIG. 3.—Series III B.

As the numbers in each of these groups are too small to bear statistical analysis it is not unreasonable to add them together and to compare the findings with the control group. From the control group (36 cases) the number of cases for a smaller group of 28 cases may be calculated and the probable number of cases during certain weeks predicted. Analysis of a larger control sample (150 cases) has shown that the control group is in fact a fair sample and the incidence of liver damage in the various periods is representative. The analysis is given in Table III.

TABLE III.—*Cases of Liver Damage during Weeks of A.S.T.*

	Total	14th–15th Weeks	16th–19th Weeks	20th–24th Weeks
Controls (36)	14·7	6·2	7·7	0·8
Treated groups (28), III A, B, D, and IV	15	1	10	4

In the groups where "protective" treatment was apparently effective the incidence of liver damage was not reduced during the second course of antisyphilitic treatment. This provided an answer to our first question. With regard to the second

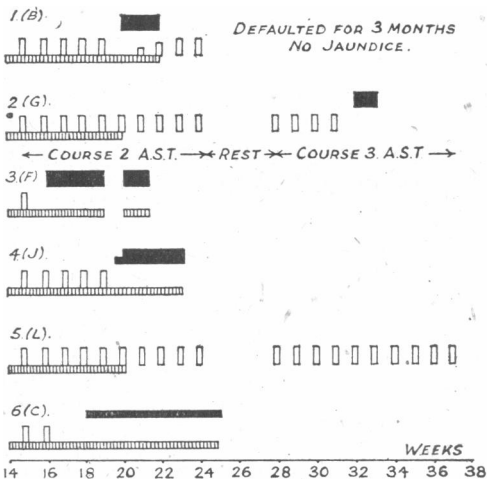

FIG. 4.—Series III C.

question, there is a tendency for more cases to appear later in the second antisyphilitic course than towards its beginning. In Series III A and B the "protective" treatment was prolonged over 3 weeks instead of over 6 weeks as in Series III D and Series IV. If this treatment had been prolonged for 6 weeks there is a possibility that the nine cases of liver damage which appeared within 21 days after the end of "protective" treatment might not have developed until the 20th–24th week period. In such an event the shift might have been more pronounced. Series III A and C are, however,

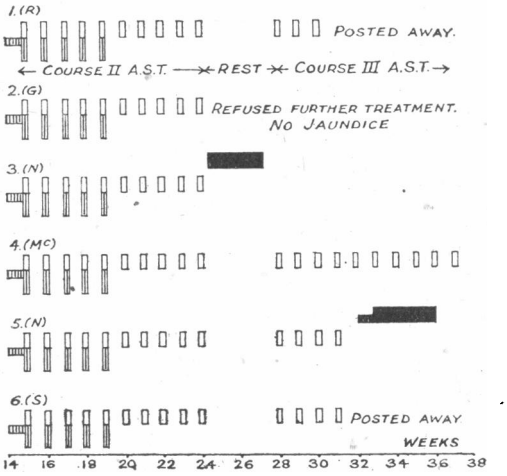

FIG. 5.—Series III D.

practically identical in daily dosage of cystine (0.5 and 0.6 g.), but differ in the length of time over which the cystine was given. Reference to Figs. 2 and 4 shows that within the first 6 weeks of the beginning of the second course of arsenical treatment each of these groups had the same number of cases of liver damage. The prolongation of treatment at the same dosage for 6 weeks apparently in no way influenced the development of liver damage in Series III C. It is a fair deduction, therefore, that such a dosage of cystine is ineffective.

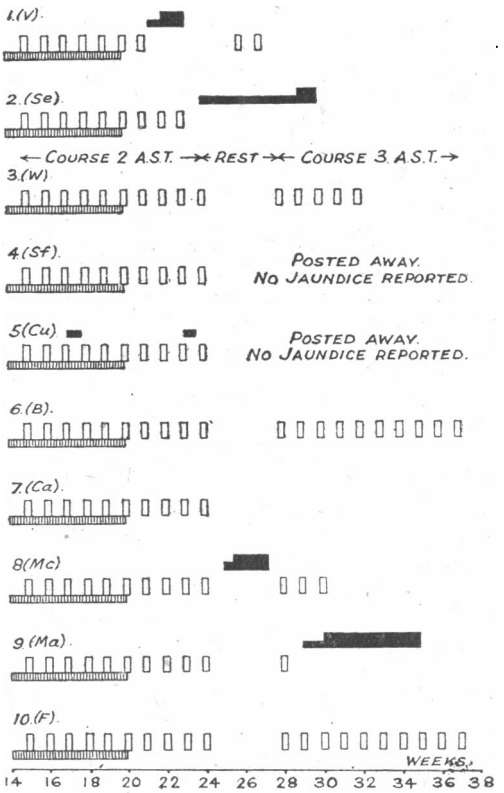

FIG. 6—Series IV.

Doubling the daily dose of cystine for a period of three weeks (Series III B) produced no cases while treatment was being given, but all the group had liver damage before the end of the sixth week from the start of the 21-day treatment.

This experiment is inconclusive in view of what has been said above regarding Series III A and C. It is possible that if treatment had been prolonged for 6 weeks a similar result to that recorded in Series III C might have been obtained; on the other hand, it is equally possible that no cases might have occurred.

Series III D treatment, in which a daily dose of 1.2 g. cystine was given during the 14th week and was followed by single doses of 2.4 g. cystine on the days on which arsenical injections were given (15th–20th weeks), was apparently effective, as no cases of liver damage appeared either during the period of "protective" treatment or during the subsequent 21 days.

Series IV seemed to show that small doses of methionine (0.6 g.) were almost as effective as the massive doses of cystine of Series III D. It should be noted that, in spite of continued full doses of arsenic, icterus did not develop in the single case of liver damage during "protective" treatment. The very significant delay in the appearance of liver damage in the remaining four cases is noteworthy.

Series I represents an attempt to increase the daily intake of methionine by using a casein digest. The results demonstrate clearly that an extra 0.3 g. of methionine present in the daily dose of digest for 14 days had no effect on the incidence or time of appearance of the post-arsphenamine jaundice. The Series II experiments, in which cystine was added to the digest, present some interesting features. During the period up to the end of the 24th week 5 patients of the 11 treated remained free from liver damage. One (R.) showed signs of liver damage at the beginning of the 20th week, but with continued "protective" treatment did not develop icterus, although full doses of arsenic were given. Two with prodromal signs and symptoms were treated by reducing the arsenical dosage and continuing the "protective" treatment. One of these (P.) did not become icteric, but a full dose of arsenic produced a reappearance of the prodromal phenomena. The other (Wf.), in spite of reduced dosage, eventually became icteric. A mild icterus in one patient (H.) was treated by reducing the arsenical dosage. At the end of 7 days icterus had disappeared and full arsenical treatment was resumed. The suspension of "protective" treatment for two weeks induced a reappearance of prodromal signs and symptoms. One patient (L.) showed no sign of liver damage until one week after the end of the protective period. Resumption of "protective" treatment, with full doses of neoarsphenamine, however, did not prevent the appearance of jaundice. He relapsed again after being without "protective" treatment for one week. Arsenic was not given during the relapse. Icterus appeared in one patient (C.) after two arsenical injections. Suspension of injections and continuation of the "protective" treatment were followed by disappearance of icterus in 3 weeks. Resumption of arsenical injections later without "protective" treatment provoked no relapse.

These results suggest that the addition of but a small quantity of cystine to the casein digest is just sufficient to protect some individuals from developing liver damage, others from the development of icterus, others also from icterus but only if the arsenical dosage is reduced; and others are not so protected. This scheme of "protective" treatment seems therefore to represent almost the balance between adequate and inadequate treatment. Assuming that the addition of 0.25 g. cystine acted by sparing methionine, it would represent the sparing of not more than 0.16 g. The total "effective" methionine supplement would therefore probably not exceed 0.4 g. per day.

Two possible explanations of these results must be considered. The protective compounds might be effective in rendering the neoarsphenamine non-toxic to the liver and presumably also to the spirochaete. Possibly post-arsphenamine jaundice is a partial deficiency disease in the sense that certain compounds, which enable the liver successfully to resist the toxic effects of virus infection combined with neoarsphenamine injections, are not present in optimal quantities in the wartime diet. Supplementation with these compounds presumably may be effective in altering the incidence, time of onset, or severity of the liver damage when it occurred.

Voegtlin (1925) showed that neoarsphenamine in the concentration found in the blood during antisyphilitic treatment was not lethal to the spirochaete until it had been oxidized to arsenoxide. Arsenoxide is rendered non-lethal, however, if it is allowed to combine with a sulphydryl-containing compound such as cysteine. Eagle (1939) suggested that arsenoxide killed the spirochaete by combining with sulphydryl groups concerned in the respiration of the cell. Glutathione is one such compound. The probability is that arsenoxide is toxic to the liver cell through a similar mechanism. It is conceivable that cystine (being readily convertible to cysteine in the liver) might be utilized in this way to detoxicate the arsenoxide. In two of the cystine-protected series the times during which "protective" treatment was continued were identical. In Series III C, however, the cystine was given daily in doses of 0.6 g./day or 4.2 g./week, whereas in III D, with the exception of the first week, the cystine was all given on the same day as the arsenical injections in a dose four times the daily dose of III C but only slightly more than half the total weekly dose. In both groups the overall incidence of liver damage was unaffected. In III C the cases occurred at the usual time or were only slightly delayed. In III D the cases were very significantly delayed in onset (Fig. 5). Such a contrasting result can best be explained by assuming that cystine given in mass doses on the day of the arsenical injection is mainly effective by acting as a chemical detoxicating agent for the arsenoxide, thus relieving the liver from the effects of arsenoxide for at least six weeks and enabling it to deal with later arsenical injections successfully for a limited period. There is only one other series in which the occurrence of liver damage was delayed to a comparable extent. In Series IV methionine was given in a manner corresponding exactly to Series III A, discussed above, but the results obtained were strictly comparable to those in III D. It seems impossible that methionine in this case could be acting as a direct detoxicating agent at a level of dosage where cystine was ineffectual. It therefore becomes necessary to assume that methionine, unlike cystine in large doses, is effective in the second of the two ways suggested above.

It must be made quite clear that Eagle's work was done *in vitro* and may therefore not be valid under *in vivo* conditions. The experimental evidence on animals is at present equivocal. In some measure this may be due to dietary variations which Martin and Thompson (1943) showed could introduce variations in the acute toxic effects of arsphenamine as great as 50%. The consensus of opinion, however, is that the sulphydryl compounds, such as cysteine, can diminish the toxic effects of the trivalent organic arsenicals—neoarsphenamine and arsenoxide. Cystine apparently can only exert such an action when it is reduced to cysteine. This reduction of cystine to cysteine can occur in the liver.

The conclusion that cystine is mainly effective as a detoxicating agent, whereas methionine remedies a specific dietary deficiency, is strengthened when actual diet figures of these men are considered. The British Army Home Forces daily ration as issued contains 118 g. protein, of which 58% is of vegetable origin. In fact, we found that the maximum these men appeared to be eating was 100 g. of protein per day. This would give a sulphur intake of about 0.96 g. daily, representing about 2 g. each of methionine and cystine. It is well known that the borderline between adequate and inadequate quantities of the various essential food constituents may be narrow. If there is a suboptimal methionine intake in these men receiving arsenical treatment an increase of 25% might easily be sufficient to raise it to an effective level. On the other hand, the quantities of a sulphydryl-containing compound sufficient to protect the liver by direct detoxication of the arsenic would have to be very much larger. One molecule of neoarsphenamine requires four sulphydryl groups for detoxication. If all the administered cystine were converted directly to cysteine and all this cysteine were then combined with arsenoxide, the weekly dose of 0.6 g. neoarsphenamine would require 0.6 g. cystine. This would need to be available during the 24 hours following the injection, as it is known that the greater part of the spirochaeticidal action of neoarsphenamine, which we assume to be similar to its hepatoxic action, occurs within this period. In view of the many ways in which cystine can be metabolized, apart from its conversion to cysteine, it is not surprising that the minimal effective dose of cystine should approach 2.4 g. when given

on the actual day of injection, or that larger quantities spread over a longer period should be relatively ineffective.

The metabolism of methionine and cystine cannot, however, be regarded as unrelated to each other. Part of the methionine in the diet is undoubtedly converted in the body to cystine and its derivatives. It is probable, therefore, that an increased daily cystine intake given to an individual with a suboptimal methionine intake would increase the amount of methionine available for those functions which cystine itself cannot perform. Such a methionine-sparing effect of dietary cystine was found to occur in growing rats by Rose *et al.* (1937). It is likely that the definite results obtained with daily doses of cystine at the lower levels (Series III A, B, C) and when it was added to the casein digest (Series II) are due to this effect.

Such theoretical discussions, however, would be better based on larger series of cases. At present it is not possible to extend this series, but the fact is clear that adequate protection in the second arsenical course can be attained. Practically it would seem desirable that men under this treatment should receive at least 2.5 g. methionine daily, and preferably 3 g. This could be done if their diet were supplemented either with the synthetic compound or with about a pint of milk daily. The use of synthetic cystine in quantities sufficient to produce the required result is not desirable, as the effect of cystine may be to detoxicate the arsenic and thus render ineffectual the antisyphilitic treatment. Moreover, we have found suggestions in some of our cases that the effect of cystine on established liver damage may be conducive to cirrhosis.

Summary

An attempt has been made to control the occurrence of liver damage in syphilitics under treatment with neoarsphenamine.

Sulphur-containing amino-acids were given during that period of treatment when the expected incidence of liver damage was maximal.

Four preparations were tested—casein digest, casein digest reinforced with cystine, cystine, and methionine.

These preparations had no effect on the overall incidence of liver damage. Certain of them, however, were markedly effective in (*a*) shifting the time of peak incidence of liver damage towards the end of the second course of antisyphilitic treatment or later; (*b*) moderating the severity of the liver damage when this did occur.

The effect of casein digest reinforced with cystine was less than that of an equivalent quantity of synthetic methionine.

Cystine produced an effect similar to that of methionine only when given in large quantity on the day of the arsenical injection.

It was concluded that men undergoing antisyphilitic treatment should receive dietary supplements as a prophylactic measure against jaundice.

These supplements should not take the form of large quantities of cystine, as this may reduce the chances of the antisyphilitic treatment being successful.

We wish to thank Brig. T. E. Osmond for putting at our disposal the facilities for carrying out this work and for his interest and encouragement. The pure amino-acids used in these experiments were presented by friends in Canada and the United States. We are grateful to them for their generous gifts. The casein digests were prepared by Mr. J. I. M. Jones of British Colloids Ltd. To him and his directors we express our thanks for their co-operation and interest, and for gifts of ample supplies of material.

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A Tsetse Fly and Trypanosomiasis Committee has been appointed by the Secretary of State for the Colonies to consider and advise on the co-ordination of action, including research, directed against human and animal trypanosomiasis, and in particular against the tsetse fly. The Dominions Office and the Sudan Government are represented on the committee, whose chairman is Mr. G. H. Creasy, of the Colonial Office. The members include Prof. P. A. Buxton, London School of Hygiene and Tropical Medicine; Dr. H. Lyndhurst Duke, lately director of the Human Trypanosomiasis Institute in Uganda, and chairman of the League of Nations Sleeping Sickness Committee; Prof. I. M. Heilbron, Imperial College of Science; Dr. E. M. Lourie, Liverpool School of Tropical Medicine; Sir Guy Marshall; Dr. S. A. Neave, director of the Imperial Institute of Entomology; and Dr. A. G. H. Smart, Medical Adviser to the Secretary of State for the Colonies.

ACUTE PERFORATED PEPTIC ULCER

FREQUENCY AND INCIDENCE IN THE WEST OF SCOTLAND*

BY

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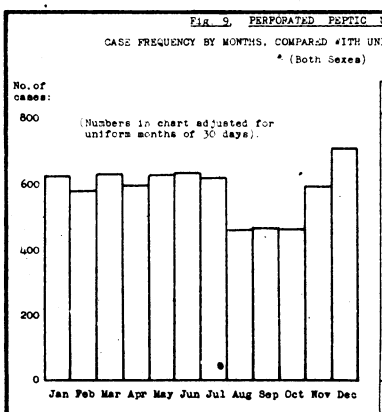
Case Frequency by Months (3 Main Hospitals; 20-year Period)

Cases occurring in like months over the 20-year period were added together. Fig. 9 shows the monthly distribution so obtained, and demonstrates the divergence between the number of perforations observed and the number which might have been expected if the distribution were uniform (due allowance being made for the number of days in the various months). It will be seen that perforations are unduly common in

Fig. 9. PERFORATED PEPTIC ULCER

CASE FREQUENCY BY MONTHS, COMPARED WITH UNIFORM DISTRIBUTION, 1924-1943

* (Both Sexes)



Month	Perforations observed	Perforations "expected"	Observed minus "expected"
Jan.	653	607.3	+45.7
Feb.	537	555.3	-16.3
Mar.	658	607.3	+50.7
Apr	600	587.9	+12.1
May	655	607.3	+47.7
June	640	587.9	+52.1
July	647	607.3	+39.7
Aug.	478	607.3	-129.3
Sep.	470	587.9	-117.9
Oct.	479	607.3	-128.3
Nov.	597	587.9	+9.1
Dec.	742	607.3	+134.7
Total	7156	7156.0	0

$X^2 = 128.5$ P is remotely small

December and relatively uncommon in August, September, and October. This distribution obtained fairly consistently throughout the 20-year period. It is highly improbable that such wide departures from uniformity have arisen by chance.

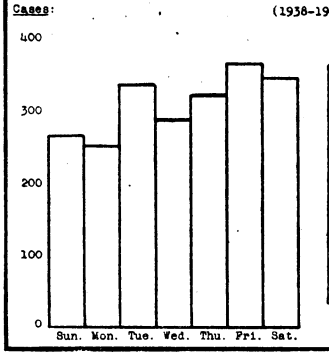
There is no obvious explanation of the December rise, which is distributed throughout that month and not related to the Christmas season. The low incidence in summer may possibly have a nutritional basis or may be related to the holiday season. In this connexion it is relevant to state that in the West of Scotland July is the popular holiday month, and it will be seen that the July incidence is not low; but it is possible that the period of rest at that time confers a measure of protection during the ensuing months.

Incidence by Days of Week (2 Hospitals; 6-year Period)

The incidence of perforation by days of the week in two hospitals (Royal and Western Infirmaries) was determined for the years 1938–43. Cases occurring on like days were added together, and the result is given in Fig. 10. It will be seen

Fig. 10. PERFORATED PEPTIC ULCER

Incidence by Days of the Week, compared with uniform distribution (1938-1943)



Day	Perforations observed	Perforations "expected"	Observed minus "expected"
Sun.	271	314.6	-43.6
Mon.	255	314.6	-59.6
Tues.	340	314.6	+25.4
Wed.	290	314.6	-24.6
Thurs.	326	314.6	+11.4
Frid.	369	314.6	+54.4
Sat.	351	314.6	+36.4
	2202	2202.2	

$X^2 = 35.31$
P very much less than 0.01

* A survey made under the auspices of the Nuffield Provincial Hospitals Trust. Concluded from page 620.