Summary

A total of 267 hospital in-patients were examined for splinter haemorrhages. These were present in 51 (19.1%).

They were found to occur more often in those patients whose occupations or activities exposed their hands to frequent trauma. They were also found more often in those patients who had recently been admitted to hospital, their incidence tending to decrease with increasing length of duration in hospital.

It is suggested that they may be traumatic in origin.

Other details which were recorded—namely, age, sex, temperature, blood-pressure, capillary fragility, and haematuria—were apparently irrelevant.

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RELATION BETWEEN CIRRHOSIS AND TRACE METAL CONTENT OF LIVER

WITH SPECIAL REFERENCE TO PRIMARY BILIARY CIRRHOSIS AND COPPER*

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The aetiology of cirrhosis of the liver is often obscure. In about 34% of cases in this country no factor can be incriminated, and these are designated as idiopathic or cryptogenic. Others we refer to by certain labels, but that does not necessarily mean that we understand what the cause is or how it produces its effect. An example of this type of obscurity is the so-called primary biliary cirrhosis. Cameron and Hou (1962) state that "we may as well frankly admit that we have little or no idea of the causation of primary biliary cirrhosis."

The knowledge that heavy metals could in some circumstances give rise to pathological changes within the liver suggested that the trace metal content of cirrhotic livers should be studied. In our own series of 450 cases we have encountered arsenical cirrhosis in 7 (1.6%), haemochromatosis (iron) in 7, Wilson's disease (copper) in 2 (0.44%), and gold cirrhosis in 2. This experience encouraged us to think that we might discover similar but more obscure evidence of some such cause for other types of cirrhosis, and perhaps a factor linking cirrhosis with primary carcinoma of the liver.

Methods of Analysis

Substantial samples of about 1 g. or more were obtained from a representative area of the liver, at operation or at necropsy, from patients suffering from a variety of pathological conditions including cirrhosis. "Normal" liver samples were taken from cases of sudden accidental death.

The concentrations of iron, zinc, cobalt, copper, and gold in liver were determined by neutron activation analysis. The procedures, by which all five elements may be determined in less than 0.5 g. of liver, are described in detail elsewhere (Parr and Taylor, 1963). The principle of this extremely sensitive method of analysis is as follows.

Weighed samples (about 0.1 g.) of the dried tissue, together with standards consisting of weighed amounts of the pure elements under study, are rendered radioactive by bombardment with neutrons in a nuclear reactor. The desired elements are then separated chemically and their radioactivity is identified and determined with a γ-ray scintillation spectrometer. The content of the element in the tissue is then calculated from the relative amounts of radioactivity in the samples and standard. With these particular techniques the overall error is less than 5%.

In order to reduce the risk of spurious results it is necessary to take great care to prevent the contamination of the sample prior to activation. Immediately after removal the tissue specimens are placed in clean polythene envelopes, sealed, and frozen until required for analysis. During subsequent processing prior to activation the specimens are handled with plastic forceps, and glass knives are used for cutting off outer surfaces of the sample. The samples are then dried and ground up in plastic vessels.

Blood samples are collected through platinum-iridium needles into specially cleaned syringes.

In a few cases it has been necessary to analyse fixed specimens, and in these instances samples of the preservative have also been analysed.

Results and Discussion

Iron

The concentration of iron in the liver of normal subjects and that of patients suffering from a variety of diseases is shown in Table I. The range of values observed is very wide, and, except for the single case of haemochromatosis in which the iron concentration is almost 1%,

TABLE I.—*Concentration of Iron in Human Liver*

Diagnosis	No. of Patients	Iron Concentration (μg. g. Wet Weight)		
		Mean	S.D.	Range
Normal adult	6	183	±86	42–252
Cirrhosis of liver (excluding cancer)	18	282	±360	39–1,570
Cancer (all sites)	40	409	±326	33–1,820
Cardiovascular disorders	10	249	±224	34–816
Haemochromatosis	1	9,100	—	—
Other disorders	13	262	±135	48–515

*Presented at the meeting of the British Gastro-Enterological Society in November, 1962.

there is no significant difference between the various disease states. The iron concentration in the liver may be raised to high levels as a result of blood transfusions, and this may well account for the wide spread of the results.

Zinc

The results listed in Table II show that in cirrhotic subjects the mean zinc concentration in the liver (49 μg./g.) is significantly less ($P<0.05$) than the normal level of 67 μg./g. A similar, equally significant reduction in zinc concentration was found in cases of primary carcinoma of the liver. Significantly increased levels of zinc were found in four cases of carcinoma of the stomach ($P<0.01$) and in five cases of leukaemia ($P<0.05$).

TABLE II.—*Zinc Concentration in Human Liver*

Diagnosis	No. of Patients	Zinc Concentration (μg. g. Wet Weight)		
		Mean	S.D.	Range
Normal adult	6	67	±20	44–92
Cirrhosis of liver	19	49	±15	24–82
Cancer (all sites)	44	88	±30	29–185
Carcinoma of stomach	4	136	±39	90–185
Carcinoma of liver	5	40	±10	29–51
Leukaemia	5	108	±31	67–149
Cardiovascular disorders	14	86	±37	39–182
Other disorders	14	94	±52	30–225

Reduced concentration of zinc in the liver of cirrhotic patients has also been reported by Vallee *et al.* (1959). The import of these abnormal levels is not at present understood.

Cobalt

The concentration of cobalt in the liver of normal subjects and in various disease states is shown in Fig. 1. Significantly reduced levels of cobalt were found in the livers of subjects suffering from a number of serious diseases, including cirrhosis ($P<0.01$), cancer ($P<0.01$), and cardiovascular disorders ($P<0.02$), but the meaning of these reduced levels has not been elucidated.

A few exceptionally high values for liver cobalt have been observed, generally more than four standard deviations from the mean value for the particular disease state. These abnormally high values have not yet been explained, but it is possible that they may have resulted from the administration of vitamin B_{12}, which contains 4% of cobalt.

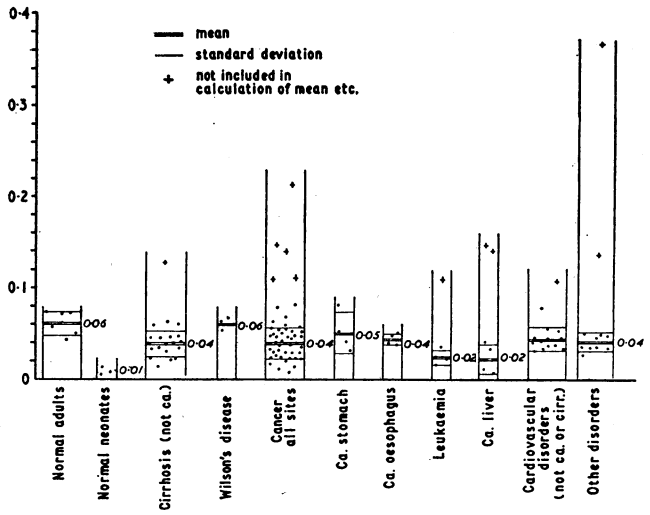

FIG. 1.—Concentration of cobalt in human liver (μg./g. fresh tissue) in various disease states. The amounts given indicate the mean concentration for each group.

Copper

Fig. 2 illustrates the copper concentration in the liver of normal subjects and of patients suffering from cirrhosis and other diseases. The most striking observation is that the mean copper concentration in the liver of the six patients suffering from primary biliary cirrhosis (220 μg./g.) is about 30 times greater than normal. In other types of cirrhosis levels ranging from one to eight times the normal mean have been observed. In three specimens of Wilson's disease studied the mean copper level was 128 μg./g. or about 18 times normal.

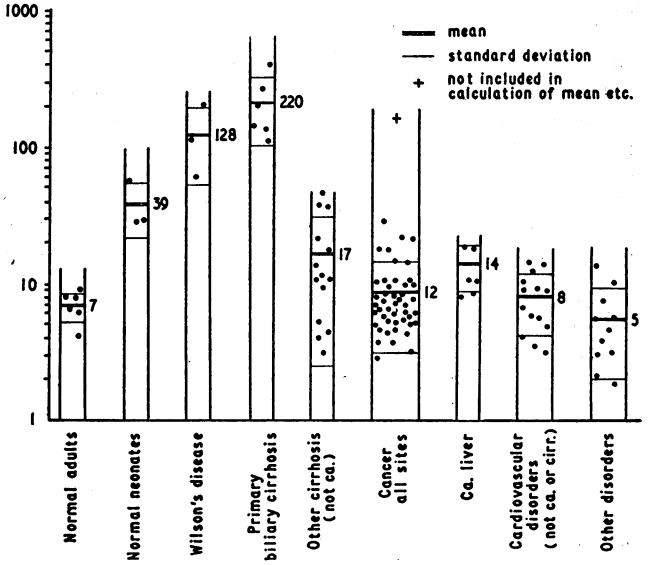

FIG. 2.—Concentration of copper in human liver (μg./g. fresh tissue) in various disease states. Concentrations are shown on a logarithmic scale; the amounts given indicate the mean concentration for each group.

This astonishing elevation in the copper concentration in the liver in primary biliary cirrhosis could be due to four possible causes: (1) increased absorption of copper from the gastro-intestinal tract, (2) obstruction of the biliary passages with retention of copper, (3) failure to excrete copper, and (4) some other disturbance of copper metabolism resulting in excessive deposition of copper in the liver. If it is assumed that these subjects have the normal dietary intake of 3 mg. of copper per day (Recommendations of the International Commission on Radiological Protection, 1959), of which between 5 and 30% is absorbed from the gastro-intestinal tract (Underwood, 1956), it can be calculated that if *all* the absorbed copper is retained in the liver it could take up to 13 years for the copper levels in the liver to rise to the concentrations observed. However, if the absorption of copper is increased, as in Wilson's disease, then these levels would be attained more rapidly.

Copper is normally excreted through the bile, and only small amounts appear in the urine. If retention of bile also results in retention of copper it might be expected that secondary biliary cirrhosis, the end-result of long-standing bile-duct obstruction, and obstructive jaundice due to other causes would also lead to retention of copper in the liver. However, in one case of secondary biliary cirrhosis of very long standing the copper concentration in the liver at death was 10.7 μg./g. and in a second case of long standing, in which the obstruction had been removed, the copper concentration was within normal limits. In a patient with obstructive jaundice due to carcinoma of the pancreas the copper concentration in the liver was only

3.2 μg./g. In these three patients bile was being reabsorbed from the bile-ducts. In primary biliary cirrhosis, on the other hand, the interference with bile flow is more proximal and reabsorption probably amounts to little. The aetiological factors, therefore, are probably different and not so simple as in secondary biliary cirrhosis. Other workers have made similar observations. Gubler *et al.* (1957) suggest "that in patients with cirrhosis and associated cholangitis the excretion of copper in the bile is impaired, and this results in retention of element in the liver." However, the mean copper concentration in the liver of their patients was only 9.4 μg./g., with a range of 3.4 to 29.7 μg./g. They also mention a very high level of copper in the liver of one patient with primary biliary cirrhosis and xanthomatosis, but give no details.

Bush *et al.* (1955) reported increased urinary excretion of copper in alcoholic cirrhosis, and in our two cases of primary biliary cirrhosis, referred to below, the urinary copper excretion, over 200 μg./day, is much in excess of normal limits (Underwood, 1956). These observations suggest that the interference with the biliary excretion of copper is partially compensated for by increased urinary output. Mahoney *et al.* (1955) have shown that when the common bile-duct is tied in dogs and pigs there is increased excretion of copper in the urine and small intestine but copper levels in the plasma are unchanged.

We have measured serum copper levels in nine cirrhotics and six normal subjects. The mean concentration in the cirrhotics was 1.56 ± 0.62 μg./g. as compared with 0.82 ± 0.15 μg./g. for normal males and 0.98 ± 0.11 μg./g. for normal females. Included in the cirrhotic group were two cases of primary biliary cirrhosis whose serum copper levels were 1.65 and 2.55 μg./g. respectively. Two cases of secondary biliary cirrhosis had serum copper levels of 1.23 and 1.65 μg./g. These raised serum copper levels in biliary cirrhosis are in marked contrast to the hypocupraemia seen in Wilson's disease (Cumings, 1962).

If it is indeed the presence of excess copper in the liver which causes the cirrhosis in Wilson's disease, it is logical to assume that it is also the cause in primary biliary cirrhosis. Treatment with a chelating agent to increase the rate of elimination of copper may therefore produce clinical improvement similar to that observed by Cumings (1951, 1962) and by Walshe (1956) in cases of Wilson's disease.

We have tried out the effect of penicillamine on the excretion of copper in two cases of proved primary biliary cirrhosis, both in an advanced stage of the disease. The results are shown in Table III. In neither case was an improvement in the clinical course anticipated and both patients have since died. The results, however, justify a more prolonged trial of the method in patients in whom the damage done to the liver is not irretrievable. Before beginning treatment the diagnosis should be proved histologically and by determination of the copper concentration. The activation analysis method is sensitive enough to allow the estimation to be made on fragments removed by needle biopsy.

TABLE III.—*Effect of Treatment with Penicillamine on the Urinary Excretion of Copper in Primary Biliary Cirrhosis*

Case 1	
Total copper output in 5 days:	
Without treatment	1,076 μg. (215 μg./day)
With 900 μg. penicillamine per day	1,848 μg. (370 ,,)
Total increase in copper output, 772 μg.	
Case 2	
Total copper output in 2 days:	
Without treatment	538 μg. (269 ,,)
With 900 μg. penicillamine per day	2,160 μg. (1,080 ,,)
Total increase in copper output, 1,622 μg.	

Gold

Thirty-two samples of liver from normal subjects were analysed in order to obtain the value for gold in the normal liver. The median value was found to be 0.057 mμg./g., with a range of from 0.013 to 0.79.

We have encountered two patients who have been treated therapeutically with gold salts and whose livers we have analysed.

The first was treated for an arthritic condition in 1939, 1940, and 1941, when he was 41 to 43 years of age. (He had a total of 1 g. of aurothioglucose ("solganal B") in 1939, 1 g. of sodium aurothiomalate ("myocrisin") in 1940, and 1.88 g. of sodium aurothiomalate in 1941). He received altogether about 1.8 g. of metallic gold. He was under treatment for portal hypertension for two years before he died in 1954, the cause of death being primary carcinoma of the liver, with cirrhosis. In the cirrhotic part of the liver the gold content was 200 mμg./g., which is about 3,500 times the normal. The carcinoma contained no gold. The whole of the liver contained 0.0116 g. of gold, which is about 0.7% of the total gold administered many years previously.

The second was a man aged 53 when he received treatment in 1943. He was given sodium aurothiomalate to the total of 0.11 g. of gold, one-sixteenth of that administered to the first patient. A biopsy of the liver at the time of operation in 1960, 17 years later, showed a gold content of 12 mμg./g. This is about 210 times the normal, also one-sixteenth of the content in the former case. A complicating factor in the second case was that antimony was also discovered, in a concentration of about 2.5 μg./g. The significance of this discovery is difficult to interpret because we found antimony in no other specimen. It is possible, however, that it was the cause of his cirrhosis. The source of the antimony was difficult to ascertain, the only possible origin being that he had been engaged in machining alloys containing this element when he had been a young man. (The atmosphere had been dusty and he remembered inhaling considerable quantities of dust.) The patient is at present in good health.

Arsenic

All seven cases of arsenical cirrhosis encountered had gross skin changes, the keratosis punctata or the raindrop pigmentation, characteristic of chronic arsenicalism. All gave a clear history of the ingestion of inorganic arsenic, in the form of liquor arsenicalis or Fowler's solution, as a tonic, for epilepsy, or for various skin disorders.

In only one case, that of a man who died of carcinoma of the lung with multiple metastases, has the liver been analysed for arsenic. In this instance the liver contained 0.11 μg. of arsenic per g. in the cirrhotic tissue and 0.09 μg./g. in the cancerous liver tissue.

Two of the seven patients died of carcinoma of the bronchus, two others received or are receiving treatment for carcinoma of the skin, one has been lost sight of, one died of septicaemia, and one is well. Arsenic is one of the recognized causes of cirrhosis of the liver and induces cancer in other parts of the body (Buchanan, 1962). Arising out of this observation it was one of our original intentions to see if we could possibly track down a relation between traces of metals, cirrhosis, and cancer. Any conclusions in respect of cancer will have to depend on a more extensive study than we have been able to carry out so far.

Summary and Conclusions

The *iron* content of the liver of a cirrhotic patient suffering from haemochromatosis was shown to be 50 times the mean value—9,100 compared with 183 μg./g. In general, iron concentrations showed a wide spread, but no significant variation from disease to disease.

The liver content of *zinc* appears to be reduced in cirrhosis and carcinoma of the liver. Similarly with *cobalt*, but there are gross irregularities which may be due to the administration of substances such as vitamin B_{12} which contain cobalt.

With *copper*, the most significant result of our investigations has been the discovery that in primary biliary cirrhosis the mean copper content of the liver is 30 times normal—220 μg./g. as compared with 6.9 μg./g. In Wilson's disease the mean copper content is 128 μg./g. A preliminary test suggests that urinary copper excretion in primary biliary cirrhosis is markedly increased when penicillamine is administered. Interference with the excretion of copper by an obstruction to the bile-ducts does not appear to be the cause of the copper retention.

In two cases of cirrhosis in which *gold* had been administered therapeutically many years previously, the gold content of the livers, normally about 0.057 mμg./g., was found to be 3,500 and 210 times this quantity. The first patient died of primary cancer of the liver.

Arsenic is another heavy metal known to induce cirrhosis. Four out of seven arsenical cirrhotics have developed cancer of the lung or skin.

These findings, so far fragmentary, suggest that the role of minute quantities of heavy metals in the aetiology of certain types of cirrhosis may be appreciable. Their part in the causation of cancer of the liver remains to be investigated.

We are grateful to the physicians and surgeons of St. Bartholomew's Hospital and the Royal Marsden Hospital who have allowed us to use material from their patients, and to others, such as Dr. J. M. Walshe and Dr. E. M. Darmady, who have sent us pieces of liver; to Dr. F. Lees, who estimated the urinary copper excretion; and to Mr. M. A. Lambert and Mr. P. Ambrose, attendants in the post-mortem departments of St. Bartholomew's and the Royal Marsden Hospitals, and Mr. F. Lawrence, Officer at the Coroner's Court, Golden Lane, for their helpful and willing co-operation in the collection of specimens from all types of cases. Professor A. Wormall, F.R.S., and Professor W. V. Mayneord originally suggested analysis by activation, and Dr. R. A. Allen carried out the early tests for gold and arsenic.

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HEPATOMA IN CIRRHOSIS

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Primary carcinoma of the liver has attracted little attention in Great Britain although extensive studies have been made in tropical countries and in the U.S.A. Stewart (1931), in a review from Leeds of precarcinomatous lesions of the alimentary tract, found only 14 cases of primary liver carcinoma in a series of 12,800 necropsies over a period of 22 years (1910–31). However, Parker (1957) and reviewers in the U.S.A. (Macdonald, 1956; Benner and Labby, 1961) have suggested a higher incidence. Experience during the past two years has supported this view and has led to an examination of records for 23 years at St. Thomas's Hospital. The results of the present series have been compared with reports from Europe and the U.S.A. Comparison with studies from other countries has not been attempted.

Material and Method

The case records and necropsy reports of primary liver carcinoma for the years 1940–62 have been studied. There were 39 cases of carcinoma (35 male, 4 female), 10 of which had been personally observed during life, and 157 cases of cirrhosis (115 male, 42 female). These figures do not reflect the experience of a unit with a special interest in liver disease. In each case the diagnosis has been confirmed by necropsy and subsequent histological review by one of us (H. S.). The investigation has been restricted to malignant hepatoma and cholangioma, excluding carcinoma of the hilar bile-ducts, and a small number of rare tumours (malignant haemangioma, sarcoma, hepatoblastoma). Cirrhosis has been accepted only where nodular regeneration with diffuse fibrosis or post-necrotic scarring was present (Fifth Pan-American Congress of Gastroenterology, 1956). Primary biliary cirrhosis, fibrosis due to chronic biliary obstruction, and cardiac cirrhosis have been excluded. A further nine cases of primary carcinoma diagnosed in life through biopsy have been omitted since necropsy was not performed.

Results

Data on the incidence of primary carcinoma of the liver and cirrhosis are presented in Tables I to V. Results from other studies are also included in Tables I and II.

Clinical Features.—The detection of malignant change in cirrhosis is notoriously difficult, and experience in this series has not brought to light any special features diag-

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