THE
ASSESSMENT OF LIVER DAMAGE
FOLLOWING
TRICHLORETHYLENE ANÆSTHESIA

A THESIS SUBMITTED FOR THE
DEGREE OF DOCTOR OF MEDICINE
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BY

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PREFACE

THE work embodied in this thesis was carried out at St. Peter's Royal Naval Auxiliary Hospital, Colombo, between October, 1944 and December, 1945, during which period I was Specialist in Anæsthetics to that establishment.

All patients studied were anæsthetised by me personally. The biochemical part of the work was done in the hospital laboratory. Surgeon Lieutenant Commander D. Shute, R.N. was in charge of this laboratory, and as he was engaged in an investigation of liver damage in amœbic dysentery with a view to submitting a thesis on that subject for the degree of M.D. of the Victoria University of Manchester, it was found convenient for us to collaborate in the work of blood collection, and in the application to batches of blood specimens of the cephalin cholesterol flocculation test. Although our collaboration was of mutual benefit, my thanks are due to Surgeon Lieutenant Commander Shute for the freely-granted use of his somewhat overtaxed laboratory facilities, and we both owe much to the technical experience and skill of Sick Berth Chief Petty Officer J. Thompson, R.N. whose help proved invaluable when difficulties were encountered in the process of cephalin

ERRATA.

p. 26, line 11. For (c) read (iii).

p. 27, table I, line 4. For "appendicetomy" read "appendicectomy".

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I am grateful, also, to the librarians of the Ceylon Medical Library, the University of Madras Medical School, the Stanley Government Medical School, Madras, and the King Institute, Guindy, Southern India. These gentlemen placed their facilities at my disposal, but were unable to supply several of the works I wished to consult, a defect which was to a great extent remedied by the kindness of Mr. W. A. Sewell, Honorary Surgeon, the Victoria Infirmary, Glasgow, who sent me photostatic copies of some of these, and by the patience of my wife, who sent others transcribed in longhand.

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INTRODUCTION

All drugs used for the induction and maintenance of general anæsthesia do so in virtue of their action on the central nervous system, which they reach via the blood-stream, whatever the route of introduction may be. It follows that all organs are exposed to the action of such drugs, and, while interest for the purpose of anæsthesia is centred on the central nervous system, it is hardly to be expected that this should be the only system involved, and that the action should be entirely selective.

A great deal of research has been done with regard to the pharmacology of anæsthetic drugs, and information is available concerning their comparative toxicities and effects on systems and organs. Such data are essential in the choice of the most suitable anæsthetic for a given case.

A striking example of toxicity to an individual organ occurring as a side-effect of general anæsthesia is seen in the case of the hepato-toxic effects of chloroform. These can be of sufficient magnitude to cause hepatic necrosis and death, and Sheehan (1940) has demonstrated in detail the morbid histology of such lesions. Partly as a result of this selective toxicity, chloroform is now eschewed as a general anæsthetic agent in hospitals, although it still finds a place in domiciliary midwifery.

Trichlorethylene, like chloroform, is a halogenated hydrocarbon, and is of comparatively recent introduction to anæsthetic practice. Other similar compounds are tetrachlorethane and carbon tetrachloride. These are highly toxic to the liver. (Willcox 1931; Cameron and Karunaratne, 1936). By reason of its composition, therefore, trichlorethylene is suspect, but the balance of evidence collected by Browning (1937) favours the view that this compound is much less toxic to the liver than are the others mentioned above.

Browning's work, in so far as it deals with trichlorethylene, consists of a summary of published reports of cases of industrial poisoning with trichlorethylene, and of results of animal experiments in which large doses of the compound were given, followed by histological study of the animals' organs *post mortem.*

Although findings with regard to liver damage were mainly negative, both in the industrial and in the experimental fields, it is

the case that a small minority of the animals subjected to trichlorethylene showed evidence of such damage, as did some of the victims of industrial poisoning.

The object of the present inquiry is to determine by biochemical methods whether there is demonstrable liver damage following the use of trichlorethylene as an inhalation anæsthetic; a secondary purpose is to draw a comparison between such damage, if shown to exist, and that which follows di-ethyl ether. It is hoped that this comparison may enhance the practical value of the work, which comprises the following sections:—

I

The History of Trichlorethylene in Anæsthetic Practice.

In 1936, the Council on Pharmacy and Chemistry of the American Medical Association published a preliminary report on the use of trichlorethylene for general anæsthesia, based on the work of Jackson (1934), Herzberg (1934), and Striker, Goldblatt, Warn and Jackson (1935). Jackson's work was both experimental and clinical, the effects of trichlorethylene inhalation on animals and human subjects being observed. Herzberg dealt with the morbid histology of organs taken from animals killed by prolonged inhalation of trichlorethylene vapour. Striker *et al* described a series of 300 administrations of trichlorethylene for surgical interventions of a minor nature.

Herzberg, in his histological studies, noted hepatic changes in dogs killed by trichlorethylene, and found similar changes in other dogs killed by electrocution. He concluded that the changes found in the first group of dogs were not specifically due to trichlorethylene because the control group showed the same appearances. An immediate criticism of this conclusion is that an electrocuted dog can hardly be regarded as a normal control, a view concurred in by the Council on Pharmacy and Chemistry in its report which stated that this evidence seemed inadequate and the conclusion unwarranted. Jackson, on the other hand, while noting the similarity in structure between trichlorethylene and chloroform, regarded them as being dissimilar in their effects on the liver, an opinion apparently founded on the work of Herzberg.

The remainder of the report dealt with the signs of trichlorethylene anæsthesia and with the occurrence of Trigeminal paralysis following trichlorethylene inhalation. Nothing more was said regarding possible effects of the drug on the liver, and the final conclusion was that available evidence did not justify the acceptance of trichlorethylene for use as a general anæsthetic.

Some years later, the possibility of using trichlorethylene for the purpose of inhalation anæsthesia was suggested to Mr. C. F. Hadfield, Honorary Secretary of the Joint Anæsthetics Committee of the Medical Research Council and the Royal Society of Medicine, and, as a result, its potentialities in this respect were investigated by Mr. C. L. Hewer, Senior Anæsthetist, St. Bartholomew's Hospital. The results of this investigation were published in 1941. (Hadfield and Hewer.)

As a preliminary, the work previously carried out in America was reviewed, and the results of animal experiments with regard to toxicity by Joachimoglu (1921), Krantz *et al* (1935) and Lande (1939) were considered, and found to be favourable with regard to liver damage, in that, in only a few of the animals killed by over-dosage of trichlorethylene was any evidence of liver damage found at autopsy, and when present, it was but slight in degree. The drug was deemed worthy of further clinicial trial as an anæsthetic, and a series of 127 administrations was performed, the series including most of the commoner major operations, and the duration of anæsthesia ranging from 10 to 190 minutes. Observations were made regarding effects on the cardio-vascular system, respiratory system, sugar metabolism and blood urea. While no clinical features of liver derangement were described as having occurred in the post-operative period, it is noteworthy that no bio-chemical means were used for the detection of possible derangement of sub-clinical degree.

Following the publication of these results, trichlorethylene became known and widely used in Great Britain as an anæsthetic in surgery, and as an anæsthetic and analgesic in obstetrical practice.

Hewer (1942 and 1944) described further observations on trichlorethylene, and, in the latter year, was prepared to regard anæsthesia with the drug as being past the experimental stage, an estimated total of 300,000 administrations having been given in Great Britain alone.

No mention was made in these papers of any bio-chemical means having been used for the detection of possible liver damage following anæsthesia with the drug, nor, up to 1944, had any clinical evidence of such damage been described. In that year, however, a report appeared (Lancet, 1944) which suggested that trichlorethylene might not be so innocuous to the liver as previous observations had seemed to indicate. This report dealt with the case of a female child, aged two years, who died 4 days after an operation for the correction of congenital ptosis of the eyelids. The duration of the operation was 30 minutes and it was stated that the " open drop " method of administration was employed, trichlorethylene being used for induction, followed by ether. Here, it is to be noted that, although trichlorethylene was used " for induction only ", it was probably inhaled throughout the administration on account of its comparatively low volatility. At autopsy, " central necrosis of the liver similar to that seen in delayed chloroform poisoning " was observed. In addition, details are given in this report of a number of deaths following trichlorethylene anæsthesia which occurred in 1944, the drug having been given in a closed circuit with carbon

dioxide absorption. In these cases, it has been established that the toxic and lethal effects were due to the interaction of trichlorethylene with the alkali in the absorber. This, in the presence of the heat of the absorption reaction, produces dichloracetylene, to which compound these deaths have been attributed. (Humphrey and McClelland, 1944; Carden, 1944). At autopsy, well-marked fatty degeneration of the liver was found in 2 cases, but this can hardly be ascribed to trichlorethylene, in view of the facts set out above. It is obviously unfortunate, however, that, in the report on the fatality following trichlorethylene and ether, no mention is made of any chemical analysis having been carried out on the samples in use. The trichlorethylene preparation concerned was "Trilene", manufactured by Imperial Chemical Pharmaceuticals Ltd. This is a product conforming to high standards of purity and incorporating 0·01% thymol, which has been shown to retard decomposition. (Hewer, 1944). It is unlikely, also, that any toxic decomposition products were present in the ether, as this drug is now commonly packed in bottles containing an anti-catalyst, and normal hospital turnover of this widely-used drug ensures that it is not kept in storage over too long a period of time. It is therefore considered to be unlikely that the liver appearances found *post mortem* in the case under discussion were due to impurities or decomposition products. The similarity between these appearances and the morbid histological findings in cases of delayed chloroform poisoning, coupled with the similarity in the chemical structures of trichlorethylene and chloroform, throw suspicion on trichlorethylene as the the cause of this fatality, although Rolleston and McNee (1929) stated that "In rare instances ether anæsthesia has been followed by the symptoms seen in delayed chloroform poisoning" These authorities referred to 9 fatal cases of acute yellow atrophy of the liver collected by Ballin (1903), the anæsthetics employed not being mentioned. The ether used therefore cannot be dismissed from consideration as an ætiological factor, but the available evidence indicates that suspicion should be directed to a greater degree against trichlorethylene.

A further fatality due to hepatic necrosis, in which trichlorethylene may have played a part, was described in 1945. (Dodds). In this case, the patient died on the fourth day of the puerperium, and, at autopsy, liver necrosis and bilateral massive suprarenal hæmorrhages, regarded as secondary to the liver lesion, were found. Three factors were instanced by Dodds as possible causes of the primary lesion, namely :—

(1) There had been clinical evidence of slight toxæmia during the last 2 weeks of pregnancy.

(2) On account of (1), the patient had been placed on a low protein diet, and it has been shown by Glynn and Himsworth (1944) that protein deprivation in rats can lead to massive liver necrosis.

(3) During labour, a small amount of trichlorethylene had been given as an analgesic.

With regard to the morbid histological findings in the case, Dodds stated that " The liver lesion did not resemble that occurring in obstetrical acute yellow atrophy as described by Sheehan (1940) ". Furthermore, " The liver lesion in this case resembled the liver lesion of chloroform poisoning described by Sheehan, and of massive necrosis due to dietary deficiency described by Glynn and Himsworth ".

The degree of protein deprivation was no greater than has been tolerated by many patients suffering from toxæmia of pregnancy and other conditions; the amount of trichlorethylene inhaled was small, certainly smaller than that administered to thousands of patients without mishap. Yet a liver lesion of a magnitude out of all proportion to the apparent exciting causes was found, and it would seem that the simultaneous action of these causes enhanced the sum of their individual hepato-toxic effects.

It is impossible to be dogmatic regarding the assessment of responsibility for this lesion. The point for the present purpose is that trichlorethylene was implicated as a possible ætiological agent in a case of fatal hepatic necrosis.

A consideration of the cases described in this section, and of the work of Browning (1937), led to the conclusion that some dubiety attached to trichlorethylene as an anæsthetic drug with reference to its effects on the liver. On the one hand, thousands of administrations had resulted in nothing untoward, while on the other, 2 cases had occurred in which death followed a liver lesion possibly due, in whole, or in part to trichlorethylene. Animals given massive doses of the drug had in most cases shown no gross or microscopic liver lesions. In a minority, liver lesions had resulted.

In endeavouring to form an opinion with regard to the hepato-toxic effects of trichlorethylene, evidence of the type derived from autopsies and animal experiments is valuable. It is definite, and the sequence of cause and effect can be observed and studied where a lesion is shown to exist, although, as in the case described by Dodds, where more than one possible cause is present, it may be difficult to apportion the responsibility among these causes. This does not complete the picture, however, in that, as has been pointed

out by Cameron (1938), "A liver which presents little evidence of structural damage may, in reality, be incompetent functionally. It becomes more and more important that sensitive methods for assessing liver damage should be sought for. Likewise it is obvious that a great deal more requires to be known about the factors which influence liver function, apart from structural damage".

It appeared, therefore, that an assessment of liver function by bio-chemical means after trichlorethylene anæsthesia might be of of value, because, while animal experiments and autopsies had shown that liver damage sufficiently gross to be histologically evident did, on occasion, occur, these means of investigation could do nothing to bring to light liver impairment in the presence of normal histological findings, or possible impairment in surgical patients other than the minority subjected to autopsy.

II.

THE SELECTION OF A SUITABLE METHOD OF ASSESSING LIVER DAMAGE IN PATIENTS FOLLOWING GENERAL ANÆSTHESIA.— THE CEPHALIN CHOLESTEROL FLOCCULATION TEST.

The selection of a test for use in this investigation presented some difficulty, on account of the multiplicity of possible tests, and of the controversy which exists regarding the value of most of them. Elaborate procedures could not be contemplated, because the work had to be done in a Naval Hospital in the Tropics where routine duties naturally took precedence over research, and where the laboratory available was adequate for the general work of the hospital, but was not to be compared, either in respect of apparatus or technical assistance, with such laboratories as are to be found in establishments normally dealing with research work. For these reasons, and for the reason that any results obtained in this type of investigation depend for their value on the soundness of the method employed in their production, the fullest possible consideration had to be given to the selection of the test to be used.

Gutman and Hanger (1941), dealing with the differential diagnosis of jaundice, included in their work a classification of liver function tests under 4 main headings, incorporating many tests, as follows :—

(*a*) Measures of disturbed metabolism or excretion of bile pigments.

- i. Determination of serum bilirubin or icteric index.
- ii. Van den Bergh Reaction.
- iii. Qualitative tests for bile pigments in urine and stools.

(*b*) Measures of the capacity of the liver to perform certain functions. (" Liver function tests ").

- i. To excrete into the bile endogenous bile pigments, (the bilirubin tolerance test), or exogenous dyestuffs (the bromsulphthalein retention test).
- ii. To utilise certain carbohydrates (the galactose tolerance test).
- iii. To detoxify or metabolise selected compounds by conjugation with substances formed by the liver (the hippuric acid excretion test).
- iv. To elaborate various substances such as prothrombin, serum albumin, and cholesterol esters.

(*c*) Measures of the formation of certain abnormal substances by the disordered liver.

i. Determination of serum globulin.
ii. Takata-Ara Test.
iii. Cephalin cholesterol flocculation reaction.

(*d*) Measures of the patency of the biliary tract.

i. Quantitative estimation of stool and urinary excretion of urobilinogen.
ii. Duodenal drainage
iii. Cholecystograms and cholangiograms.
iv. Determination of free cholesterol in the serum.
v. Serum phosphatase determination.
vi. Several of the tests mentioned under (*a*) and (*b*).

The object of the work done by Gutman and Hanger was to evolve a satisfactory laboratory technique for the differentiation of hepatic from obstructive jaundice, and they came to the conclusion that this could best be done by applying to each jaundiced patient tests for both types of lesion.

Where hepatogenous jaundice exists, liver damage is present. Therefore, interest for the purpose of this study of the effect of trichlorethylene on liver parenchyma, was centred on the tests selected by Gutman and Hanger as being of the greatest value in determining that a given case of jaundice was of hepatogenous origin. These were the estimation of serum cholesterol esters, the hippuric acid excretion test, the galactose tolerance test, and the cephalin cholesterol flocculation test. It was, of course, appreciated that Gutman and Hanger were dealing with cases in which sufficient liver damage existed to cause jaundice, and that the degree of liver impairment (if any) to be expected in the post-anæsthetic cases under consideration, was unlikely to be of such magnitude. If a test were to be selected from among those instanced by Gutman and Hanger, it would therefore require to be the one adjudged to be the most delicate, regard being paid to the fact that the most delicate test might well be technically impossible in view of the limited facilities available.

Considering in turn each of the tests named in the preceding paragraph, it was found that controversy existed as to the behaviour of the serum cholesterol in the presence of liver damage. Rennie (1942), in a review of tests of hepatic efficiency, stated that, where parenchymatous liver damage exists, the total blood cholesterol may

be subnormal in amount, and that the ester fraction may be below the normal 60% of the total. Greene, Hotz and Leahy (1940) claimed that estimations during the course of a case of parenchymatous disease of the liver could be used as an indication of the progress of the disease. Jones (1942) failed to confirm this finding.

The hippuric acid excretion test depends on the fact that, when sodium benzoate is ingested, the liver synthesises glycine, which is conjugated with benzoic acid to form hippuric acid. This is excreted in the urine, and the excretion of subnormal amounts of hippuric acid following the intake of sodium benzoate is taken as an indication that the capacity of the liver to synthesise glycine is defective. The degree of defect is regarded as an index of parenchymatous liver damage. The test dose of sodium benzoate may be given intravenously. (Quick, Ottenstein and Weltchek. 1938).

This test was considered to be unsuitable for use in the work contemplated, because the day temperature of the wards in which the patients to be studied were located, ranged from 85° to 95° Fahrenheit. Urinary output was therefore low, and while it was appreciated that this need not necessarily have affected the excretion of hippuric acid, the practical point remained that it might have been difficult to have secured specimens of urine at the times required. This difficulty would have been especially marked in patients whose fluid intake had been impaired by post-operative nausea or vomiting, and in patients who had been subjected to operations on the lower abdomen, such being liable to cause post-operative difficulty of micturition. In addition, the technique of the test is time-consuming, and a further complication has been added by Scurry and Field (1943) who observed that hippuric acid excretion varied as body weight and surface area, and who produced formulæ for the prediction of normal excretion for given body weights, to be applied to each subject tested.

The galactose tolerance test has for its basis the fact that the liver removes galactose from the blood stream following absorption of that sugar from the alimentary tract, or following intravenous injection thereof. Figures for blood galactose above those found in normal individuals after a standard test dose of galactose are regarded as an indication of liver damage, as is the excretion of excessive amounts in the urine.

The intravenous route is held to be the more reliable on account of possible delay or irregularity in the absorption of galactose from the gut, and this was used by King, Harrison and Delory (1940) in an assessment of the value of the test, utilising for this purpose rabbits

subjected to liver damage with carbon tetrachloride. King and Aitken (1940) applied the test clinically, and, in their investigation, a 50 gm. intravenous dose of galactose was given. Blood galactose estimations were made 5 minutes after injection, and thereafter half-hourly for 2 hours. These workers concluded that the test was useful in the differential diagnosis of hepatogenous and obstructive jaundice, but that it was of no value in the detection of minor degrees of liver damage in the absence of jaundice. It was therefore held to be unsuitable for an investigation dealing with non-jaundiced patients. The multiplicity of venepunctures required was regarded as an additional disadvantage, as were the amount of chemical analysis required by each set of blood samples, and the high cost and scarcity of galactose. Similar criticisms apply to the lævulose tolerance test.

The cephalin cholesterol flocculation test was the one selected by Gutman and Hanger as being most applicable to their investigation. This test was first described by Hanger in 1938 and is sometimes referred to as the Hanger Test. It is based on the observation that the serum of patients suffering from parenchymatous liver lesions brings about flocculation in cephalin cholesterol emulsions.

A survey of work done in connection with this test showed that it had been investigated and applied by several workers with impressive results.

Hanger, in his original paper, described the application of the test to over 900 cases, including normal individuals and patients taken at random from the medical wards. Normals gave consistently negative results, denoted in his paper by the symbol "o". Where active disease of the liver parenchyma was present, flocculation almost always occurred, and various degrees thereof were denoted by the symbols "+", "++", "+++", "++++", Serial observations on cases of catarrhal jaundice showed that the amount of flocculation diminished as clinical improvement took place. Flocculation was encountered also, in some patients suffering from diseases not primarily hepatic. These cases will be discussed later along with similar findings made by other observers.

In his second paper (1939), Hanger described the successful use of his test in the differential diagnosis of hepatogenous and obstructive jaundice.

The work of Gutman and Hanger (1941) was an elaboration on Hanger's work of 1939, in that, as has already been mentioned, a test for the presence of biliary obstruction was incorporated. The procedure chosen for this purpose was the estimation of serum

phosphatase values, and this was complementary to the cephalin cholesterol flocculation test in the differential diagnosis of hepatogenous and obstructive jaundice, the rationale being that, in a given case of jaundice ascribable to either liver damage or biliary obstruction, one test should give positive, and the other negative results. In tabular form, results with the cephalin cholesterol flocculation test were as follows :—

		Cephalin cholesterol flocculation test results.				
JAUNDICE	No. of cases	++++	+++	++	+	— or ±
Obstructive	77	0	0	1	6	70
Catarrhal	60	37	11	6	2	4

It is evident from these figures that the test proved its value for the required purpose.

Emphasis has already been given to the fact that hepatogenous jaundice has a basis in parenchymatous hepatic damage. This being so, positive flocculation reactions indicate that such damage is present, and it would seem, from the serial observations made by Hanger, that the degree of flocculation is parallel to the amount of damage. Post-anæsthetic cases, however, are very seldom the victims of sufficient liver damage to become jaundiced, and it was apparent that, if the cephalin cholesterol flocculation test were to be satisfactory for the investigation of such cases, it would have to show considerably more delicacy than had been required of it in the work concerning jaundice described above.

Evidence that such delicacy might indeed be a feature of the test was obtained from the work of Kopp and Solomon (1943) who investigated liver damage in therapeutically induced benign tertian malaria.

In naturally-acquired malaria there may be jaundice, enlargement and tenderness of the liver, diarrhœa and dark-coloured urine, these being signs indicative of possible liver involvement. Parenchymatous liver lesions are not uniformly present *post-mortem*, but, according to Rolleston and McNee (1929), Manson-Bahr (1940), Beattie and Dickson (1943), and Strong (1943), both degenerative changes and fatty infiltration are sometimes found.

Kopp and Solomon carried out exhaustive tests for liver damage on a group of 9 men, aged 32 to 55 years, all undergoing malarial therapy for the relief of general paralysis of the insane. Of these patients, 6 had had no previous arseno-therapy, 3 had had tri- and penta-valent arsenical drugs. None of the patients showed evidence of disease other than neuro-syphilis.

The therapeutic technique in use was to infect the men with benign tertian malaria. After an incubation period of 3 to 7 days, they developed a quotidian or mixed type of fever. This was terminated after 4 to 12 paroxysms by quinine sulphate gr. 30 daily for 7 days.

Liver function was investigated before, during, and after malaria by the following methods :—(i) The bromsulphthalein test. (ii) Estimation of serum cholesterol. (Total, free, and ester). (iii) Estimation of blood phospholipoids. (iv) Hippuric acid excretion test. (v) Cephalin cholesterol flocculation test. (vi) Estimation of plasma fibrinogen. (vii) Estimation of serum bilirubin. (viii) Van den Bergh Reaction. (ix) Icterus index. It will be appreciated from this that the investigation was thorough and comprehensive.

The tests, when applied before malaria, returned essentially normal results in 8 patients. In only one patient were abnormal results encountered, and he had had no less than 14 years of intensive arsenotherapy, with toxic hepatitis in 1928.

Significant results after malaria were moderate bromsulphthalein retention, a marked fall in total cholesterol and cholesterol, ester values, a diminution in hippuric acid excretion and a strongly positive cephalin cholesterol flocculation test. The other tests were found to be of little or no value.

Figures obtained, based on average values in 8 patients were as follows :—

		Normal range	Before malaria	After malaria
Bromsulphthalein retention	(i) at 18 min. (%)	0	3·5	9·4
	(ii) ,, 30 ,, ,,	0	0	0
Fibrinogen	(mgm. per 100 cc.)	190-330	303·3	367·8
Cholesterol, total	(mgm. per 100 cc.)	140-230	219	126
ester	(,, ,, ,, ,,)	69-77	71·1	47·2
Cephalin cholesterol flocculation test		0	0	+++
Phospholipoids	(mgm. per 100 cc.)	8-9	8·25	7·53
Hippuric acid excretion test	(mgm. per 100 cc.)	4-6	4·9	3·5

Following quinine therapy, results in the tests regarded as significant reverted to normal in 3 to 6 weeks, except in the case of the cephalin cholesterol flocculation test, which remained positive over a longer period.

The persistence of a positive result with this test suggested that it was capable of detecting liver damage of very mild degree, an essential feature if the test were to be effective in the study of such sub-clinical lesions as might follow general anæsthesia.

Rosenberg (1941) applied the cephalin cholesterol flocculation test to the study of digestive disorders, his opinion being that minor degrees of liver damage are seldom clinically recognisable. He held that a test for such disease should be incorporated in all upper alimentary investigations as are, for example, gastric analysis and cholecystography. He described the test under discussion, on account of its simplicity and sensitivity, as the ideal test for the purpose.

Dick (1945) assessed the value of the test by applying it to sera of 3 different groups, namely, (i) sera of 100 normal individuals, (ii) sera of 164 patients suffering from a variety of systemic disorders, (iii) sera of 77 patients with hepatic or biliary disease.

From his results in this work, Dick concluded that flocculation occurred where parenchymatous liver disease was present, and that the degree of flocculation varied directly as the severity of the disease, the sensitivity of the test being of a high order. He considered that the test was of value in the differential diagnosis of hepatic and obstructive jaundice, but that it was of no value in the early diagnosis of arsenical hepatitis. In normally healthy subjects negative results were invariably obtained, but a number of positive findings were made in patients suffering from illnesses not primarily hepatic. These will be discussed later.

The work of Pohle and Stewart (1941), Lippman and Bakst (1942), Kirschner and Glickman (1943), and Mateer, Baltz, Marrion and Macmillan (1943) was on similar lines to that already described and afforded confirmation of the value of the test. Lippman and Bakst commented particularly on its relative cheapness and simplicity and advocated its use as a routine test in the study of patients with suspected hepatic lesions. Yardumian and Weisband (1943) described the test as the best available means of investigating the integrity of the liver.

It was evident, at this stage, that the cephalin cholesterol flocculation test had been subjected to thorough investigation in many laboratories, with results which proved the test to be a sensitive one for the detection of parenchymatous liver damage. As a final step in the assessment of its value, the three criteria laid down by Higgins, O'Brien, Stewart and Witts (1944) for use in the evaluation of tests of liver function, were applied. These are :—

(i) That the test should be applied to large numbers of healthy men and women of different ages, in order to assess the extent of normal variations.

(ii) That the test should be applied in proved hepatic disease to demonstrate sensitivity. Results, to be satisfactory, should differ materially from those obtained from healthy subjects.

(iii) That the test should be applied in other diseases in order to measure its specificity.

The first criterion has been applied by all workers investigating the test, and while some simply described normal subjects as giving negative flocculation reactions, others described single plus reactions (+), or doubtful reactions (±), as being frequently encountered in normal subjects and therefore as being within normal limits. It appears that this discrepancy arose from the the fact that samples of cephalin vary in sensitivity, and that flocculation may indeed occur when normal serum is added to a cephalin cholesterol emulsion made with highly sensitive cephalin. A rational procedure would seem to be to include, with each group of possibly abnormal sera to be tested, one, or more than one specimen from subjects in normal health. Should these normal sera produce flocculation, similar flocculation occurring in the case of possibly abnormal sera is discounted, and they are regarded as normal. Any increment of flocculation above that given by the normal is estimated in terms of "+" symbols and accepted as a positive reaction. With this proviso, the first criterion is held to be adequately fulfilled.

Regarding the second criterion, results in the work done on the differential diagnosis of jaundice and on liver damage in malaria demonstrated a high degree of sensitivity, and showed, moreover, that readings in liver lesions, even of slight degree, were markedly different from the normal.

The third criterion, which is concerned with specificity, introduces matter open to discussion in that the application of the test to other diseases has shown that flocculation can occur in certain conditions not primarily hepatic. Hanger, for example, in his original (1938) work, encountered positive results in 72 instances in 893 selected hospital cases. Conditions giving a high proportion of positive findings included bacterial endocarditis, the pneumonias, uræmina, the leukæmias and idiopathic anæmias, serous tuberculosis and Prontylin idiosyncrasy. Dick described positive flocculation in 13 out of 164 patients suffering from a variety of diseases and tabulated them thus :—

Case	Disease	Degree of flocculation
1	Pernicious anæmia	++
2	Iron deficiency anæmia ..	++++
3	Malaria	++++
4	,, ...	++++

Case	Disease	Degree of flocculation
5	Lung cancer	+
6	Pleurisy with effusion	++++
7	Diaphragmatic pleurisy	+
8	Asthma	++
9	Diabetic coma	+++
10	Cancer of stomach. (Liver not involved). ..	+
11	Nephritis and hæmolytic anæmia due to sulphonamide therapy	++
12	Rheumatoid arthritis	++++
13	,, ,, ...	++++

Lippman and Bakst, out of a total of 83 cases with no clinical or laboratory evidence of liver disease, found that 4 returned positive cephalin cholesterol flocculation reactions, the diseases in these 4 cases being hyperthyroidism, pernicious anæmia plus hyperthyroidism, Cooley's anæmia with liver enlargement, and bronchial asthma with peri-arteritis nodosa. Comparing the total number of cases of disease not primarily hepatic with the total number of positive results reported by these workers, it is found that, in all, 7·8% were positive. This figure would be above that acceptable, if it were not for the fact that a secondary liver lesion is a recognised pathological feature of some of the conditions listed, notably the anæmias, leukæmias and malaria. In others, a strong toxic element is present, for example, diabetic coma, nephritis and uræmia, or a septicæmic and toxic element as in the pneumonias. A severe toxæmia or septicæmia can cause secondary liver damage, and this is the probable explanation of the occurrence of positive reactions in such cases. Deducting these from the total number of positive results in other diseases, it is found that 1·3% remain, and for these explanation is not easy. It may be that these conditions do cause alterations in the serum of such a kind as to bring about flocculation in cephalin cholesterol emulsions for reasons not connected with the liver, or it may be that a secondary alteration in the liver is responsible.

In this connection it is of interest that the occurrence of liver damage in rheumatoid arthritis has been commented upon by Rawls, Weiss and Collins (1937), in hyperthyroidism by Beaver and Pemberton (1933), Shaffer (1940), and Schmidt, Unruh and Chesky (1942), in pneumonia by Curphey and Solomons (1938), in various blood dyscrasias by Barker (1938), in diabetes mellitus by Lowe (1938-39), in advanced pulmonary tuberculosis by Levinson and Siegal (1938), and in infective mononucleosis by Davis, Macfee, Wright and Allyn (1945). These workers demonstrated, in some cases histologically, and in others by bio-chemical means, that liver damage could occur in a wide variety of unrelated conditions, and it may be that the sum of such conditions will increase as knowledge

of pathology in general, and of the pathology of the liver in particular, increase. The salient point for the present purpose is that there is evidence that secondary hepatic lesions do occur in many diseases, and that such lesions are detected by the cephalin cholesterol flocculation test.

If it is borne in mind that no claim is made that the test differentiates between primary and secondary parenchymatous liver lesions, it appears that the criterion of specificity is adequately fulfilled, especially when it is realised that reactions such as the Kahn and Wassermann, on which great reliance is placed, similarly fail to achieve the ideal 100% specificity, which, as experience has shown, is hardly to be expected of any biological test.

The mechanism of the cephalin cholesterol flocculation reaction has been a subject for speculation since Hanger's original work appeared in 1938 and 1939. At that time, theories advanced by Hanger as to the factors responsible for flocculation were, (1) that flocculation was due to an interaction between defective fibrinogen, a possible product of diseased liver, and colloidal cephalin ; (2) that a serum producing flocculation contained a nitrogen-bearing constituent in the globulin fraction which became attached to the surface of the cephalin cholesterol particles.

Recently, Moore, Pierson, Hanger and Moore (1945) have published a paper based on work done in the electrophoresis laboratory, College of Physicians and Surgeons, Columbia University, regarding the mechanism of the reaction, and their conclusions were as follows :—" In disease, a positive flocculation may be obtained with a serum due to any of the following alterations :—(1) Increase of gamma globulins in such quantity that there is insufficiency of the normal components of the serum albumin fraction to inhibit the reaction ; (2) diminution of the serum albumin fraction below initial levels necessary to inhibit the reaction ; (3) diminution in the flocculation-inhibiting properties of the albumin fraction (such as has been demonstrated above). The positive test observed in hepatitis, etc. is probably due to a combination of all these factors, especially to modification of the albumin fraction ".

The conclusion reached as a result of this survey of the application and rationale of the cephalin cholesterol flocculation test was that it seemed to be sufficiently sensitive for the detection of possible post anæsthetic liver damage, and that the technique of the test compared favourably in respect of simplicity with other possible procedures. It was therefore decided to employ the test in the problem to be considered, provided the extraction of cephalin proved feasible, and a preliminary trial gave satisfactory results.

III.

A DESCRIPTION OF PRELIMINARY WORK DONE IN CONNECTION WITH THE CEPHALIN CHOLESTEROL FLOCCULATION TEST.

In general terms, this work comprised the extraction of cephalin from sheep's brains, its combination with cholesterol to form the antigen used in the test, and an application of the test, using the material so prepared, to sera of normally healthy individuals and of patients suffering from diseases proved by previous workers to yield positive results.

The results of this preliminary investigation, when some technical difficulties had been overcome, agreed very well with those of previous work carried out elsewhere, which indicated that the cephalin had been efficiently extracted, and that the technique followed in the application of the test was sound.

In greater detail, the work carried out was as follows :—

(*a*) The extraction of cephalin.

This was prepared from sheep's brains supplied by an army abattoir in which sheep, imported from India, are killed as food for Indian troops. It was providential that this source of supply existed, because no sheep are reared in Ceylon.

The method used was that described by Hanger (1939), and followed by Dick (1945). Hanger's concise account of this is as follows :—" Sheep brains were dehydrated by three extractions with acetone and the dry tissue powder was three times extracted with ether. (Free of peroxides.) The ether extracts were concentrated *in vacuo* and the crude cephalin was precipitated by the addition of four volumes of absolute alcohol. The resulting precipitate was dissolved in the minimum amount of ether, the accompanying cerebroside impurities were precipitated by chilling and removed by centrifugation. The supernatant ether solution was again precipitated with four volumes of absolute alcohol, chilled, and the precipitate filtered, washed with alcohol and acetone, and desiccated. The cephalin preparation is a brown, powdery material containing traces of other lipids. These, however, do not feature in the reaction ".

(*b*) The preparation of the cephalin cholesterol antigen.

This was done by dissolving 100 mgm. of sheep brain cephalin and 300 mgm. of cholesterol in 8cc. of ether. This solution is stable and remains effective if well stoppered and stored in a refrigerator.

(*c*) The technique of the cephalin cholesterol flocculation test.

Here again, the method of Hanger was followed, and his account reads thus :—" An emulsion of a cephalin cholesterol complex may be prepared by adding (slowly and with stirring) 1cc. of the stock ether solution to 35cc. of freshly distilled water warmed to 65°-70° and then heated slowly to boiling. The mixture is allowed to simmer until the final volume is reduced to 30cc. During the heating, all coarse granular clumps are dispersed to a stable, milky, translucent emulsion and all traces of ether are driven off. After cooling to room temperature the preparation is ready for the test which consists of adding 1cc. of the emulsion to a test-tube, preferably a centrifuge tube, containing ·2cc. of the patient's serum diluted with 4cc. of normal (·85 %) saline. After thorough shaking and stoppering with cotton, the tube is allowed to stand undisturbed at room temperature and notation is made at the end of 24 and 48 hours as to the amount of flocculation and precipitation that have taken place. With normal human sera, the emulsion remains as a stable, homogeneous suspension, but with sera from patients with diffuse hepatitis, the lipoid material tends to flocculate and precipitate to the bottom of the tube. A ++++ reaction indicates a complete precipitation leaving the supernatant liquid water-clear. Gradations of the reaction between negative and ++++ are designated in terms of +, ++, +++ ".

(*d*) The application of the cephalin cholesterol flocculation test to sera of normally healthy individuals and of patients suffering from diseases shown by previous workers to produce positive reaction.

The technique described was followed, with one slight modification made in the light of experience. It was found that many normal sera gave a positive result in 48 hours, a finding which agreed with those of Clay and Moore (1942), and of Dick (1945). The practice of leaving the test in being for 48 hours was therefore discontinued, and readings were made at 24 hours only. The notation " ± " was used to cover cases in which there was a doubtful departure from the normal.

The sera investigated were obtained from members of the medical, sick berth and nursing staffs. Asiatic ratings were excluded on account of the high incidence of chronic malaria, dysenteric disorders and helminth infestation among these people. In this connection, it is of interest that Karunaratne (1940) has described cirrhosis of the liver in association with hookworm disease. A recent history of malaria, jaundice or dysentery led to exclusion

in the case of Europeans. With sera from these supposedly normal subjects, sera from patients suffering from catarrhal jaundice and malaria were studied, the work of Hanger (1938-39), of Gutman and Hanger (1941) and of Dick (1945) having shown that consistently positive results were to be expected in catarrhal jaundice, and that of Kopp and Solomon (1943) and of Fredericks and Hoffbauer (1945) that similar results were to be expected in malaria.

A convenient method was found to be to deal with sera in batches of 10 to 12 samples, a typical batch comprising, for example, 6 normal sera, 3 from cases of malaria, and 3 from cases of catarrhal jaundice. The geographical situation of the hospital ensured that there was a plentiful supply of patients suffering from these diseases from whom serum could be obtained.

It was found, when a satisfactory cephalin had been prepared, that sera of normal individuals gave negative results in all but a very small percentage of cases, and that sera of patients with catarrhal jaundice and malaria gave consistently positive results, the degree of flocculation varying with the severity of the disease. Three positive results were encountered among the presumable normals. One of these was a petty officer who had had jaundice some 6 months before for the third time. The other was a chief petty officer subject to migraine. There was nothing relevant in the medical history of the other man. In all, 45 normal sera were tested in this way, as were 20 from catarrhal jaundice cases and 14 from cases of malaria.

These encouraging results were not obtained with the first sample of cephalin prepared, however. This cephalin proved satisfactory over a period of 2-3 weeks, then it was found that flocculation occurred immediately the test was set up, both in normal and abnormal sera. The material was therefore discarded, and a careful scrutiny of the technique followed in its preparation showed the ether which had been used to be grossly contaminated with aldehydes and peroxides, which was hardly surprising, as it was part of a consignment which had been in store since 1942. This was considered to be a possible cause of the erratic behaviour of the cephalin in question. A fresh supply was prepared with ether tested for, and found to be free from decomposition products, the tests employed being those recommended by Hewer (1944). This sample of cephalin was put into use immediately on preparation, and it was found to behave in a similar fashion to the first sample in its later stages, that is, immediate flocculation occurred both with normal and abnormal sera. This over-sensitivity was corrected by exposing the material to sun and air for a period of 2 weeks,

this ripening process having been followed by Hanger and Patek (1941) with the cephalin they used in a study of a series of hepatic cirrhosis cases. These workers allowed a 6 weeks ripening process, by which time the original powdery material had become dark brown and gummy in consistency. After 2 weeks, the cephalin prepared here remained as a powder, but had become darker in colour. This was found to give satisfactory results, and a number of serial tests made in cases of catarrhal jaundice and malaria seemed to indicate a sufficient sensitivity. This cephalin was stored in a stoppered bottle in a refrigerator, and while Mateer, Baltz, Marrion and Macmillan (1943) are of the opinion that cephalin in storage gradually increases in sensitivity, this need not jeopardise the value of the material, provided the degree of flocculation observed in tubes containing known normal sera is regarded as a negative reading, and only tubes showing an increment of flocculation above that shown by the normal are regarded as containing abnormal sera. Over a long period, using the same cephalin, an increase of flocculation might be observed with serum from the same individual. This does not imply that the subject concerned has fallen a victim to parenchymatous liver disease, but simply indicates an increase in the sensitivity of the cephalin, and therefore a raising of the datum line for the recording of positive flocculation readings. This point has already been discussed in connection with the criteria regarding tests for liver damage laid down by Higgins *et al* (1944). (Section II.).

It may be added that the cephalin prepared and ripened as described, was in use over a period of 9 months without any noticeable increase in sensitivity taking place. The process, if it does occur, is therefore very slow.

IV

The application of the cephalin cholesterol flocculation test to the anæsthetic problem.

The primary purpose was to determine whether liver damage could be demonstrated after anæsthesia with trichlorethylene. A secondary purpose was to compare the degree of damage, if any such were detected, with that following anæsthesia with di-ethyl ether.

The reason for the inclusion of this secondary investigation was that, while the primary investigation might enable a statement to be made as to whether liver damage does, or does not occur as a result of trichlorethylene anæsthesia, such knowledge is of little practical value unless it can be correlated with findings regarding liver damage occurring as a result of the administration of other anæsthetic drugs. It was clearly impossible to extend the investigation to include all such drugs, but it was considered that a comparative series of results following di-ethyl ether anæsthesia would be of value in this connection, for the reason that di-ethyl ether is a widely used anæsthetic agent familiar to all anæsthetists, which has been investigated with regard to liver damage following its use, and compared in this respect with other anæsthetic drugs. (Rosenthal and Bourne 1928). Furthermore, a comparison between results obtained with the cephalin cholesterol flocculation test following ether anæsthesia, and results of other workers using other methods, gave promise of serving as a useful check on the validity of the test in this type of investigation.

At the outset, it must be emphasised that no group of patients can possibly present the uniformity to be found in selected experimental animals. Patients vary with regard to age and the lesion from which they suffer. In any ward it would be easy to find patients quite useless as subjects for an investigation of the effects of anæsthetic agents on the liver, for example, hyperthyroid patients and cases of jaundice or malaria. In addition, it had to be borne in mind that the patients available for study were in hospital for the cure of the conditions from which they suffered, and that the anæsthetic technique carried out had to embody premedication and induction of anæsthesia with drugs other than those being subjected to study. In this respect, patients differ from experimental animals, it being quite inadmissible to subject them to the inhalation of irritant vapours without ensuring their comfort and post-operative well-being by employing some of the niceties of anæsthetic technique.

These considerations led to the adoption of the following rationale :—

(i) The selection of patients.

These were all young European Naval ratings, whose presence in hospital was attributable to lesions of a mechanical, as opposed to a metabolic or septic nature, for example hernia, hydrocœle and non-septic orthopædic conditions. Young patients were selected because Raffsky (1943) demonstrated impairment of hepatic function as a constant finding in the aged. Few aged patients are to be found in Naval Hospitals, but this finding was regarded as of sufficient importance to lead to the choice of the younger patients available. Relatively minor surgical interventions were preferred to major ones on account of the possible effect of surgical shock on the liver.

A history of malaria, jaundice or dysentery led to exclusion from the series of cases studied. Sufficient has already been said regarding liver damage in malaria and in jaundice (Section II.) to render it unnecessary to elaborate further the advisability of rejecting such cases, and Heilig and Vesveswar (1944) have shown that a high incidence of liver damage occurs in amœbiasis, a condition which is hyper-endemic in Ceylon and which accounts for many of the cases of dysentery seen there. This finding of Heilig and Vesveswar has been confirmed by Shute (1945) using the cephalin cholesterol flocculation test as a criterion of liver damage. It is of interest, in this connection, to recall a case of acute appendicitis whose serum was included in one of the preliminary batches examined prior to this investigation. This returned a "++++" result with the cephalin cholesterol flocculation test before operation, and shortly afterwards, in the post-operative period, a sharp attack of diarrhœa led to the performance of stool examinations which revealed the presence of the *Endamœba histolytica* in large numbers. Appropriate treatment led to a speedy resolution of the patient's symptoms, and to the cessation of the discharge which had developed from his abdominal incision. This case illustrates the wisdom of excluding acute abdominal cases and patients giving a history of dysentery from the series studied.

Diet was another factor of importance in the selection of cases, Glynn and Himsworth (1944) having proved that protein deprivation could cause hepatic necrosis, and Goldschmidt, Ravdin, and Lucke (1937) having shown that a high carbohydrate diet offered some protection against the necrotising effect of certain anæsthetic agents on the liver, whereas malnutrition enhanced this effect. Here again, it will be appreciated that relatively fit men with lesions

of the type mentioned above were obviously most suitable, in that they were able to be on a full, mixed diet up to the time of operation, and neither malnutrition in general, nor protein lack in particular, were likely to have been present in men on Naval victualling.

(ii). The anæsthetic technique used.

This subject is best considered in terms of :—

(*a*) Premedication.—All patients were given morphine sulphate gr. $\frac{1}{4}$ and atropine sulphate gr. 1/100 by subcutaneous injection, $1\frac{1}{4}$ hours before operation. Atropine had to be given to check excessive salivation and bronchial secretion which are marked with di-ethyl ether, and are present to a lesser degree with trichlorethylene. Morphine could perhaps have been omitted, but, on careful consideration it was decided to exhibit this narcotic, because induction of anæsthesia without pre-operative sedation, especially in young robust men, is apt to be stormy, and to be accompanied by struggling, breath-holding and anoxæmia as evidenced by cyanosis. The avoidance of anoxæmia was very important, because it has been shown by Goldschmidt *et al* (1937) that anoxæmia, acting alone, can cause liver damage. These considerations led to the conclusion that a much more accurate impression of the effect of trichlorethylene, (and di-ethyl ether) on the liver would be gained from a study of patients adequately premedicated and, as a result, smoothly anæsthetised, than could have been derived from a direct exhibition of these drugs alone, which would certainly have been accompanied by the features mentioned, namely struggling and breath-holding in the second stage of anæsthesia, with hyper-secretion throughout the administration. Under these conditions, it would have been extremely difficult to have avoided anoxæmia, either from mechanical obstruction to respiration by secretions, or by glottic spasm. A further indication for sedation lay in the fact that pre-operative fear and apprehension might have led to hyper-secretion of adrenalin with depletion of glycogen stores in the liver.

It may be argued that it was inadmissible to draw conclusions regarding the action of a particular drug when that drug had been given only as one member of a sequence of drugs, and that no means were available of assessing the possible hepatic effects of morphine and atropine, and of estimating what proportion of the total effects should have been attributed to these drugs. This is not regarded as a valid objection to the rationale followed, it having been shown by Rosenthal and Bourne (1928) that, in dogs, doses of morphine, proportionally 30 to 50 times as great as the therapeutic dose in man,

produced only transient evidence of liver impairment of slight degree. These observers concluded that the therapeutic dose in man could not have any significant effect on the liver. This conclusion is borne out, to some extent, by the observation, made during this study, that negative cephalin cholesterol flocculation test results were encountered in patients who had had fairly heavy dosage of morphine in the period preceding testing, for example gr. 2 in 7 days. These were patients who required the drug for the relief of post-operative pain.

With reference to possible hepatic effects of atropine, consultation of works on pharmacology by Meyer and Gottlieb (1926), Dixon (1929), Edmunds and Gunn (1936), Clark (1937) and Adriani (1942) revealed no mention of any such effects having been observed. Sollmann (1937) cited Okada (1915) and Yanagawa (1916) as authorities for the statement that " The secretion of bile is sometimes slightly diminished by atropine ", and noted that Doyon and Kareff (1904-06) had described the formation of an anti-coagulant substance on perfusion of the liver with atropinised blood. No suggestion was made that atropine had any toxic effect on liver parenchyma.

It would appear that the drugs given as premedication were extremely unlikely to have had any significant effect on the liver, whereas the features it was hoped to obviate by their use, namely anoxæmia and pre-operative liver glycogen depletion, had they been allowed to occur, would have completely vitiated the investigation.

(*b*) Induction and maintenance of anæsthesis.—Induction was carried out with nitrous oxide and oxygen, great care being taken to avoid anoxæmia. The arguments for, and against the use of nitrous oxide are similar to those advanced regarding premedication, and it was considered justifiable to employ the gas for similar reasons, especially as Rosenthal and Bourne (1928) had shown it to be non-toxic to the liver in the presence of adequate oxygenation.

A Marrett's apparatus was used for all administrations. This is a simple contrivance in which the patient's own inspirations serve to volatilise the anæsthetic drug in use by drawing air over it. As an added precaution against anoxæmia, oxygen was added at the rate of 500cc. per minute during the maintenance of anæsthesia. Both the trichlorethylene and the di-ethyl ether in use were tested for impurities, the methods followed being those recommended by Hewer (1944).

The requirements of the surgeon in dealing with the conditions represented in the series studied, were adequately met by maintenance in the first plane of surgical anæsthesia. Therefore, although there was variation in the duration of anæsthesia from case to case, the depth of anæsthesia was constant. The fact that relatively light anæsthesia sufficed and that relatively minor surgical interventions were selected, excluded, to a great extent, the variable factor of surgical shock and the possible hepatic effects thereof, major causes of such shock being prolonged, deep anæsthesia, and extensive surgical intervention.

(*c*) The timing and technique followed in the application of the test.—Under ideal conditions, the test would have been applied to all patients before operation, immediately after operation, and thereafter at daily intervals until normal results appeared, in such cases as yielded abnormal results with the first post-operative test.

Owing to pressure of other duties and the fact that no assistance was available for such routine work as the collection of blood specimens and the maintenance of necessary equipment, this ideal could not be attained, and it was decided that the best distribution of tests in each case would be pre-operative, 24 hours after operation, and again 7 days later, that is, 8 days after operation. A further test was carried out 7 days after the second post-operative test in such cases as showed a persistently positive result in that test.

The technique described in Section III was followed. Two normal sera were included with each batch tested, these being necessary for the accurate interpretation of results, and a specimen of known abnormal serum from a case of catarrhal jaundice or malaria was also included as an aid to this end. In most batches of sera there were specimens both from patients anæsthetised with trichlorethylene, and from patients anæsthetised with di-ethyl ether.

V. Tables of Results

TABLE I

CEPHALIN CHOLESTEROL FLOCCULATION TEST RESULTS BEFORE AND AFTER ANÆSTHESIA WITH TRICHLORETHYLENE

No.	Patient	Age	Operation	Duration	Test Results*			
					1	2	3	4
1	J.A.	27	Excision of toe-nails	.. 35 min.	—	++	+	—
2	S.D.	26	Interval appendicectomy	.. 32 ,,	—	—	—	
3	F.C.F.	21	Orchidectomy	.. 37 ,,	—	+	—	
4	W.T.	18	Interval appendicetomy	.. 43 ,,	—	±	—	
5	R.A.J.	19	Excision of toe-nails	.. 20 ,,	—	+	—	
6	A.S.C.	32	Herniotomy (bilateral)	.. 75 ,,	—	+	—	
7	T.M.	24	Meniscectomy	.. 55 ,,	—	+	—	
8	D.D.	31	Tonsillectomy	.. 45 ,,	—	+	—	
9	G.J.	20	Orchidectomy	.. 35 ,,	—	±	—	
10	L.B.	21	Tonsillectomy	.. 40 ,,	—	++	±	—
11	A.C.	36	,,	.. 45 ,,	—	+	—	
12	P.V.M.	25	,,	.. 35 ,,	—	+	—	
13	J.H.	30	Excision of toe-nails	.. 25 ,,	—	±	—	
14	T.E.L.	20	Excision of pilonidal sinus	.. 40 ,,	—	++	—	
15	J.J.H.	22	Meniscectomy	.. 50 ,,	—	±	—	
16	L.M.	20	Herniotomy	.. 50 ,,	—	+	—	
17	P.D.W.	22	Tonsillectomy	.. 50 ,,	—	+	—	
18	D.V.	25	Excision of cyst of jaw	.. 20 ,,	—	+	—	
19	G.W.	26	Hæmorrhoidectomy	.. 25 ,,	—	+	—	
20	R.A.J.	19	Excision of toe-nails	.. 25 ,,	—	+	—	

*1 Pre-operative.
2 24 hours post-operative.
3 8 days post-operative.
4 15 days post-operative.

CEPHALIN CHOLESTEROL FLOCCULATION TEST RESULTS BEFORE AND AFTER ANÆSTHESIA WITH TRICHLORETHYLENE—*Contd.*

No.	Patient	Age	OPERATION	Duration	Test Results*			
					1	2	3	4
21	V.P.	27	Open reduction of fracture	.. 95 min.	—	+	—	
22	C.R.L.	19	Tonsillectomy	.. 40 ,,	—	++	±	—
23	H.R.	19	Circumcision	.. 25 ,,	—	++	—	
24	G.M.	19	,,	.. 25 ,,	—	+	—	
25	J.W.H.	25	Herniotomy (bilateral)	.. 90 ,,	—	+	—	
26	G.L.	25	Circumcision	.. 25 ,,	—	±	±	—
27	G.M.H.	19	,,	.. 25 ,,	—	++	±	—
28	R.W.B.	21	Herniotomy	.. 36 ,,	—	+	—	
29	J.D.R.	21	,, (bilateral)	.. 65 ,,	—	++	—	
30	C.W.P.	22	Interval appendicectomy	.. 60 ,,	—	+	—	
31	J.B.M.	19	Circumcision	.. 20 ,,	—	+	—	
32	R.F.H.	19	,,	.. 25 ,,	—	±	—	
33	G.W.H.	19	Excision of toe-nails	.. 20 ,,	—	+	—	
34	H.J.S.	35	Tonsillectomy	.. 40 ,,	—	+	—	
35	A.S.	23	,,	.. 35 ,,	—	±	—	

*1 Pre-operative.
2 24 hours post-operative.
3 8 days post-operative.
4 15 days post-operative.

TABLE II

CEPHALIN CHOLESTEROL FLOCCULATION TEST RESULTS BEFORE AND AFTER ANÆSTHESIA WITH DI-ETHYL ETHER

No.	Patient	Age	Operation	Duration	Test Results*			
					1	2	3	4
1	J.H.	22	Interval appendicectomy	.. 45 min.	—	+	—	
2	A.W.	26	Circumcision	.. 30 ,,	—	+++	—	
3	F.B.	20	Meniscectomy	.. 64 ,,	—	++	—	
4	D.S.	20	Interval appendicectomy	.. 45 ,,	—	+	—	
5	F.E.L.	23	Meniscectomy	.. 50 ,,	—	+++	—	
6	D.M.	19	,,	.. 60 ,,	—	+++	+	—
7	G.G.	20	Fixation of epicondyle	.. 45 ,,	—	+++	—	
8	W.L.	23	Excision of toe-nails	.. 35 ,,	—	++	—	
9	B.R.	31	Circumcision	.. 25 ,,	—	+++		
10	P.T.	18	Tonsillectomy	.. 50 ,,	—	++++	+	—
11	H.C.	24	Excision of pilonidal sinus	.. 35 ,,	—	++	—	
12	C.R.	21	Tendon repair	.. 70 ,,	—	++	+	—
13	A.B.S.	20	Radical cure of hydrocœle	.. 50 ,,	—	+	—	
14	A.P.	22	Amputation of thumb	.. 26 ,,	—	++	—	
15	F.J.	26	Meniscectomy	.. 49 ,,	—	+++	+	—
16	G.E.C.	18	Circumcision	.. 25 ,,	—	+	—	
17	D.W.	33	Herniotomy	.. 44 ,,	—	+	—	
18	J.S.	34	Hæmorrhoidectomy	.. 21 ,,	—	+	—	
19	E.M.W.	33	Extraction of impacted teeth	60 ,,	—	+++	±	—
20	D.E.	20	Circumcision	.. 20 ,,	—	++	—	

*1 Pre-operative. 3 8 days post-operative.
2 24 hours post-operative. 4 15 days post-operative.

VI

Analysis and discussion of results.

Table I. The trichlorethylene series comprised 35 patients. The age range was from 18 to 36 years. The duration of anæsthesia was from 20 to 95 minutes, the average being 40 minutes. The conditions leading to operation in no case involved acute sepsis or any clinical metabolic upset. General pre-anæsthetic examination showed no abnormality apart from the lesion which required operative treatment, and a careful history was taken in all cases to exclude malaria, jaundice and dysentery.

The pre-operative cephalin cholesterol test result was negative in all cases.

Post-operative results were :—

Test No. 2. (24 hours after operation)
The sera of 7 patients produced ++ flocculation.
,, ,, ,, 20 ,, ,, + ,,
,, ,, ,, 7 ,, ,, doubtful (±) results.
,, serum ,, 1 patient ,, no flocculation.

Test No. 3. (8 days after operation.)
The serum of 1 patient produced + flocculation.
The sera of 4 patients produced doubtful (±) results.

Test No. 4. (15 days after operation.)
There were no positive results.

These figures show that there was evidence of liver impairment, as measured by the cephalin cholesterol flocculation test, in 34 out of 35 patients when the test was applied 24 hours after operation. This was transient, as shown by the results on the eighth and fifteenth days. In no case was there any clinical evidence of liver damage.

The average duration of anæsthesia, where a ++ result was encountered, was 39 minutes, which is just below the average for the series as a whole. In the case of the doubtful and negative results it was 34 minutes. The 3 cases anæsthetised for the longest periods all returned + results; the 3 cases anæsthetised for the shortest periods returned + results likewise. No correlation can therefore be demonstrated between duration of anæsthesia and degree of hepatic impairment. The results show no evidence of the delayed onset of hepatitis described by Roholm (1933).

Table II. Twenty patients comprised the series subjected to di-ethyl ether anæsthesia. The ages of the patients ranged from 18 to 34 years, the duration of anæsthesia from 20 to 70 minutes, with

an average duration of 40 minutes. The 2 series are therefore strictly comparable except in respect of numbers.

The pre-operative cephalin cholesterol flocculation test was negative in all cases.

TEST NO. 2. (24 hours after operation.)

The serum of	1 patient	produced	++++	flocculation.
,, sera	,, 7 patients	,,	+++	,,
,, ,,	,, 6 ,,	,,	++	,,
,, ,,	,, 6 ,,	,,	+	,,

There were no negative results.

TEST NO. 3. (8 days after operation.)

The sera of 4 patients produced + flocculation.

The serum of 1 patient produced a doubtful (±) result.

TEST NO. 4. (15 days after operation.)

There were no positive results.

These figures show that there was evidence of liver impairment, as measured by the cephalin cholesterol flocculation test, in all patients in the series, 24 hours after operation. This was transient, as witness the results on the eighth and fifteenth days. In no case was there any clinical evidence of liver damage. There is no correlation between duration of anæsthesia and degree of hepatic impairment, on application of the criteria used in the case of trichlorethylene.

These results following di-ethyl ether agree with those of Borgstrom (1943), who noted a distinct impairment of liver function, often lasting more than 24 hours. Information is not available regarding the tests used in the investigation on which the observation was made. Rosenthal and Bourne (1928) observed a definite depression of hepatic efficiency ascribable to ether anæsthesia, detectable by the bromsulphthalein test immediately after operation but not 24 hours later. This discrepancy between the results obtained by these workers and the results set out above is probably due to a difference in the sensitivity of the tests used, the cephalin cholesterol flocculation test being much the more sensitive of the two procedures. The work of Kopp and Solomon (1943) amply demonstrated this fact. (Section II.)

The difference between the results obtained with the two tests lies in the length of time in the post-anæsthetic period during which hepatic impairment could be demonstrated. Both indicate that this does follow di-ethyl ether anæsthesia, and this agreement confirms that the primary purpose of this investigation, namely the assessment of the effect on the liver of trichlorethylene anæsthesia, was based on a sound method.

Comparing results in the two tables, it is evident that the degree of impairment caused by trichlorethylene is less than that caused by di-ethyl ether. This is a fact of some importance to the anæsthetist in making his choice of anæsthetic for a given case, but it must be appreciated that this is in no sense an index of the absolute toxicities of the two drugs, because these clearly depend on the sum of the effects observed on all organs and systems.

The work of Rosenthal and Bourne, taken in conjunction with the present results, shows that chloroform, di-ethyl ether, and trichlorethylene should be placed in that order of descending hepato-toxicity. Rosenthal and Bourne estimated the toxicity to the liver of ethylene as being less than that of ether, but no evidence is available to show how ethylene compares with trichlorethylene in this respect. Valuable knowledge, however, lies in the fact that both drugs are less toxic to the liver than is di-ethyl ether.

The cephalin cholesterol flocculation test is not a sufficiently quantitative procedure for any more exact statement to be made than that trichlorethylene is less toxic to the liver than di-ethyl ether.

A clue as to why this should be the relationship of the two drugs in this particular, is to be found in the results of investigations of sugar metabolism. Hadfield and Hewer (1941) reported no significant alteration in blood sugar levels when estimations were made before, during and after trichlorethylene anæsthesia, and commented on the contrast between this finding and the constantly raised blood sugar levels which occur when chloroform and di-ethyl ether are administered. Cantarow and Gehret (1931) observed this hyperglycæmia during ether anæsthesia, and advanced the view that it is due to increased hepatic glycogenolysis, caused either by a direct action of ether or by the increased plasma hydrogen ion concentration associated with ether anæsthesia. Pancreatic depression, in their view plays no part in this hyperglycæmia. Adriani (1942) described the liver glycogen as falling by 50% during the first hour of di-ethyl ether anæsthesia.

Davis and Whipple (1919), Ravdin (1929) and Goldschmidt *et al* (1937) have demonstrated that the capacity of the liver to withstand injury by toxic agents is dependent to some extent on its glycogen content, and it seems very probable that the maintenance of this protective store under trichlorethylene anæsthesia, and its dispersal under di-ethyl ether anæsthesia, account for the difference in the hepato-toxic effects of the two componnds

VII.

Summary and Conclusions.

(1) Trichlorethylene was administered as an inhalation anæsthetic to 35 patients, and di-ethyl ether to 20 patients selected for their general physical fitness. The technique employed was such as to exclude, as far as possible, extraneous factors capable of causing liver damage. Maintenance, in all cases, was in the first plane of surgical anæsthesia.

(2) The cephalin cholesterol flocculation test before operation was negative in all patients. Twenty four hours after operation the test returned positive results in 27, and doubtful results in 7 of the patients given trichlorethylene. There was one negative result. The test was positive 24 hours after operation in all patients given di-ethyl ether, flocculation occurring to a greater degree than it did with trichlorethylene. All positive and doubtful results became negative early in convalescence.

(3) Hepatic impairment is caused by trichlorethylene anæsthesia in the majority of patients subjected to it. This is slight in degree, and is less than that due to di-ethyl ether. In the case of both drugs the impairment is transient.

(4) Knowledge of the relative hepato-toxicities of trichlorethylene and di-ethyl ether is a valuable addition to the criteria on which the choice of anæsthetic for a given case is made.

(5) It is probable that the greater toxicity exhibited by di-ethyl ether is due to the hepatic glycogenolysis which has been shown to occur during anæsthesia with that drug.

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APPENDIX

Rosenberg (1941) advocated the use of the cephalin cholesterol flocculation test as a routine method for the detection of subclinical liver lesions.

Experience with the test in this work concerning post-anæsthetic patients leads to agreement that it could be so applied, providing certain conditions were fulfilled.

The first of these is that there should be continuity in its application. This could be the case in any large hospital in which comprehensive investigation of patients is constantly being carried out, and the reason why this point is stressed is that the estimation of degrees of flocculation is a matter in which practice is essential to accuracy, and in which the skill of the observer increases with experience. In this, the test falls into line with the Kahn and Wassermann tests which are invariably applied to batches of sera by technicians skilled in these procedures.

The second condition is that a stabilised, standardised cephalin should be made available. There is a commercial preparation of cephalin on the market, produced by the Difco Laboratories, Detroit, and attempts were made, without success, to obtain some of this from Messrs. Baird and Tatlock, London, and from the makers via the United States Army Medical Corps, for comparison with the samples extracted here. It is not known, therefore, whether the Difco product has the desirable features mentioned, but the preparation of such cephalin should be within the scope of a commercial laboratory of repute.

The third feature which might with advantage be added, is that the test should be made quantitative. In this connection it will be recalled that trichlorethylene and di-ethyl ether could be compared with reference to hepato-toxicity, only in terms of "less" and "more". Bruger (1943) claimed to have rendered the test quantitative by a method involving successive dilutions of the sera to be tested. Mirsky and von Brecht (1943) could not confirm this, and a small series of tests carried out here also failed to demonstrate any merit in the procedure. It does seem possible that an advance in this direction might be made if a series of cephalin preparations standardised to, (and stabilised at) different degrees of sensitivity could be produced, for the reason that, using a highly

sensitive cephalin, maximum flocculation results from comparatively minor liver lesions. Any more gross lesion can cause no more strongly positive reading, and, above a certain "threshold" level of liver damage, the test ceases even to be comparative, let alone accurately quantitative.

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