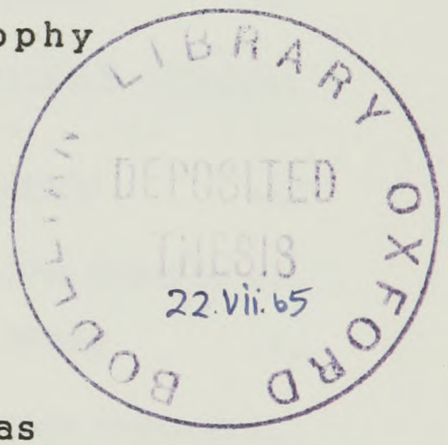
STUDIES ON IRON ABSORPTION IN DISEASES OF THE LIVER

A Thesis
submitted for the Degree
of
Doctor of Philosophy



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Chapter 1

INTRODUCTION

Iron is present in most living organisms in minute amounts and its biological role is of considerable importance. Mammalian life could not be continued without the iron-containing compounds which carry oxygen and are concerned with oxidation processes in the tissues. The way in which iron enters the body is as yet incompletely understood, but it is well recognised that excessive absorption of this normal component of the diet may lead to disease and even death.

The association of an increased amount of iron in the body with morbid conditions was first indicated by Trousseau (1865) and later Trossier (1871). Hanot and Chauffard (1882) gave the first full descriptions of what we now know as primary haemochromatosis to cirrhosis of the liver, diabetes and pigmentation, and used the term diabète bronzé. Von Recklinghausen (1889) introduced the term haemochromatosis, and the development of the condition as a clinical entity was reviewed by Sheldon (1935) in his classical monograph.

In recent years primary haemochromatosis has been considered as only one of a larger group of iron-storage diseases which have been reviewed by Finch and Finch (1955). There is some confusion in terminology because some authors have referred to any kind of iron overload as haemochromatosis; hence the necessity to refer to the

familial inherited type as primary haemochromatosis. Other iron-storage diseases are best referred to as secondary haemochromatosis or haemosiderosis. Among the latter group may be included patients with alcoholism or idiopathic cirrhosis who develop iron overload. This condition, though recognised many years ago, has only recently attracted attention.

Von Recklinghausen (1889) described deposits of iron in the liver, spleen and pancreas in patients who had various forms of anaemia, tuberculosis and carcinoma of the stomach. A noteworthy feature was the presence of cirrhosis of the liver in all these patients.

In 1895 Richardière described a patient with pigmented cirrhosis without diabetes; but this patient may have been in the early stages of classical haemochromatosis. In 1897 Letulle described three patients with cirrhosis and pigmentation but without diabetes. One of these, a 58-year-old man, had evidence of portal hypertension, had iron present in the liver, but had little pancreatic damage and was most probably a true instance of cirrhosis with haemosiderosis.

Villaret, Justin-Besançon and Even (1932) described 12 patients with pigmented cirrhosis in whom five had diabetes, but in whom there were a number of distinguishing features from classical haemochromatosis.

Their finding of all degrees of haemosiderosis in association with portal cirrhosis has been confirmed and extended by several other groups of workers (Herbut and Tamaki, 1946; Finch and Finch, 1955; Bell, 1955; MacDonald and Mallory, 1960).

The apparent overlap between pigmented cirrhosis and haemochromatosis both with regard to clinical features and histological findings has prompted MacDonald and Mallory to regard these conditions as part of a spectrum of disease in which classical haemochromatosis is one extreme. They reject Sheldon's hypothesis that haemochromatosis is an inborn error of metabolism, stating that the infrequency of familial occurrence in Sheldon's review and that of Finch and Finch deny a genetic cause. They emphasise alcoholism as an aetiological factor.

Caroli and his associates have, however, drawn a very clear distinction on pathological grounds between primary haemochromatosis and cirrhosis with haemosiderosis (Caroli and Etévé, 1954; Caroli and André, 1964). They point out that in the former the iron deposition precedes the cirrhotic changes, whereas in the latter the cirrhosis antedates heavy iron deposition.

Furthermore, in countries with a low incidence of alcoholism haemochromatosis is apparent. This finding, together with the family studies of Williams, Scheuer and Sherlock (1962), supports

Sheldon's concept of haemochromatosis being an inborn error of metabolism.

The questions which naturally arise in all states of iron overload are where does the iron come from and how does it get into the body? In transfusion haemosiderosis the iron overload is clearly iatrogenic, the body being unable to excrete the iron derived from the breakdown of transfused red cells. In primary or secondary haemochromatosis the picture is far less clear. The excessive amounts of iron present in these patients must come from iron absorbed from the intestine.

Before the introduction of radioactive isotopes of iron the technical difficulties in measuring absorption of iron from the gut were formidable. Robscheit-Robbins gave the current views on the absorption of iron in a review in 1929 in which she stated:

> "Our present ideas as to iron absorption and excretion may be said to be as follows. Varying portions of iron are absorbed. The site of absorption is the entire intestinal tract although the duodenum and a portion of the upper small intestine probably play a major role.... Elimination is almost entirely by the intestine especially the colon, very slight amounts are found in the urine and the bile."

The introduction of chemical balance studies was soon to throw doubt on the role played by excretion in the metabolism of iron. Basset, Killip and McCann (1931) showed that a patient with polycythaemia rubra vera who was being treated with acetylphenylhydrazine experienced during administration of the haemolytic agent a fall in total blood volume, red-cell volume and haemoglobin. This considerable increase in endogenous iron metabolism did not result in an increased faecal excretion of iron and the amount of iron that appeared in the urine could be accounted for by the haemoglobinuria.

This finding was confirmed in a polycythaemic woman studied by McCance and Widdowson (1937). They clearly showed that the implication of these findings was that iron is not excreted in any large amounts by the body and hence the regulation of the total iron content of the body cannot be achieved by regulation of excretion. Iron, once it has been absorbed, must be stored within the body and McCance and Widdowson therefore postulated that the onus for regulation must reside with the intestinal mucosa.

In the next few years a number of careful balance studies using chemical methods were done. Josephs (1939) and Leverton and Roberts (1937) studied children and adults respectively. It is interesting to note that a small positive balance greater per kilogram of body weight in children than in adults was found.

In 1939 isotopic iron was introduced and an investigation of McCance's hypothesis was rendered easier. Hahn, Bale, Ross, Balfour and Whipple (1943) studied three anaemic dogs. When ^{59}Fe-labelled iron was given 28 per cent. of the 14-mg. dose appeared in the blood. If the labelled iron was preceded by 100 mg. of inorganic iron, only 4 to 8 per cent. of the dose could be accounted for in the blood. They interpreted this result to mean that the absorption of the tracer dose was being impaired by the iron already given, and suggested that if iron was present in the mucosal cells then any further iron received later into the intestinal lumen could not gain entrance, i.e., a mucosal 'block' had occurred.

Histological supports for the hypothesis came from the work of Granick (1946). He studied scrapings from lengths of duodenum obtained from guinea pigs and was able to demonstrate crystallised ferritin. A qualitative difference existed between the animals previously given iron and the control animals. The formation of ferritin from apoferritin was presumed to be responsible for prevention of further iron absorption.

Although there is no doubt that ferritin does play an important part in iron absorption, the importance of the mucosal cell as the prime regulator of absorption is not so certain. Wohler, Heilmeyer, Emrich and

Kang (1957) demonstrated that iron absorption in guinea pigs continued even when their mucosae were fully saturated with iron by prolonged dosage. Brown, Dubach and Moore (1958), in studies on human subjects, showed that a large dose of ferrous iron will produce a partial temporary block; nevertheless, some absorption occurs. As they point out, such large doses of iron are not physiological and it would be impossible for food iron to produce such an effect.

The introduction of the use of radioactive iron in research on iron metabolism has stimulated many investigators to look at other factors outside the mucosal cell which may influence the absorption of iron. These may be classified broadly into intraluminary factors, i.e., dietary or secretory factors within the gut, and changes in the internal metabolism of iron. Into the former group come, for example, the effect of low-phosphate or phytate diets, diets low in protein but with increased fat, all of which tend to enhance absorption, or high-phosphate and phytate diets which reduce absorption (Hegsted, Finch and Kinney, 1949; Moore and Dubach, 1951; Moore, 1955; Kaufman, Klavins and Kinney, 1958). Factors in the latter group are increased erythropoiesis or diminished iron stores which enhance absorption, and decreased erythropoiesis or increased iron stores which in general depress absorption (Bothwell, Pirzio-Biroli and

Finch, 1958; Pirzio-Biroli and Finch, 1960).

One of the most cogent arguments against the mucosal block theory is the occurrence of pathological iron overload such as occurs in primary haemochromatosis and in some cases of cirrhosis. It is clear that in these conditions the normal regulating mechanisms have broken down but little is known of the possible factors involved.

When the present investigation was started there was practically no information available on the absorption of iron in cirrhosis of the liver and only a very limited amount of data on primary haemochromatosis.

It was therefore planned to investigate the absorption of iron in acute and chronic liver disease and in classical haemochromatosis using radioactive iron as a tracer. This thesis is concerned with the results of these investigations together with some preliminary studies into the factors responsible for the increased iron absorption which was found in patients with primary haemochromatosis and cirrhosis of the liver.

Chapter 2

PATIENTS STUDIED WITH IRON ABSORPTION TESTS

The absorption of iron was studied in normal control subjects, patients with iron-deficiency anaemia, haemochromatosis, cirrhosis of the liver, acute hepatitis, transfusion siderosis and chronic alcoholism. The number of patients in each group is given in Table 1 below.

TABLE 1

Diagnosis	Number
Normal control subjects	61
Iron-deficiency anaemia	46
Haemochromatosis	8
Cirrhosis of the liver	17
Acute hepatitis	8
Transfusion siderosis	4
Chronic alcoholism	2

Normal Control Subjects and Patients with Iron-Deficiency Anaemia

Many observations on iron absorption in normal control subjects and patients with iron-deficiency anaemia were available from previous studies in the Department. The results of these studies are given in Appendix I. These results were taken in conjunction with those made in the present study. The normal control subjects consisted of medical staff, students and patients with no evidence of organic disease and who were known to be haematologically normal.

In the patients with iron deficiency the cause of the anaemia was varied and included menorrhagia and hiatus hernia. In patients with menorrhagia care was taken that if more than one absorption test was done it was carried out in a similar part of the menstrual cycle. In patients who had become anaemic from recurrent bleeding from hiatus hernia no studies were made until at least three weeks after bleeding had ceased. Patients with intestinal malabsorption were not included in the study. Confirmatory evidence of iron deficiency included an estimation of the haemoglobin, mean corpuscular haemoglobin concentration, a study of a stained blood film and a serum iron estimation. A prompt response to the administration of iron was also taken as evidence of iron deficiency.

Patients with Haemochromatosis

Eight patients with this diagnosis were under the care of the hospital at the time of these investigations. The clinical case histories are given in Appendix II. One new case was diagnosed during the period of the study. Two other patients were referred, one from Princess Mary's Hospital, R.A.F., Halton, and one from Stoke Mandeville Hospital.

The diagnosis was based on a family history of pigmentation, diabetes or liver disease and on the presence of the characteristic

changes on liver biopsy which was done on all the patients. Estimations of the serum iron-binding capacity and percentage saturation of the iron-binding capacity were made. A glucose tolerance test was done on any patient who was not frankly diabetic.

One patient (B.J.) was known to have had an increased saturation of his iron-binding capacity some years before the present investigation (Appendix II). He gave a history of recurrent gastro-intestinal bleeding and at the time of the study he was anaemic and had a low percentage saturation of his iron-binding capacity (Table 3).

Patient E.P. had been successfully treated by venesection (Appendix II). He was not anaemic but had a serum iron level at the lower end of the normal range and a decrease in the percentage saturation of the total iron-binding capacity (Table 3). Only minimal changes in the tests of liver function were noted in the haemochromatotic patients with the exception of R.F. (Table 4).

Cirrhosis of the Liver

Seventeen patients with cirrhosis of the liver were studied. Their clinical case histories are given in Appendix II. This condition is not common in the Oxford Region and opportunity was taken of studying every patient with this diagnosis who had been admitted to the Radcliffe Infirmary in the last few years and in addition patients

who were referred, particularly from Cheltenham Hospital and Kettering Hospital during the course of the study.

Six of the 17 patients had a background of alcoholism; in the remainder the aetiology was unknown. The diagnosis was made on clinical grounds aided by appropriate liver function tests, radiological demonstration of oesophageal varices and assessment of liver size and surface contour by pneumoperitoneum. Liver biopsy was undertaken only in those few cases in which the diagnosis remained in doubt.

Details of the haematological and biochemical findings in these patients are given in Tables 5 and 6. Most of the patients were ambulatory and well compensated at the time of study but three were ill. In two of these, H.B. and A.S., studies were done following recovery from an episode of encephalopathy provoked by an alcoholic bout. The third patient, E.R., was jaundiced and ill at the time of the absorption test. A further three patients, though feeling well, showed deterioration in their liver function tests with a raised plasma bilirubin, a fall in the serum albumin level below 3 g. per 100 ml. and a pseudocholinesterase level of less than 30 units. Dibucaine numbers obtained in these patients (but not reported) showed that the low pseudocholinesterase levels were not due to a congenital deficiency of the enzyme.

Five of the cirrhotic patients were anaemic with levels of haemoglobin below 11 g. per 100 ml. One patient was not anaemic but was sideropaenic with a level of serum iron of 60 µg. Three patients were found to have occult blood in their faeces on routine random testing. The remaining eight patients had either normal or increased levels of serum iron and some showed slight increase in the amount of iron in their bone marrow or liver biopsy (Table 14).

Patients with Acute Liver Disease

Most of the eight patients who were studied were under the care of the Department when the investigations were carried out. Their case histories are given in Appendix II. However a few patients who had been admitted to other wards agreed to co-operate. The diagnosis was made clinically and with the aid of liver function tests. Five patients probably had acute infectious hepatitis, the history of one strongly suggested serum hepatitis and in two the clinical history and result of the serological studies supported a diagnosis of lupoid hepatitis. The results of haematological and liver function tests are given in Tables 7 and 8.

Six of the eight patients were ill when first studied. One (G.P.) was recovering from her hepatitis and another (I.P.) was already receiving steroid hormones for her proven lupoid hepatitis.

Two of the eight patients (G.P. and J.T.) had iron-deficiency anaemia as a complication of their hepatitis.

Patients with Transfusion Siderosis

These four patients had received their transfusions for refractory anaemia (Appendix II). All had received more than 50 units of blood and showed either very high serum iron levels or saturation of their iron-binding capacity. Apart from the necessity for transfusion they were all in reasonably good health at the time of the studies. If they were anaemic on admission to hospital they were transfused to a level of haemoglobin between 10 and 11 g. before absorption studies were started. One patient (A.W.) showed a reticulocytosis in the peripheral blood and abnormal liver function tests at the time of the study (Table 9).

Patients with Chronic Alcoholism

A number of cirrhotic patients were also alcoholic and it was thought to be of interest to see how absorption of iron was affected in patients who were alcoholic but had minimal evidence of liver damage. The opportunity arose to study two such patients who gave a history of long-continued excessive intake of alcohol (Appendix II). They were well at the time of the studies and both were attempting to

reduce their intake of alcohol.

One (W.M.) had had a total gastrectomy for carcinoma of the stomach some 12 years previously (Table 10). There was no suggestion on clinical examination or barium meal of liver disease or portal hypertension nor of recurrence of the carcinoma. He was not anaemic; his liver function tests were normal and liver biopsy showed no fibrosis but fat vacuoles in the parenchyma. The second patient (L.J.) was quite well at the time of the study apart from oedema of the ankles, which was due to local circulatory disturbance. He was not anaemic. With the exception of a slightly raised bromsulphthalein retention his liver function tests were completely normal. His liver biopsy showed no abnormality (Table 10).

Chapter 3

METHODS

Methods of Studying Iron Absorption

In the present study the inorganic iron used in the tests was ferrous sulphate to which ascorbic acid had been added. The food iron was rabbit haemoglobin labelled with ^{59}Fe. All doses were adjusted to contain a standard amount of 5 mg. of iron.

To determine the absorption of either of these forms of iron the radioisotopic balance technique introduced by Dubach, Callender and Moore (1948) was employed. The principle of the method is simple in that, knowing the amount of activity administered and determining the activity appearing in the faeces, the amount of iron retained can be calculated and equated to the amount absorbed.

Preparation of Labelled Ferrous Sulphate and Labelled Haemoglobin for Use in the Absorption Studies

Labelled ferrous sulphate: Four doses of labelled ferrous sulphate and a standard were prepared at a time. 99.2 mg. of Analar grade ferrous sulphate ($FeSO_4 7H_2O$) containing 20 mg. of elemental iron was put into a 100-ml. graduated flask and 200 mg. of ascorbic acid added. ^{59}Fe-ferric citrate solution of high specific activity was added so as to give an activity of 3 to 5 μc. for each dose. The

graduated flask was filled with distilled water to about 2 ml. above the graduation mark. The solution was thoroughly shaken and 25-ml. portions were pipetted into four separate containers. The few ml. remaining were kept as a standard.

All the doses and the standard were kept in the -20° C. refrigerator until required. When they were required they were allowed to thaw gently. The standard was prepared by taking 1 ml. and making up to 200 ml. with water in a 500-ml. polythene container of similar shape to the cartons used for stool collection.

Labelled haemoglobin: About four to six doses of labelled rabbit haemoglobin together with a standard were prepared at a time. A rabbit was given intravenously 100 μc. of ^{59}Fe-labelled ferric citrate in sterile isotonic saline. On the fourth or fifth day following injection it was bled from an ear vein. A number of doses each containing 5 mg. of elemental iron and containing 3 to 5 μc. of activity were prepared as described by Callender, Mallett and Smith (1957).

In both preparations therefore equal amounts of elemental iron were given and the radioactivity of the preparations was comparable.

Administration of the Test Dose and the Collection of the Faeces

The subjects fasted overnight and were given the dose of radioactive iron in the morning. They were allowed nothing to eat or drink for a further two hours. The beaker used for administration and the dose container were preserved and the residue of the dose counted.

Patients were instructed to collect each stool in a 500-ml. capacity wax carton with a screw-on lid. Every stool carton was labelled by the patient with his or her name, the date on which the stool was passed and the number of the stool in sequence. This information was also noted by the patient on a special card given for this purpose. Stool specimens and the patient's card were checked to ensure that the collection was complete. Collection of the stools continued until less than 1 per cent. of the dose appeared in successive specimens.

The stool specimens were counted in a plastic phosphor well counter (Warner and Oliver, 1962) which was large enough to accommodate a whole carton and the total activity excreted found. The dose given was determined from the standard after deduction of the activity left in the beaker and container. Absorption was equated with the percentage of the dose which was not recovered from the faeces.

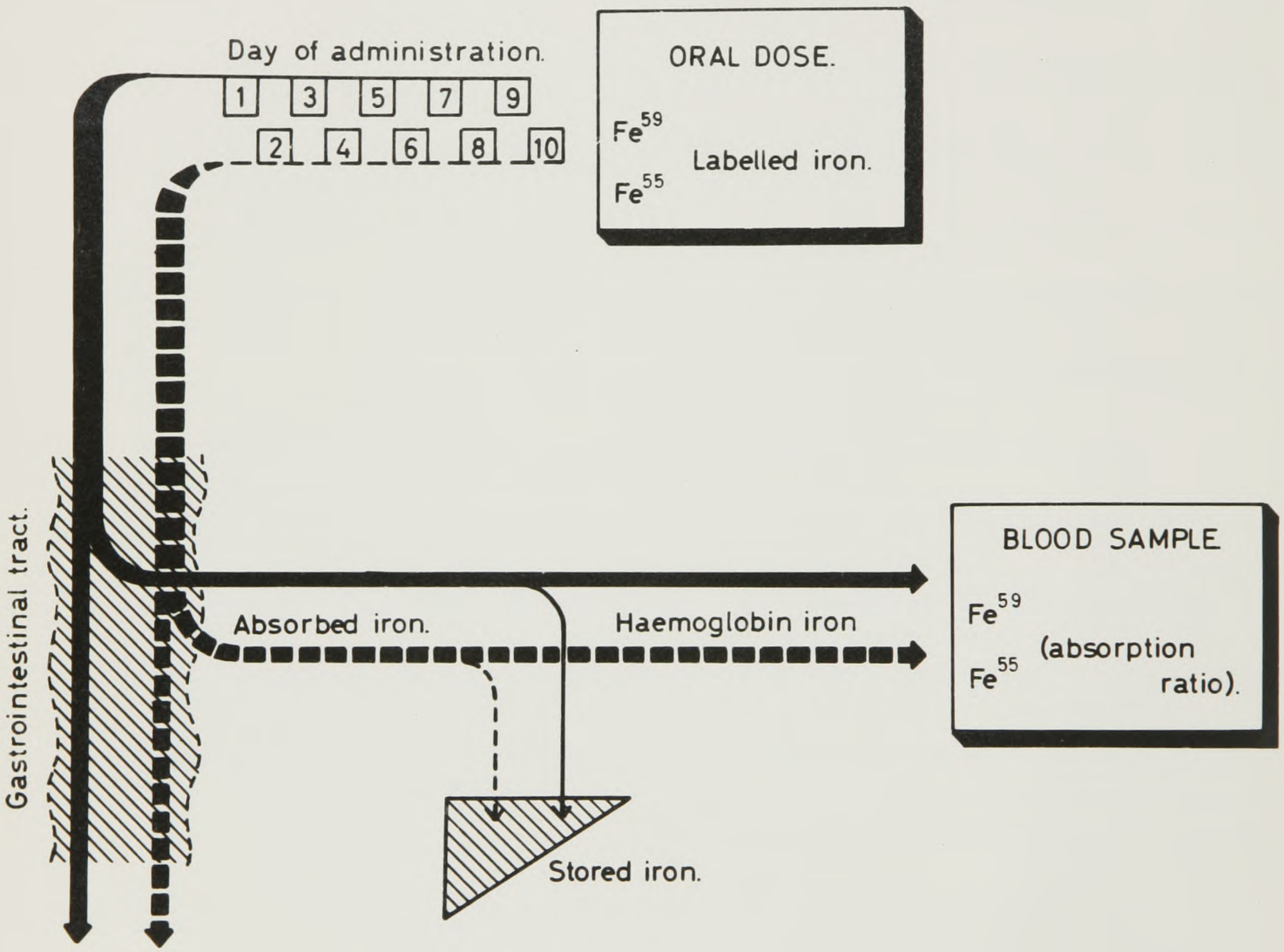
The red-cell utilisation of the radioactive iron which was used was determined on a blood sample taken at the 11th to the 15th day after the administration of the test dose. 20-ml. samples of blood were taken, haemolysed with saponin and counted on a scintillation counter (type 1186) employing a sodium iodide crystal (Oliver, 1953). Blood volumes were calculated from the patient's height and weight, using the tables of Nadler, Hidalgo and Bloch (1962). The total activity present in the blood was calculated and this was expressed as a percentage of the dose given.

The radioisotopic balance technique seemed ideally suited to the present study. The phosphor well counter allowed determination of the radioactivity of the faecal specimens to be made without homogenisation. There was also wide experience with the technique in the Department, not only with regard to the absorption of labelled ferrous sulphate but also labelled haemoglobin.

The chief criticism of the technique is the difficulty in assuring complete collection of the faeces. Any loss of the faecal specimens results in an apparent increase in absorption. When there was any doubt about the satisfactory completion of a faecal collection or when there was a discrepancy between the record of the collection kept by the patient and the actual number of specimens, the absorption test was repeated, or the results discarded.

Fig. 1:

Double isotope technique using Fe^{55} and Fe^{59}.



AFTER BRISE AND HALLBERG.

A disadvantage of the isotopic balance technique is that some three or four weeks are necessary to complete two absorption tests. During this time there may be an alteration in the patient's condition, which may affect his or her ability to absorb iron. This may happen either during the control test or when the test run in which the effect of a particular factor is being studied. For example, even the occurrence of a menstrual period during one of the absorption tests may influence absorption. The effect of this kind of factor can be eliminated in paired absorption studies by randomising the sequence in which the test substance is given and timing the successive collections so that they occur in a similar part of the menstrual cycle.

In an effort to overcome the disadvantage of sequential radioisotope balance studies, Brise and Hallberg (1962) have devised a method using the isotopes ^{59}Fe and ^{55}Fe to compare the effect of various conditions on the absorption of iron. One isotope is given under condition A on days 1, 3, 5, 7 and 9 and the other isotope under condition B on the even days (Figure 1). The ratio of the isotopes in the blood is measured two weeks later.

This method gives no absolute measure of absorption except perhaps in the iron-deficient subject when close to 100 per cent. of

the iron absorbed should be utilised for haemoglobin formation. Assuming that utilisation remains constant throughout the study, Brise and Hallberg's method gives comparative figures for the conditions being tested. It was used in four subjects in the present study (vide infra). The technique used was as follows:-

Comparative studies on iron absorption using two radioisotopes of iron after Brise and Hallberg (1962): The doses of ferrous sulphate were prepared in exactly the same way as for the radioisotope balance technique. ^{59}Fe or ^{55}Fe isotopic iron was added to give a level of activity of 3 to 5 μc. of ^{59}Fe and 25 μc. of ^{55}Fe.

The amount of ^{59}Fe and ^{55}Fe activity in blood taken 11 to 15 days after the last oral dose of iron was determined. At this time optimal utilisation of the absorbed dose for haemoglobin synthesis could be expected to have occurred. The ^{59}Fe and ^{55}Fe were counted on the same samples. Separation of the iron was done by wet digestion of whole blood using sulphuric and nitric acid. Iron was precipitated as ferric hydroxide. The precipitated ferric hydroxide was redissolved in a hydrochloric acid-ascorbic acid mixture (Perry and Warner, 1963).

The solution was added to the scintillator containing phenoxazolyl benzene and diphenyloxazole and the activity determined in a Nuclear Enterprises Scintillation Counter NE.8301 maintained at 10° C. The counting rates of the two isotopes could be determined by altering the discriminator bias voltage and the gate width.

The total amount of activity appearing in the blood is calculated for each isotope. The result is expressed as a percentage of the dose administered.

Serum Iron Estimation and Estimation of the Total Iron-Binding Capacity

Serum iron: A colorimetric method using aa-dipyridyl was used (Bothwell and Mallett, 1955). The results were reported as µg./100 ml.

Total iron-binding capacity: Three methods were used: (1) A colorimetric method using aa-dipyridyl (Peters, Giovanniello, Apt and Ross, 1956). (2) A radioisotope method employing ^{59}Fe in a modification of the method of Bothwell, Jacobs and Kamener (1959), to be reported (Stojceski, Malpas and Witts, 1964). (3) A diffusion method using a strong specific anti-human transferrin (Soothill, 1962). The results were reported as µg. per 100 ml.

Estimation of Faecal Fat

In patients on a hospital diet actual fat balances are not

required (Cooke, 1956).

A four-day collection of stools was taken for the estimation. The faecal fat was determined by the wet method of van der Kamer, Huinink and Wejers (1944) and the results expressed as grammes of fat excreted per day.

Haematological Methods

Estimation of the haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, total leucocyte count, platelet count, reticulocyte count, detection of lupus erythematosus cells, erythrocyte sedimentation rate and the examination of the bone marrow were done as described by Dacie (1963).

Liver Function Tests

Estimation of plasma bilirubin, proteins, alkaline phosphatase, transaminase, flocculation tests and bromsulphthalein excretion was done as described by Varley (1963).

The plasma pseudocholinesterase estimation and the sensitivity to Dibucaine was estimated by the spectrophotometric technique of Kalow and Genest (1957) with benzoylcholine as the substrate.

Examination of Faeces for Occult blood

The faeces were tested for occult blood by the test employing

Ortho Tolidine described by Varley (1963). Tests for occult blood were also done with haematest (Ames).

Chapter 4

IRON ABSORPTION IN NORMAL CONTROLS AND IRON-DEFICIENT PATIENTS

A number of radioisotope balance studies on normal controls and iron-deficient subjects had been done in the Department before the commencement of the present study. These results were used in conjunction with the values obtained during the course of the present investigation.

Results of Absorption Tests using Labelled Ferrous Sulphate in Normal Control Subjects

Results of radioisotope balance studies were available from 37 normal control subjects after giving labelled ferrous sulphate. The amount appearing in the blood was estimated in 28 of these subjects. The details of the results are given in Appendix I.

The mean absorption in 45 observations in this group was 30 per cent. and the range of absorption was from 7 to 70 per cent. The mean percentage of the dose administered which appeared in the blood was 23 per cent. and ranged from less than 1 per cent. to 68 per cent.

Results of Absorption Tests using Labelled Heemoglobin in Normal Control Subjects

The results from studies on 24 normal subjects were available. Values for the amount appearing in the blood were obtained in each patient. The detailed results are given in Appendix I.

The mean absorption in 24 studies was 11 per cent. with a range of absorption from 0 to 32 per cent. The mean percentage of the dose given which appeared in the blood was 6 per cent. and the range 0 to 21 per cent.

Results of Absorption Tests using Labelled Ferrous Sulphate in Iron-Deficient Patients

Twenty-two observations were available from 19 iron-deficient patients, of whom eight were studied in the present investigation. In 18 patients the amount of isotope appearing in the blood was determined. Details of the results are given in Appendix I.

The mean absorption in this group was 54 per cent. and absorption ranged from 14 to 94 per cent. The mean percentage of the dose appearing in the blood was 53 per cent. and the range was from 8 to 90 per cent.

Results of Absorption Tests using Labelled Haemoglobin in Iron-Deficient Patients

Twenty-seven patients had absorption studies using this form of iron. Twelve estimations were done in the course of the present investigation. In 24 the activity appearing in the blood was estimated. Details of the results are given in Appendix I.

The mean percentage of the dose absorbed was 20 per cent.,

with a range of from 1 to 56 per cent. The mean percentage appearing in the blood was 18 per cent., with values ranging from 0 to 40 per cent.

Chapter 5

IRON ABSORPTION IN PATIENTS WITH HAEMOCHROMATOSIS

TABLE 2

Results of Iron Absorption Studies in Idiopathic Haemochromatosis

Authors	Method	Dose of iron	Number of patients studied	Percentage absorption of iron	Normal range for controls and numbers studied
Howard and Stevens (1917)	Chemical balance	Food iron	1	2.5mg. in 5 days	Nil
McClure (1918)	Chemical balance	Food iron	1	48mg. in about 5 days	Nil
Dubach, Callender and Moore (1948)	Isotope balance after ferrous salts	1 mg./kg.	1	21.5	2-11 (10)
Alper, Savage and Bothwell (1951)	Isotope balance after ferrous salts	34 mg.	1	60	14 (1)
Bothwell, van Doorn-Wittkampf, Du Preez and Alper (1953)	Isotope balance after ferric salts	20 mg.	2	8, 24, 24, 72	9-25 (4)
Peterson and Ettinger (1953)	Isotope balance after ferric salts	30-60 µg.	2	20, 44	1.5-4.4 (4)
Chodos, Ross, Apt, Pollycove and Halkett (1957)	Isotope balance, ferrous salts,	5 mg.	9	0.5-11.1	4-12 (10)
	iron in food	Food iron	2	20, 27	0.5-10 (2)
Pirzio-Biroli, Bothwell and Finch (1958)	Blood levels using ^{55}Fe and ^{59}Fe	Labelled meal	4	4.8, 7, 1.4, 17	0.5-16.6 (24)
Biggs and Davies (1963)	Total-body counting	5 mg.	8	Mean 51	Not given

The previous studies on iron absorption in patients with haemochromatosis are given in Table 2. The first tentative attempt to investigate the obviously deranged metabolism of iron is related by Gaskell, Mackenzie Wallis, Sladen and Garrod (1914). The patient was a pharmaceutical chemist who was admitted to St. Bartholomew's Hospital with diabetes. He was pigmented and he had a large liver and a diagnosis of haemochromatosis was made. Although no chemical iron balance studies were done on this patient the investigators were surprised to note that no iron was found in his urine or faeces even though he was heavily laden with iron at the post-mortem examination.

Howard and Stevens (1917) carried out chemical balance studies on a 54-year-old carpenter with pigmentation and glycosuria. They estimated the amount of iron taken in the diet and the amount that appeared in the faeces and urine. The patient's intake was 29.3 mg. of iron and the amount excreted in the faeces was 26.8 mg. during a four-day period. This indicated a total retention during this time of

2.5 mg. The amount of iron in whole blood was 45 mg. per 100 ml. There was clinical evidence that the patient was iron laden. This study is probably the first demonstration that iron absorption continues to take place in spite of tissue iron overload in patients with haemochromatosis.

Another haemochromatotic patient studied by McClure in 1918 by a chemical balance technique showed continued absorption of iron but the patient was anaemic and may possibly have had gastro-intestinal bleeding as he had a large spleen which would suggest portal hypertension.

Dubach, Callender and Moore (1948), in a carefully controlled absorption study on a number of patients with various causes for anaemia, studied a haemochromatotic patient. Radioisotopic iron was used to label inorganic iron which was given as ferrous chloride in a dose of 1 mg. of iron per kg. body weight. The absorption was estimated from the amount of activity not appearing in the faeces. In 10 estimations on eight normal subjects the range of absorption was 2 to 11 per cent. A mean absorption of 7 per cent. was noted. In the patient with haemochromatosis who had a normal blood count (values for the serum iron were not given, but a liver biopsy was

heavily laden with iron) absorption was 21.5 per cent. and of that 2 per cent. of the dose appeared in the blood.

This increased absorption was confirmed by Alper, Savage and Bothwell (1951), who studied a patient with haemochromatosis using labelled inorganic iron. They found that 60 per cent. of a 34-mg. dose of iron was absorbed and some 4 per cent. of the iron utilised for red-cell formation. This patient was unusual in that the condition pursued a relentless course and he died aged 21 years. Liver biopsy and autopsy confirmed the diagnosis and that there was considerable iron deposition, but the fulminating course made the absorption findings difficult to compare with those in other patients with haemochromatosis.

The first larger series of absorption studies in patients with haemochromatosis was done in seven studies on five patients with haemochromatosis diagnosed on clinical grounds and by liver biopsy by Bothwell, van Doorn-Wittkampf, Du Preez and Alper (1953). They studied the absorption of ^{59}Fe and ^{55}Fe-labelled ferric chloride given in 20 mg. dosage to patients on standard hospital diets. Absorption was estimated from the percentage of activity not appearing in the faeces. Four control absorption studies were done in patients with diabetes and duodenal ulcer. The range in these normal controls was 8 to 25 per cent. The absorption of iron was 8, 24, 24 and 72

per cent. on tests on their four haemochromatotic patients.

Three of these patients showed levels of absorption at the upper level or above of those found in the normal controls. This represented a markedly increased absorption in patients who were iron-laden.

It was pointed out by Peterson and Ettinger (1953) that studies of iron absorption had involved the use of iron in amounts greater than those normally present in the diet. Using 30- to 60-μg. doses of iron, they showed that absorption in their two patients with haemochromatosis was 20 and 21.2 per cent. in the first patient and 44.7 per cent. in the second patient. Their normal subjects showed an absorption of between 1.5 and 4.4 per cent. These figures for normal controls are surprisingly low, in view of the small doses of iron employed. It is notable that the patient showing 20 per cent. absorption had actually been rendered iron-deficient at the time when the absorption studies were carried out and that the patient with 44.7 per cent. was iron-laden. Utilisation of the administered radioactive iron was 100 per cent. in the first case, whereas it was reduced to 33 per cent. of the dose absorbed in the second.

This increased absorption in haemochromatosis was not confirmed by Chodos, Ross, Apt, Pollycove and Halkett (1957). They were the first to study the absorption of labelled foodstuffs in

this condition. They used biologically labelled beet and eggs in addition to 5-mg. doses of ferrous iron. In nine patients with idiopathic haemochromatosis absorption of labelled ferrous chloride varied from 0.5 to 11.1 per cent. The range of absorption in normal controls was 4 to 12 per cent. The absorption of inorganic iron in these iron-laden patients therefore fell within the normal range.

They found that although inorganic iron absorption was not increased, two of the patients given labelled beet absorbed 20 per cent. and 27 per cent. of the iron present as compared with 0.5 to 10 per cent. in normal control subjects.

The preparation of a number of biologically labelled foodstuffs to make up a balanced meal is exceedingly difficult. To give only one item of diet labelled with an isotope is an artificial situation. In order to avoid this, Pirzio-Biroli, Bothwell and Finch (1958) have added labelled ferric chloride to a standard meal shortly before it is eaten, and assume that the meal is completely labelled.

They employ a double isotope technique, using ^{55}Fe and ^{59}Fe. One of these is used to label the meal. The other is injected ingravenously. The amount of the two isotopes appearing in the blood is estimated from a sample taken from 11 to 15 days later. This is the time when maximum utilisation for haemoglobin formation would have

been expected to occur.

It is assumed that the proportion of the isotope coming into the plasma from the gut which is utilised for red-cell formation is the same as the proportion utilised when given intravenously. On the basis of this assumption it is possible to calculate how much iron has been absorbed from the meal.

This method has the obvious advantage of avoiding faecal collections. Particularly good results can be obtained in normal or iron-deficient patients when 70 to 100 per cent. of both isotopes are utilised and appear in the red blood cells. However, in conditions of iron overload the amount of the radioisotope which eventually appears in the blood is small. Slight errors in estimating the amount of activity may therefore markedly affect the results.

Using this double isotope technique, Pirzio-Biroli et al. estimated the absorption of iron as 4.8, 7, 11.4 and 17 per cent. in four patients with haemochromatosis. The mean absorption was 10 per cent. This compares with a group of normal control subjects who absorbed 0.5 to 16.6 per cent. with a mean of 5.3 per cent. The absorption in Pirzio-Biroli's patients is high for iron-laden subjects.

One of the largest series of haemochromatotic patients studied is that reported by Biggs and Davis (1963). They studied eight

TABLE 3

Haematological Findings in Patients with Primary Haemochromatosis

Patient	Age	Hb, g./100ml.	MCHC	Retics. %	Serum iron, µg./100ml.	TIBC, µg./100ml.	Saturation of TIBC %
A.C.	53	17.2	38	--	296	314	94
E.P.*	59	14.8	33	--	66	279	24
J.E.	61	14.5	34	--	274	396	69
L.D.	70	13.8	32	1	172	272	63
K.E.	33	15.4	35	0.6	228	287	79
B.J.†	46	9.5	32	4	39	228	17
R.F.	53	14.6	--	--	152	--	--
E.T.	57	15.9	34	5	200	240	83

*After a long course of venesections.

†Recent gastrointestinal bleeding.

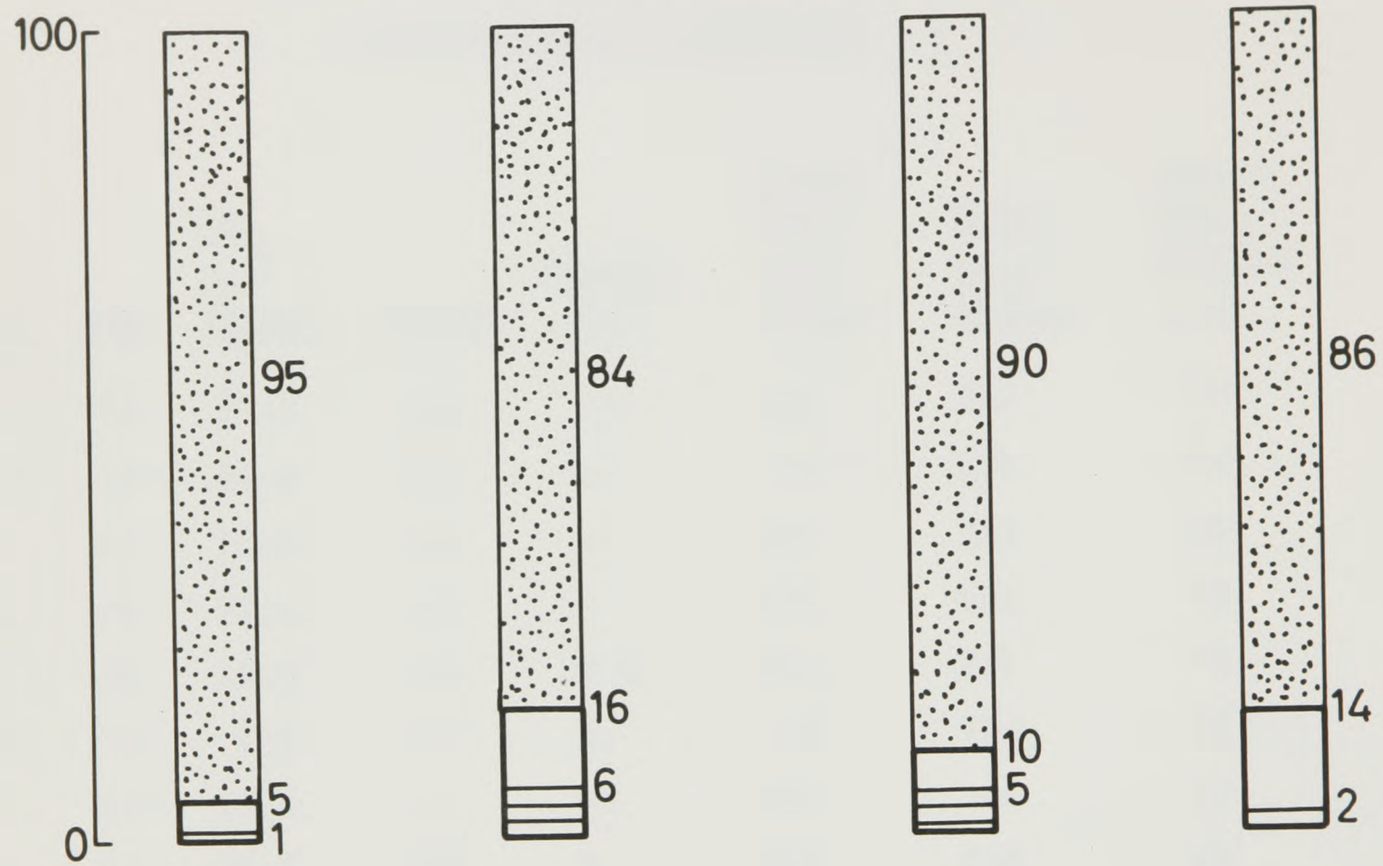
K.E., B.J., R.F. and E.T. had associated alcoholism.

All patients were males.

None had positive occult blood in stool.

Fig. 2:

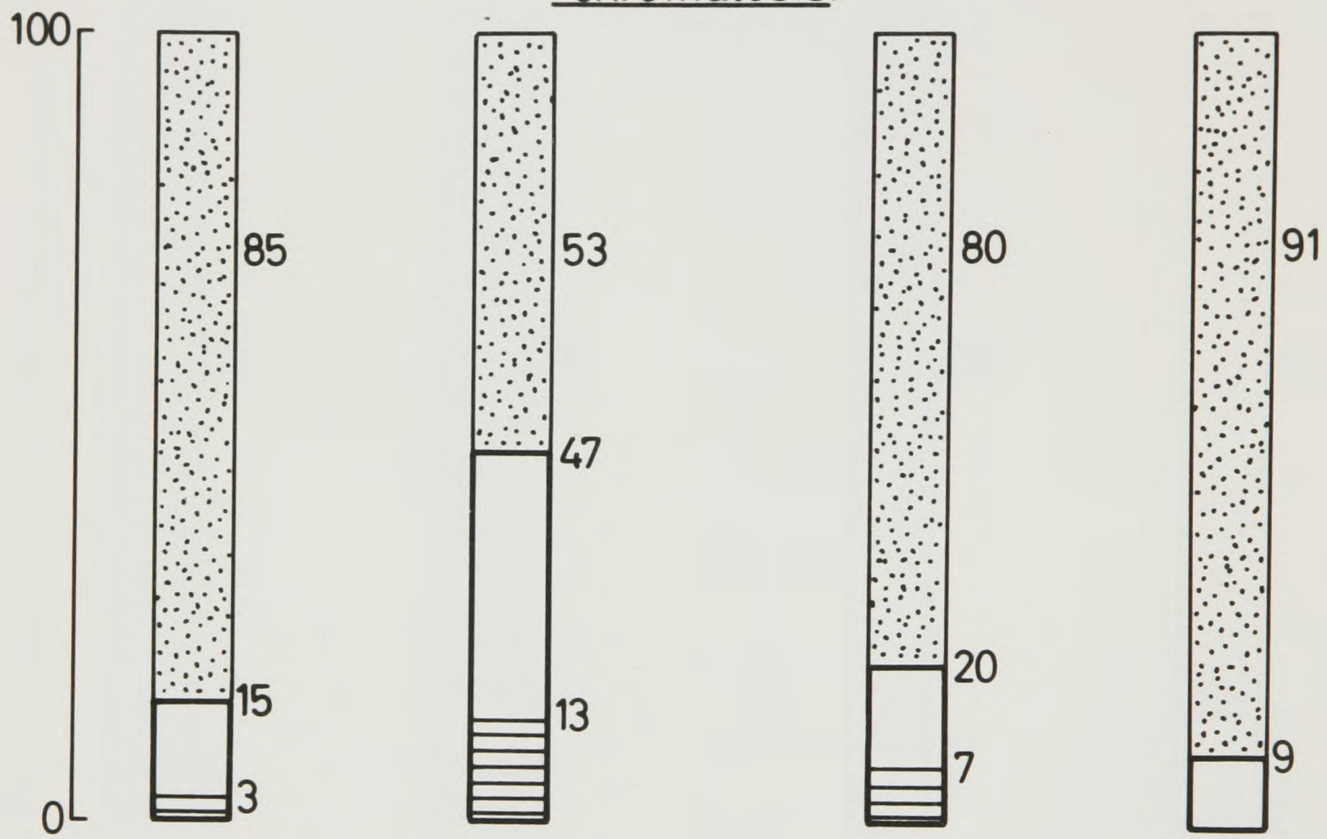
Absorption of labelled haemoglobin by patients with haemochromatosis.



Patient.	KE	BJ	RF	ET
Hb.	15·4	9·5	14·6	15·9
MCHC.	35	32	-	34
Retics %.	0·6	4	-	5
Serum Fe.	228	39	152	200
% sat. of IBC.	79	17	-	83

Fig. 3:

Absorption of labelled haemoglobin by patients with haemo-chromatosis.



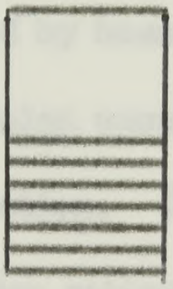
Patient.	AL	EP	JE	LD
Hb.	17·2	14·8	14·5	13·8
MCHC.	38	33	34	32
Retics %.	-	-	-	1
Serum Fe.	296	66	274	172
% sat. of IBC.	94	24	69	63

THIS KEY APPLIES TO

ALL SUBSEQUENT FIGURES



Percentage in faeces



Percentage unaccounted for

Percentage in blood

} Percentage absorbed

patients using a whole-body counting technique. 5 mg. of ferrous iron was given and a mean absorption of 51 per cent. of this was noted. This result is considerably higher than the absorption found in normal control subjects given ferrous iron in radioisotope balance techniques when the mean absorption is 30 per cent. However, some of Biggs et al's patients had already been partly treated by venesection when they were studied and their tissue iron overload was reduced. This would tend to increase the mean level of absorption.

Results of the Present Study

The present study was designed to assess the absorption of food iron as represented by haemoglobin. Patients with haemochromatosis who were iron-laden were studied, and one patient who had been treated by venesection. (Tables 3 and 4.)

In seven iron-laden patients the mean absorption was 12 per cent. and the range was 5 to 20 per cent. (Figures 2 and 3) (mean value in normal controls, 11 per cent., range 0 to 32 per cent.). The amount of iron utilised for haemoglobin formation was 4 per cent. and the range was from 1.5 to 7 per cent. (mean value in normal controls 6 per cent., range 0 to 21 per cent.).

In one patient whose iron stores had been reduced by a full

TABLE 4

Liver Function Tests in Patients with Haemochromatosis

Patients	Plasma bilirubin mg./100ml.	Plasma alkaline phosphatase units	Plasma proteins g./100ml. Total	Albumin	Pseudo-cholinesterase units	Bromsulphthalein retention, %:
A.C.	0.9	22	6.3	4.0	85	--
E.P.	0.2	12	6.1	3.6	44	--
J.E.	0.2	12	6.8	5.5	--	--
L.D.	0.7	21	6.2	3.6	--	--
K.E.	0.3	12	6.7	--	51	14
B.J.	0.7	15	6.8	--	41	6
R.F.	1.3	13	5.6	2.9	10	--
E.T.	0.3	10	7.4	--	56	11

course of venesection absorption was 47 per cent. and the patient utilised 13 per cent. of the dose.

The absorption of labelled haemoglobin in the iron-laden patients was therefore very close to that seen in normal controls. This could be considered as an increased absorption in the presence of iron overloading. It is interesting to note that when tissue iron is reduced to normal levels in these patients a high absorption of iron occurs compared to normal control subjects.

The utilisation of iron for haemoglobin formation in the subjects studied showed low values in all except the patient treated by venesection.

In conclusion, the studies so far on iron absorption in haemochromatosis show a level of absorption of both inorganic and food iron similar to that seen in normal subjects. When these patients are treated and their iron stores are reduced to within normal limits their absorption increases to greater than normal.

Chapter 6

IRON ABSORPTION IN PATIENTS WITH CIRRHOSIS OF THE LIVER

Compared with the studies of iron absorption that have been done on patients with haemochromatosis, relatively few results on patients with cirrhosis of the liver have been published. Bothwell, van Doorn-Wittkampf, Du Preez and Alper (1953) studied one patient with cirrhosis and secondary haemosiderosis attributed to malnutrition. Although iron absorption was not studied directly, counting of radioactivity by means of a surface counting technique in the region of the liver showed that a considerable proportion of the iron had been absorbed. The level of activity was much higher than in normal control subjects.

In 1961 Van Hoek, using a whole-body counting technique, noted that iron absorption was greater in five out of nine patients with cirrhosis of the liver than in his normal control subjects. Two of the patients with increased absorption had increased levels of serum iron.

Conrad, Berman and Crosby in 1962 studied 10 patients with Laennec's cirrhosis. Three had increased iron stores, three were studied after recent gastrointestinal bleeding and four were chosen at random. Iron absorption was determined using a whole-body counting technique. In their group of normal controls the mean absorption of iron was 10 per cent. In the three patients with normal or increased levels of serum iron the absorption was 30, 41

and 64 per cent. It was noted that these patients were chronic alcoholics, which was considered to be a cause of their increased absorption of iron although the mechanism by which this occurred was not known.

Sherlock (1964), reporting studies on iron absorption, found increased absorption in four out of five patients with well-compensated cirrhosis of the liver. Deposition of iron in the liver was noted in only one patient and he was an alcoholic.

Recently Greenberg, Strohmeyer, Hine, Keene, Curtis and Chalmers (1964), using a whole-body counting technique, showed an increase of inorganic iron absorption in about a third of their 24 cirrhotic patients. Some of the petients who had increased absorption had had porta caval anastomoses for portal hypertension.

In any study of the absorption of iron in patients with cirrhosis a number of factors which may influence absorption must be taken into account. The way in which the iron is presented either as inorganic iron or a food iron will be of importance as in all absorption studies. The presence of sideropaenia or evidence of recent gastrointestinal bleeding which may commonly complicate cirrhosis of the liver will tend to increase the absorption of iron.

In presenting the results of this study these influences on iron absorption have been noted and the patients have been subdivided

TABLE 5

Haematological Findings in Patients with Cirrhosis of the Liver

Patient	Sex	Age	Hb, g./100ml.	MCHC	Retics. %	Serum iron µg./100ml.	TIBC, µg./100ml.	Saturation of TIBC, %	Occult blood in faeces
Non-alcoholic cirrhosis									
S.M.	♀	39	10.0	--	1	78	260	30	Negative
V.S.	♀	49	12.8	32	--	60	292	21	Negative
E.R.	♀	52	15.4	34	--	212	278	76	Negative
V.H.	♂	54	14.9	33	1.6	292	380	79	Negative
E.B.W.	♀	59	14.5	33	--	144	294	49	Negative
R.A.	♀	61	10.0	31	0.8	48	344	14	Negative
F.C.	♀	65	10.3	31	1.6	160	--	--	Trace
E.W.	♀	73	13.4	36	1.8	184	322	57	Negative
W.W.	♂	74	13.7	30	--	384	485	80	Trace
K.W.	♀	74	10.9	32	--	130	260	50	Negative
A.J.	♂	76	17.4	33	1.2	264	330	79	Negative
Alcoholic cirrhosis									
A.S.	♂	43	10.2	--	--	72	242	30	Negative
C.H.	♀	43	9.3	--	--	116	296	39	Negative
H.B.	♂	46	12.6	34	2	200	--	--	Negative
A.D.	♀	47	12.0	--	--	212	292	72	Negative
M.K.	♀	49	14.0	--	--	202	--	--	Trace
P.Q.	♂	53	14.2	33	3	252	281	90	Negative

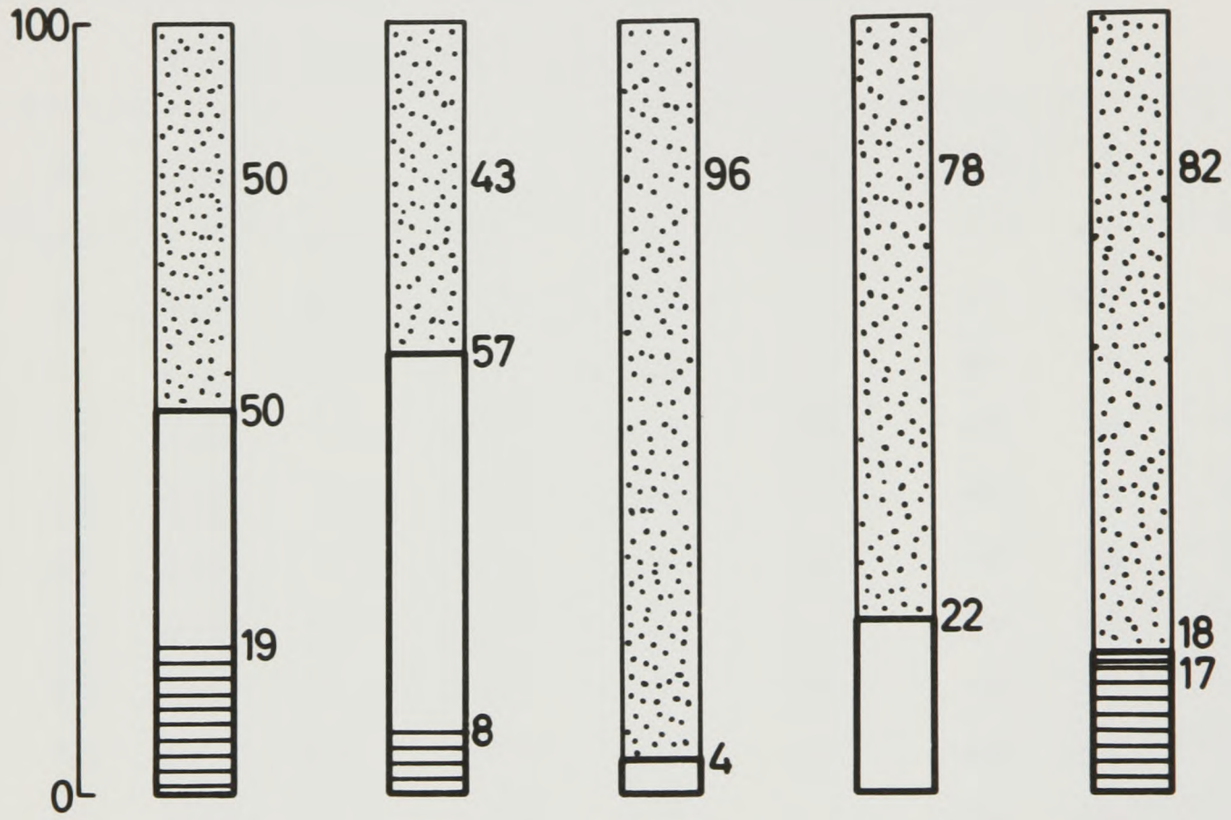
TABLE 6

Liver Function Tests in Patients with Cirrhosis of the Liver

Patient	Sex	Age	Bilirubin mg./100ml.	Alkaline phosphatase units	Plasma proteins g./100ml. Total	Plasma proteins g./100ml. Albumin	Pseudocholinesterase units	Transaminases SGOT	Transaminases SGPT	Bromsulphthalein retention %
Non-alcoholic cirrhosis										
S.M.	♀	39	0.2	--	6.6	4.2	36	4	4	22
V.S.	♀	49	1	16	7.1	3.2	16	--	--	--
E.R.	♀	52	9.5	30	8.2	2.6	9	--	80	--
V.H.	♂	54	0.5	10	6.3	3.6	41	--	--	39
E.B.W.	♀	59	1.6	5	6.9	4.2	30	--	--	--
R.A.	♀	61	1.8	8	7.3	3.1	--	--	--	25
F.C.	♀	65	0.8	7	5.9	2.4	19	--	18	46
E.W.	♀	73	0.8	16	6.9	3.6	--	--	44	23
W.W.	♂	74	0.6	--	7.5	--	43	--	--	--
K.W.	♀	74	3	80	7.4	2.6	--	--	80	--
A.J.	♂	76	1.8	21	8.2	4.2	16	--	--	63
Alcoholic cirrhosis										
A.S.	♂	43	3.5	11	7.5	3	24	70	28	--
C.H.	♀	43	0.2	7	7.1	3	24	--	18	22
H.B.	♂	46	1.8	9	6.3	2.7	13	--	26	33
A.D.	♀	47	1.2	22	7	3.9	28	--	8	--
M.K.	♀	49	10.0	10	--	2.8	--	--	120	--
P.Q.	♂	53	0.2	10	9	3.6	16	--	--	39

Fig. 4:

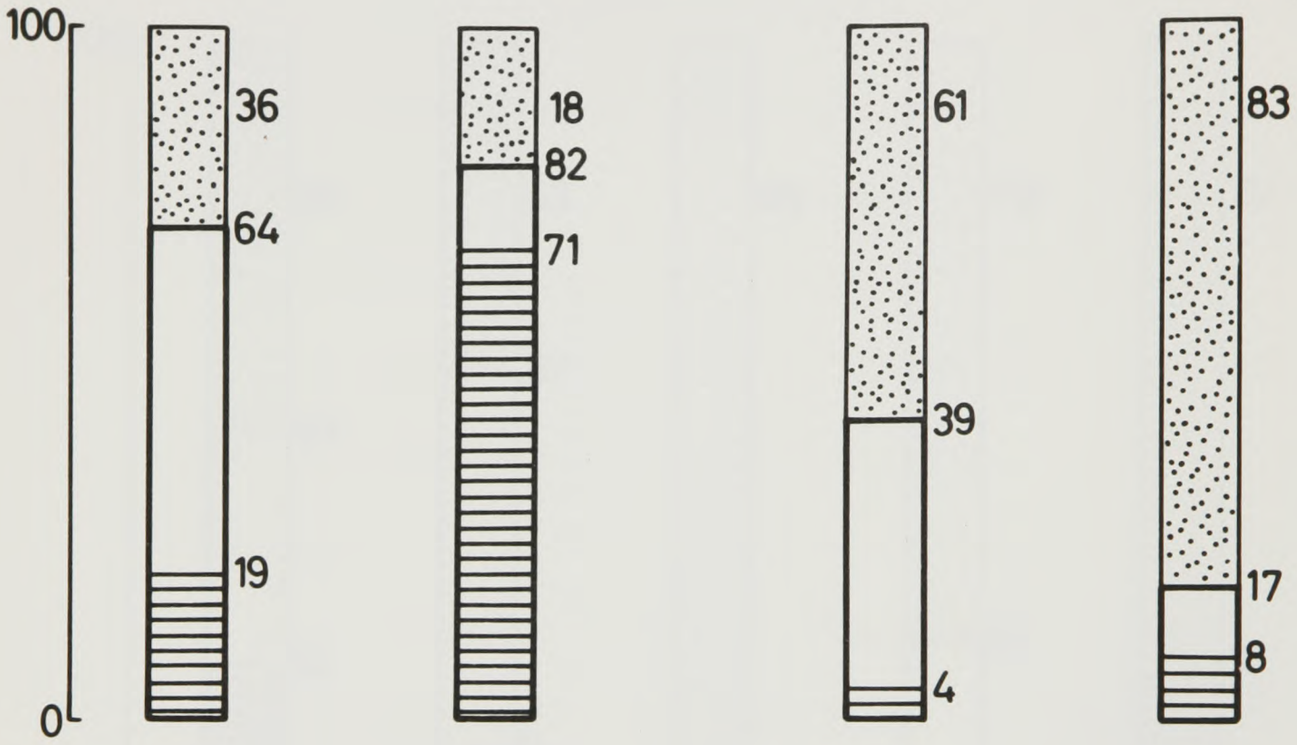
Absorption of labelled haemoglobin by anaemic patients with cirrhosis of the liver



Patient.	KW	RA	AS	SM	CH
Hb.	10·9	10·0	10·2	10·0	9·3
MCHC.	32	31	33	-	-
Retics %.	-	0·8	-	1·0	-
Serum Fe.	130	48	72	78	116
% sat of IBC.	50	13	30	30	39

Fig. 5:

Absorption of labelled ferrous sulphate and labelled haemoglobin by patients with cirrhosis of the liver who have recently bled or are sideropaenic.



Patient	VS	WW	MK	FC
Dose.	$Fe^{59}Hb$	$Fe^{59}SO_4$	$Fe^{59}Hb$	$Fe^{59}SO_4$
Hb.	12·8	13·7	14	10·3
MCHC.	32	30	-	31
Retics %.	-	-	-	1·6
Serum Fe.	60	384	202	160
% sat. of IBC.	21	80	-	-
Occult blood in stool.	neg.	trace.	trace.	trace.

into groups accordingly.

Results of the Present Study

A total of 17 patients with cirrhosis were studied. Most were well-compensated cirrhotics. In contrast to previous series the majority of these patients had cirrhosis which was not associated with chronic alcoholism.

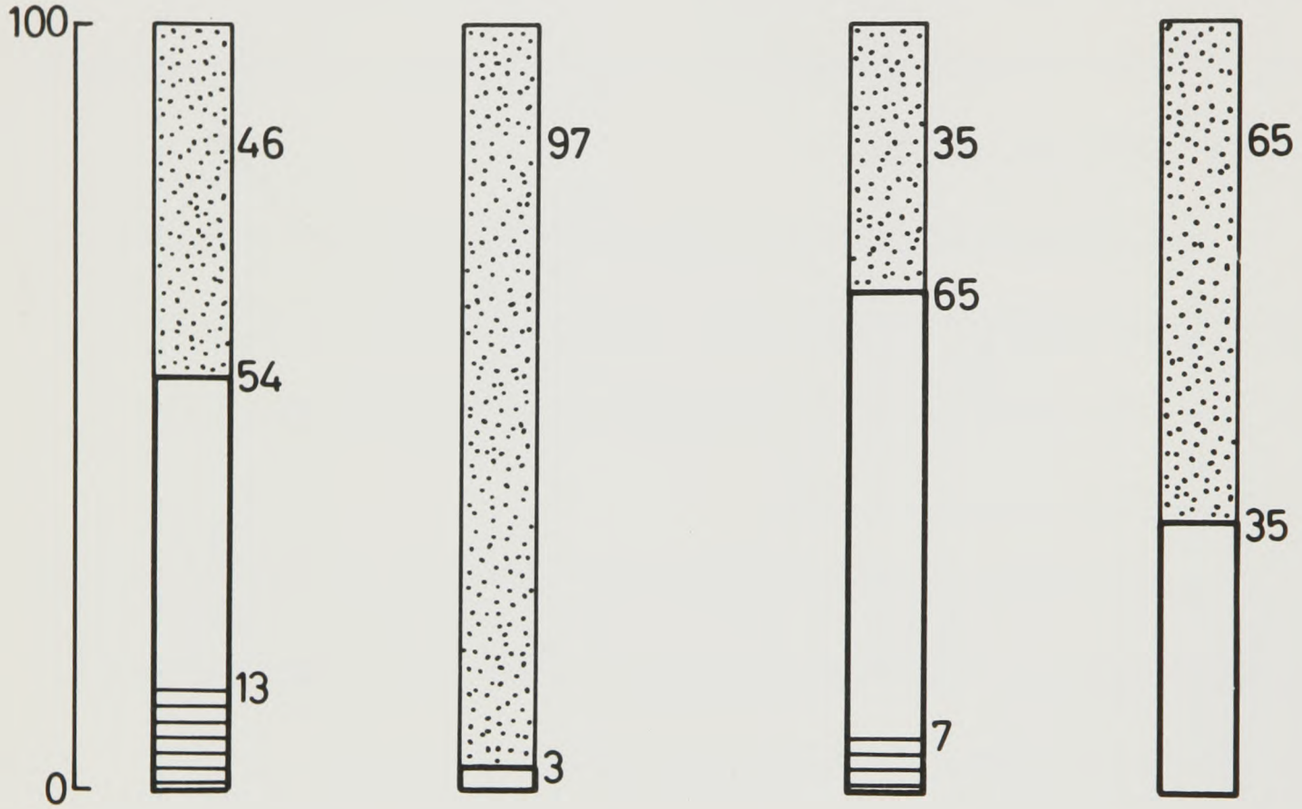
Absorption studies using either labelled ferrous sulphate or labelled haemoglobin were done. The results of some of these studies have been reported in a preliminary communication by Callender and Malpas (1963).

Nine of the patients studied were anaemic, or had evidence of recent bleeding. Eight showed neither of these features and had normal or increased levels of serum iron. (Table 5.)

The results of the absorption studies in the cirrhotic patients with anaemia or bleeding are shown in Figures 4 and 5. All except two patients showed increased absorption of iron whether it was presented as ferrous sulphate or haemoglobin iron. There were two exceptions to this finding. One was the patient A.S., noted in Chapter 2 (Selection of Patients), who had recently recovered from an episode of liver failure. The other was the patient F.C., who had evidence of markedly disturbed liver function at the time of the absorption study (Table 6). In the remainder the increased

Fig. 6:

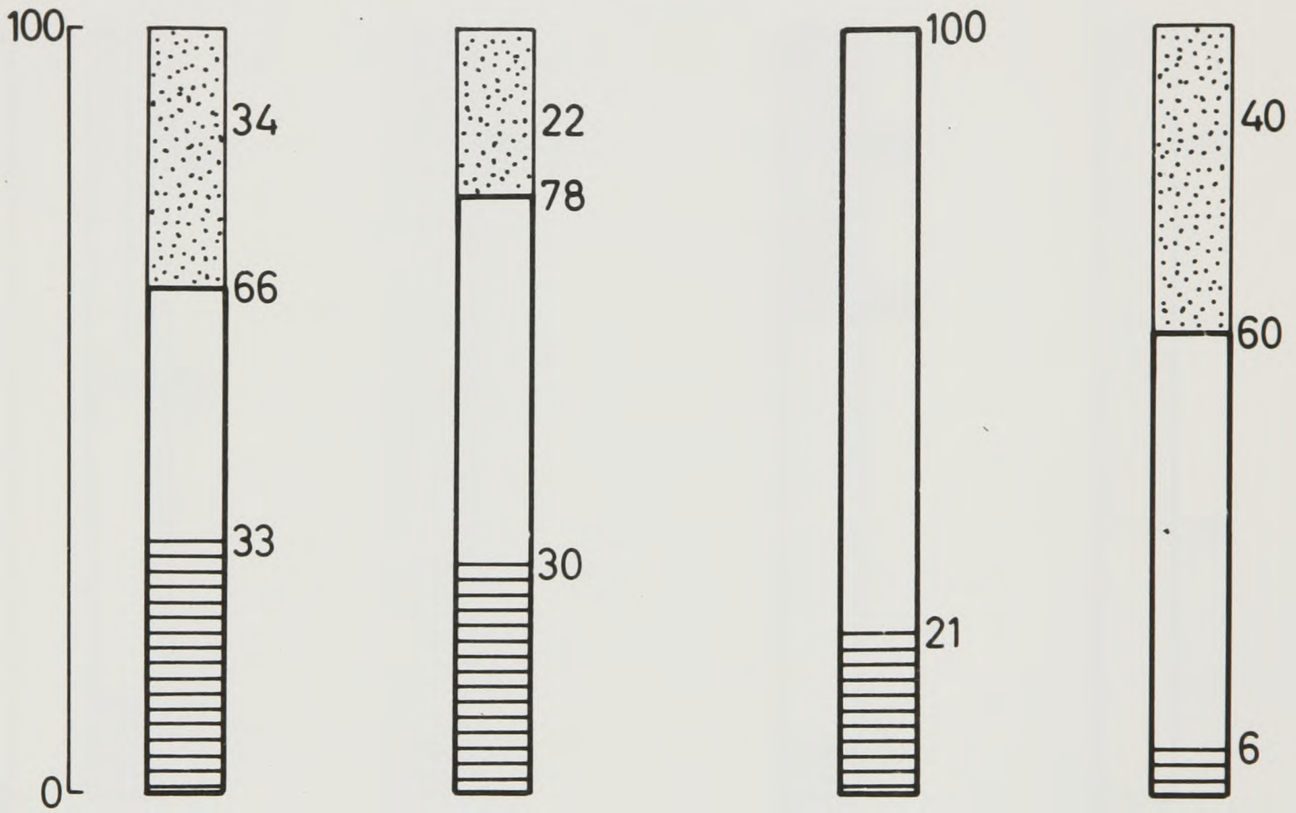
Absorption of labelled ferrous sulphate and labelled haemoglobin by patients with cirrhosis of the liver who have normal levels of serum iron.



Patient.	EBW	ER	EW	HB
Dose.	$Fe^{59}Hb$	$Fe^{59}Hb$	$Fe^{59}SO_4$	$Fe^{59}SO_4$
Hb.	14·5	15·4	13·4	12·6
MCHC.	33	34	36	34
Retics %.	-	-	1·8	2
Serum Fe.	144	212	184	200
% sat. of IBC.	49	76	57	-

Fig. 7:

Absorption of labelled ferrous sulphate and labelled haemoglobin by patients with cirrhosis of the liver with increased levels of serum iron.



Patient.	VH	PQ	AJ	AD
Dose.	$Fe^{59}SO_4$	$Fe^{59}SO_4$	$Fe^{59}SO_4$	$Fe^{59}Hb$
Hb.	14·9	14·2	17·4	12·0
MCHC.	33	33	33	-
Retics %.	1·6	3	1·2	-
Serum. Fe.	292	252	264	212
% sat of IBC.	79	90	79	72

absorption was not unexpected in view of the blood loss and anaemia.

The proportion of iron used for red-cell formation varies quite markedly. Nearly 100 per cent. of the dose absorbed was utilised in this way by the anaemic patient (C.H.). The amount appearing in the red cells was usually less than this and in some patients (R.A., M.K.) it was between 10 and 20 per cent.

In eight patients with normal or increased levels of serum iron the absorption of inorganic or haemoglobin iron was markedly increased (Figures 6 and 7). This was noted in all patients with the exception of the patient E.R., noted in Chapter 2 to be jaundiced and in liver failure at the time of the study.

Not only is iron absorption higher than that seen in normal control subjects but in some cases it exceeds that seen in patients with simple iron-deficiency anaemia.

Statistical examination of these results shows that the absorption of both forms of iron is significantly increased compared with normal control subjects. Details of the analysis are given in the statistical appendix.

In the group of patients with normal or increased levels of serum iron the proportion of absorbed iron which is utilised for red-cell formation is low. In some patients (E.W. and A.D.) it

is about 10 per cent. and in only one patient (V.H.) did it approach 50 per cent. This proportion is comparable with the findings in patients with tissue iron overload such as primary haemochromatosis or transfusion haemosiderosis. These results correlate quite closely with the increased saturation of the total iron-binding capacity seen in these patients (Table 5).

In conclusion this study demonstrates the presence of increased absorption of inorganic iron or haemoglobin iron in a number of patients with cirrhosis of the liver in whom none of the usual causes for increased absorption were present. The levels of absorption may be of the order seen in iron-deficient patients. In marked contrast to iron-deficient patients, however, the cirrhotic patients show in general poor utilisation of the absorbed iron for haemoglobin formation. The increased absorption of iron was seen not only in alcoholic cirrhotics but in patients with no associated alcoholism.

Chapter 7

IRON ABSORPTION IN ACUTE LIVER DISEASE

TABLE 7

Liver Function Tests and Faecal Fat Excretion in Patients with Acute Liver Disease

Patient		Diagnosis	Bilirubin mg./100ml.	Alkaline phosphatase units	Plasma proteins g./100 ml. Total	Plasma proteins g./100 ml. Albumin	Pseudocholinesterase units	Transaminase SGOT	Transaminase SGPT	Faecal fat excretion g./day
K.M.	(1)	Infectious hepatitis	19.5	28	5.9	2.9	37	22	22	47.6
	(2)	Improving; on steroids	0.9	29	6.9	--	66	--	82	5.3
J.T.	(1)	Infectious hepatitis	2.5	48	6.9	3.5	27	--	100	--
J.P.	(1)	Infectious hepatitis	1.8	12	7	3.9	30	--	460	6.4
	(2)	Recovered	0.7	6	8	5.3	74	--	--	--
G.P.	(1)	Infectious hepatitis; improving	0.3	28	7.6	5.1	52.5	--	--	3
N.C.	(1)	Infectious hepatitis	11.6	18	7.5	3.5	39	>2000	>1500	5.3
	(2)	Improving; jaundiced	9	13	--	--	--	300	130	1.7
	(3)	Recovered; on steroids	2	8	6.3	4	--	--	10	1.0
M.W.	(1)	Serum hepatitis	3.2	10	8	2.2	14.5	--	260	18.9
	(2)	Improving; on steroids	2.4	10	--	--	--	--	106	17.1
M.D.	(1)	Lupoid hepatitis	8	18	8.1	3.2	19	460	400	13.6
	(2)	Improving; on steroids	2	13	--	--	--	32	20	4.8
I.P.	(1)	Lupoid hepatitis	3	--	7.2	2.6	--	--	160	7.8

TABLE 8

Haematological Findings in Patients with Acute Liver Disease

Patient		Sex	Age	Diagnosis	Hb, g./ 100ml.	MCHC	Retics. %	Serum iron, µg./ 100ml.
K.M.	(1)	♀	29	Infectious hepatitis	12.5	--	--	472
	(2)			Improving on steroids	--	--	--	128
J.T.	(1)	♂	33	Infectious hepatitis	9	29	3.2	101
J.P.	(1)	♂	36	Infectious hepatitis	14.4	--	--	172
	(2)			Recovered	--	--	--	128
G.P.	(1)	♀	56	Infectious hepatitis	14.8	33	2	52
N.C.	(1)	♀	63	Infectious hepatitis	12.3	32	--	256
	(2)			Improving; jaundiced	--	--	--	140
	(3)			Recovered; on steroids	--	--	--	100
M.W.	(1)	♀	35	Serum hepatitis	10.9	32	1.6	200
	(2)			Improving; on steroids	11.7	32	3	184
M.D.	(1)	♀	43	Lupoid hepatitis	14.9	34	--	164
	(2)			Improving; on steroids	--	--	--	60
I.P.	(1)	♀	56	Lupoid hepatitis	13.9	34	--	120

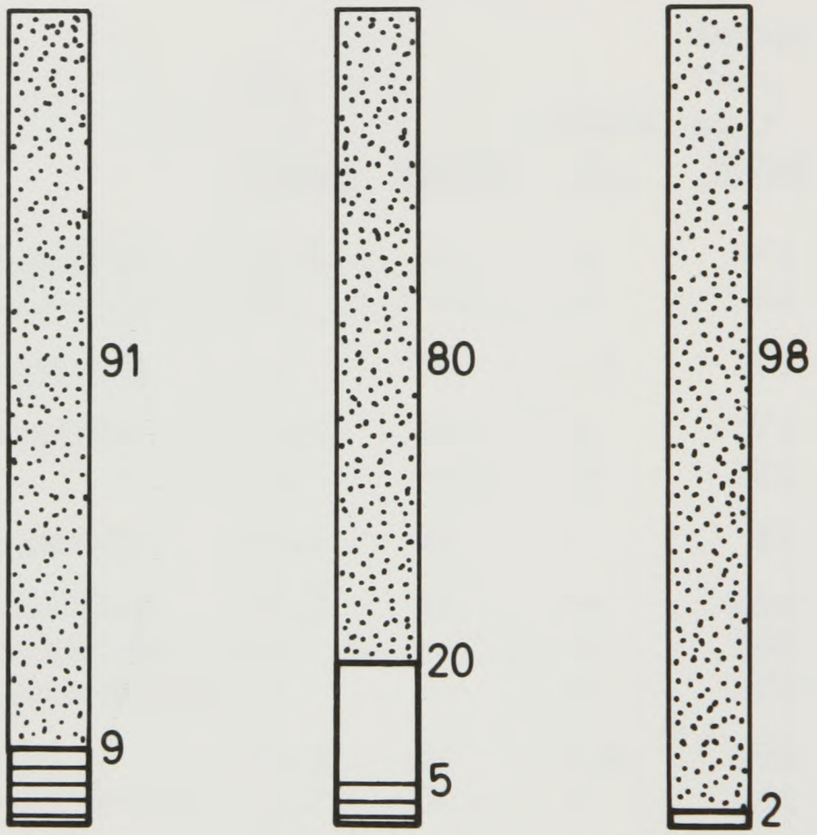
No patient had positive occult blood in the faeces.

Fig. 8:

Absorption of labelled ferrous sulphate and labelled haemoglobin by patients with acute liver disease.

Infectious hepatitis.

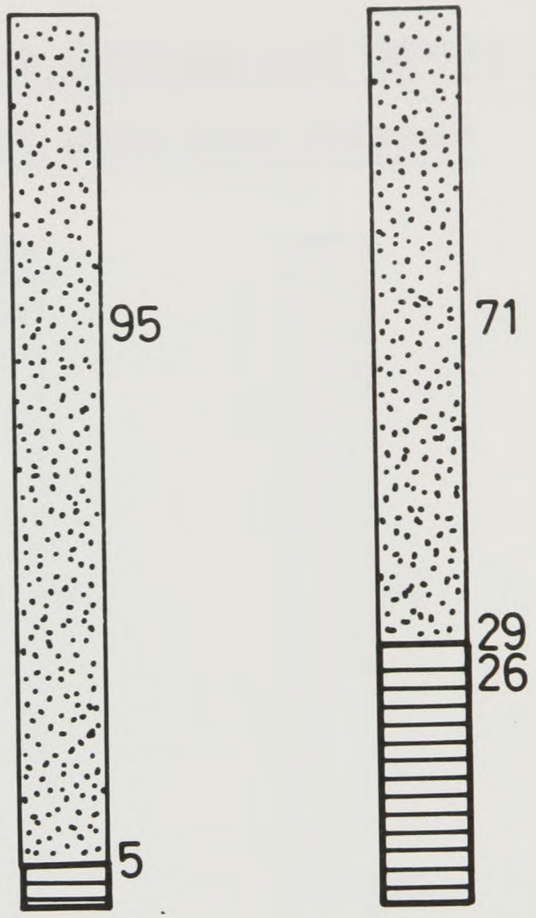
Patient NC.



Condition of patient.	Ill.	Improving. Jaundiced.	Recovering. On steroids.
Haemoglobin.	12·3	-	-
Serum iron.	256	140	100
Bilirubin.	11·6	9	2
Albumen.	3·5	-	4
Transaminase SGPT.	1500	130	10
Faecal fat excretion.	5·3	1·7	1·0

Fig. 9:

Serum hepatitis.

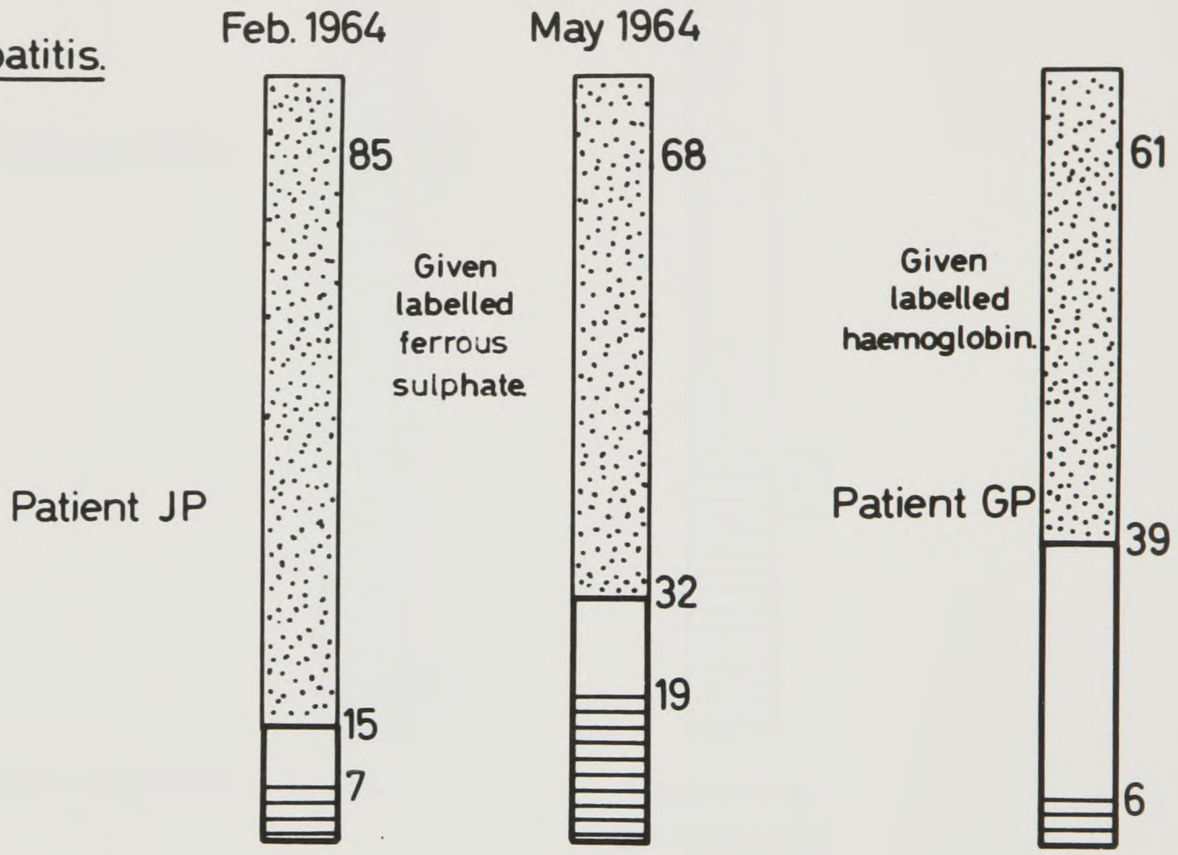


Condition of patient.	Ill.	Improving. On steroids.
Haemoglobin.	10·9	11·7
Serum iron.	200	184
Bilirubin.	3·2	2·4
Albumen.	2·2	-
Transaminase SGPT.	260	106
Faecal fat excretion.	18·9	17·1

Fig. 10:

Absorption of labelled ferrous sulphate and labelled haemoglobin by patients with acute liver disease.

Infectious hepatitis.

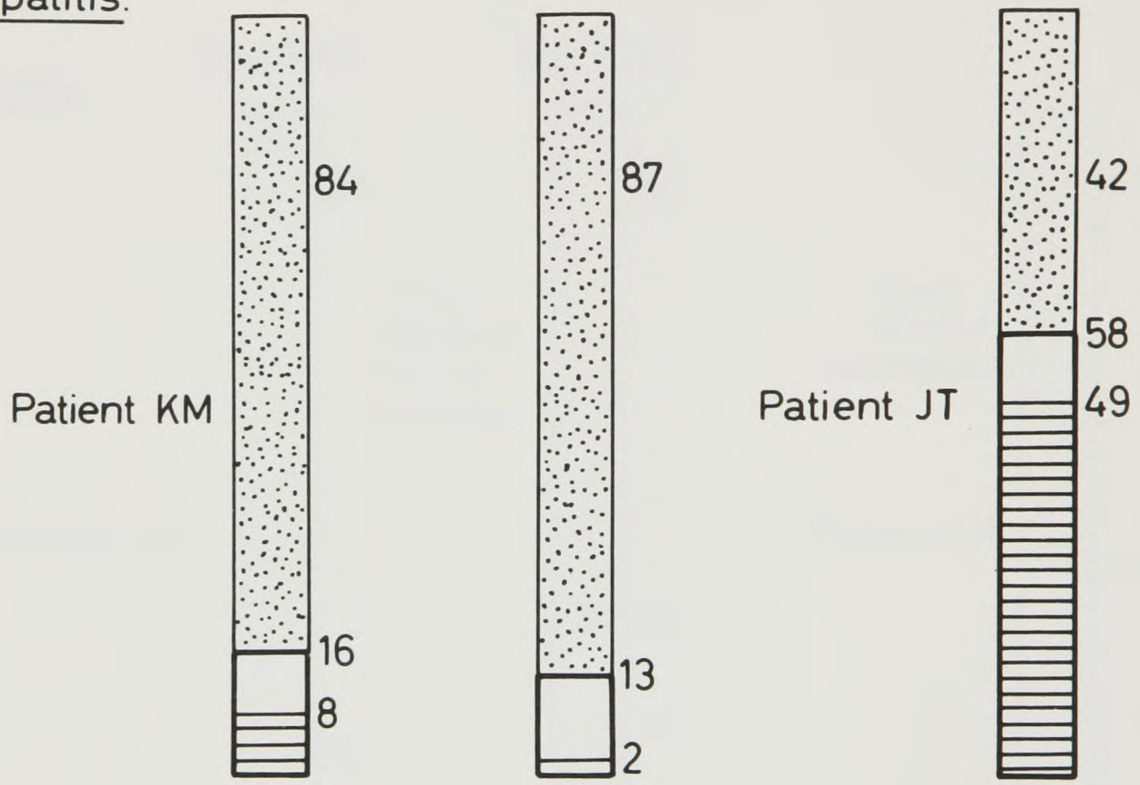


Condition of patient.	Ill. No steroids.	After recovery.	During recovery.
Haemoglobin.	14·4	-	14·8
Serum iron.	172	128	52
Bilirubin.	1·8	0·7	0·3
Albumen.	3·9	5·3	5·1
Transaminase SGPT.	460	-	-
Faecal fat excretion.	6·4	-	3

Fig. 11:

Absorption of labelled ferrous sulphate and labelled haemoglobin by patients with acute liver disease.

1. Infectious hepatitis.

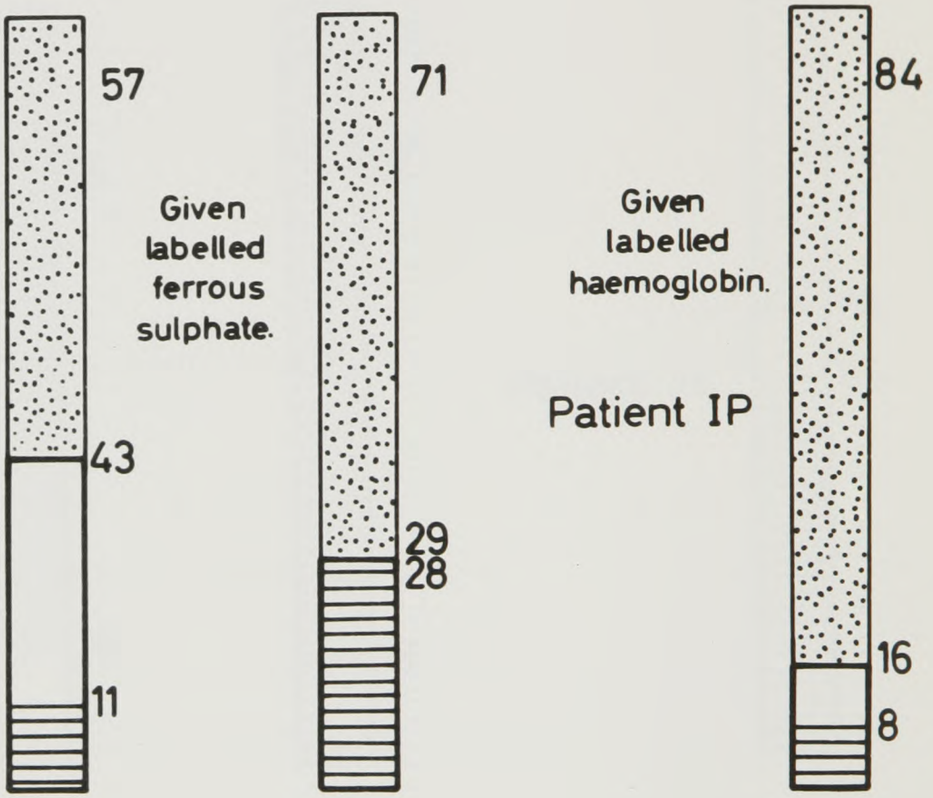


	Oct. 1963	May 1964	
Condition of patient	Ill. No steroids.	Improving. On steroids.	Ill. No steroids.
Haemoglobin.	12·5	-	9·0
Serum iron.	472	128	101
Bilirubin.	19·5	0·9	2·5
Albumen.	2·9	-	3·5
Transaminase SGPT.	22	82	100
Faecal fat excretion.	47	5·3	3·8

Fig. 12:

Absorption of labelled ferrous sulphate and labelled haemoglobin by patients with acute liver disease.

Lupoid hepatitis



Patient MD

Patient IP

Condition of patient.	Ill.	Improving. On steroids.	Recovering. On steroids.
Haemoglobin.	14·9	-	18·9
Serum iron.	164	60	120
Bilirubin.	8	2	3
Albumen.	3·2	-	2·6
Transaminase SGPT.	460	32	-
Faecal fat excretion.	13·6	4·8	7·8

It has not been possible to find any published work on the absorption of iron in patients with acute liver disease. There is, however, some unpublished work by Williams (1964, personal communication) who has studied absorption in a variety of liver diseases by the double isotope technique of Bothwell and Finch. In acute liver disease he has found in general decreased absorption except in patients with lupoid hepatitis.

Results in the Present Study

It was intended initially to study the absorption of both labelled inorganic iron and labelled haemoglobin. Pilot studies, however, showed markedly low values of haemoglobin iron absorption and the remaining investigations employed inorganic iron (Tables 7 and 8).

Results of Absorption Studies in Infectious Hepatitis, Presumed Serum Hepatitis and Lupoid Hepatitis

The absorption of labelled ferrous sulphate given to five patients in the various stages of their illness is shown in Figures 8, 9, 10, 11 and 12. During the acute stage absorption is low but

with recovery or the administration of steroids it tends to rise. In two patients whose infectious hepatitis complicated pre-existing iron-deficiency anaemia (G.P. and J.T.) there was an increased absorption similar to that seen in patients with uncomplicated iron-deficiency anaemia. Details of these patients' clinical histories can be seen in Appendix II.

Two patients with lupoid hepatitis were studied (Figure 12). One patient (M.D.) was given an iron absorption test before she was started on steroid therapy. Another patient (I.P.) was already on steroid therapy at the time of the absorption test. The untreated patient (M.D.) showed an increased absorption of labelled ferrous sulphate which fell with the administration of steroids. I.P. showed a rather low level of absorption.

Although a number of patients with acute liver disease showed steatorrhoea (Table 7), this was not considered to be an important factor in the low absorption of iron. In one patient (M.W.), for example, steatorrhoea continued to the same marked degree without influencing the increase in the absorption of iron which occurred during her recovery.

In conclusion low iron absorption was seen in patients with acute liver disease with the exception of one patient who had untreated lupoid hepatitis.

Chapter 8

IRON ABSORPTION IN TRANSFUSION SIDEROSIS

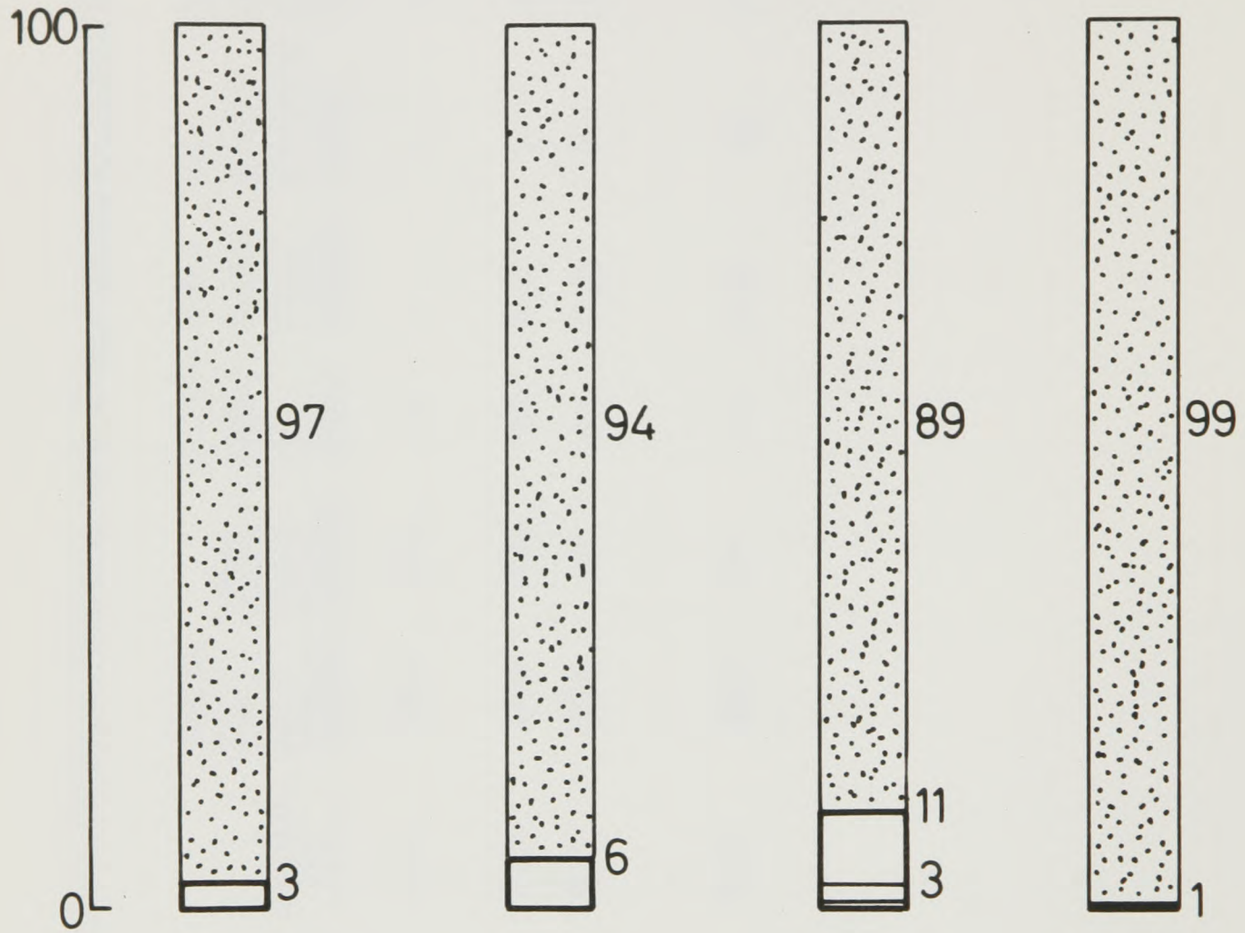
TABLE 9

Haematological Findings and Liver Function Tests in Patients with Transfusion Haemosiderosis

Patient	Sex	Age	Diagnosis	Hb, g./100ml.	MCHC	Retics. %	Serum Fe, µg./100ml.	TIBC, µg./100ml.	Saturation of TIBC	Bilirubin mg./100ml.	Alk. phosphatase units	Total protein g./100ml.	Albumin, g./100ml.	Bromsulphthalein retention
F.D'A.	♀	70	Aplastic anaemia	10.1	32	2.6	250	261	96	1	5	6.1	3.5	9
F.A.	♂	54	Ditto	10.4	32	0.8	244	264	92	0.7	8	6.6	4.7	--
A.Sa.	♂	44	Ditto	10.6	34	0.4	358	--	--	0.8	9	7.4	Normal electrophoresis	--
A.W.	♀	83	Ditto	10.6	--	7.6	184	--	--	0.5	25	--	--	23

Fig. 13:

Absorption of labelled haemoglobin by patients with transfusional haemosiderosis.



Patient.	FD'A	FA	AW	AS'R
Hb.	10·1	10·4	10·6	10·6
MCHC.	32	32	-	34
Retics %.	2·6	0·8	7·6	0·4
Serum Fe.	250	244	184	258
% sat. of IBC.	96	92	-	-

In their studies on iron absorption in various types of tissue iron overload Bothwell, van Doorn-Wittkampf, Du Preez and Alper included a small number of patients with transfusion haemosiderosis. Using a double isotope technique, they studied the absorption of a 20-mg. dose of labelled ferric chloride. Two patients had absorptions of 7 per cent. and 8.4 per cent. In four control subjects studied in the same way the range of absorption was from 8 to 25 per cent.

Cappell, Hutchison and Jowett (1957) found that all the iron in the bodies of patients with transfusion haemosiderosis could not be accounted for on the basis of their well-documented blood transfusions. They postulated that these patients must have been absorbing an excessive amount of iron from their diet. It was therefore of particular interest to see whether absorption of food iron as labelled haemoglobin was increased in these patients.

Absorption was low in three patients (Figure 13). In a fourth (A.W.), who showed evidence of regeneration of the bone marrow, it was within normal limits (Table 9). There was therefore no indication that absorption of haemoglobin iron was increased in this small group.

Some of the lowest values of absorption seen in this study occurred in these patients (Figure 26). In the light of work which

will be discussed later (Chapter 10) it is noteworthy that F.D'A., who showed a low level of iron absorption, had marked pancreatic fibrosis at post-mortem.

Chapter 9

IRON ABSORPTION IN PATIENTS WITH CHRONIC ALCOHOLISM

TABLE 10

Haematological Findings, Liver Function Tests and Liver Histology in Chronic Alcoholic Patients

Patient	Sex	Age	Diagnosis	Hb, g./ 100ml.	MCHC	Retics. %	Serum iron, µg./ 100ml.	TIBC, µg./ 100ml.	Saturation of TIBC %	Bilirubin, mg./ 100ml.	Alk. phosphatase units	Total protein g./ 100ml.	Albumin g./ 100ml.	Bromsulphthalein retn.	Liver biopsy
L.K.	♂	56	Alcoholic	14.1	--	--	130	338	38	0.2	11	6	4.1	17	Normal
W.M.	♂	58	Alcoholic; gastrectomy	16.6	32	1.4	124	--	--	0.2	3	6.8	4.4	3	Fat vacuoles in liver cells

Whilst there are no reports of iron absorption studies in chronic alcoholism in man, some work has been done on the effect of alcohol on iron absorption in animals.

Brown (1964) has shown that rats maintained on a drinking supply containing 20 per cent. alcohol showed increased absorption of ferrous iron compared with normal control rats. When after some weeks distilled water was substituted for the diluted alcohol the absorption of iron in the rats returned to normal. It is of interest to note that no change in hepatic histology was noted in the 'alcoholic' rats.

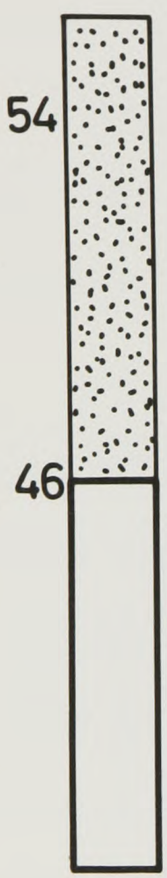
In a further study on the acute effect of alcohol, administration of ferrous iron with alcohol did not increase iron absorption. There was, however, an increase in iron absorption when ferric iron and alcohol were given together. This is particularly interesting since Bothwell (1964) showed that when alcohol and ferric iron were given together absorption of iron was greatly increased in human subjects.

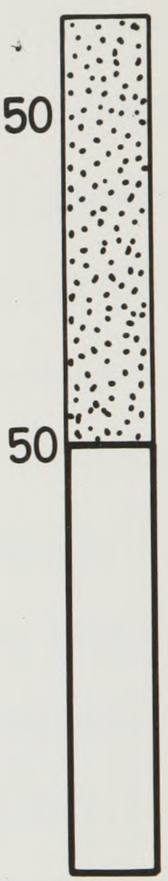
In discussing absorption of iron in cirrhosis of the liver (Chapter 6) it was noted that several workers had emphasised the association between chronic alcoholism and increased iron absorption in their patients with alcoholic cirrhosis. In view of this and Brown's findings in animals, it was of interest to study absorption in chronic alcoholic patients who, as yet, showed only minimal evidence of liver damage.

The absorption of labelled haemoglobin was high in both patients

Fig. 14:

Absorption of labelled haemoglobin by chronic alcoholic patients.





Patient.	LK	WM
Hb.	14·1	16·6
MCHC.	-	3·2
Retics %.	-	1·4
Serum Fe.	130	124
% sat. of IBC.	38	-

(Figure 14). It was thought that the absorption of haemoglobin in W.M. would be low as the result of his total gastrectomy. However in the patients with total gastrectomy given labelled haemoglobin the mean absorption was shown to be 11 per cent. with a range of 3 to 20 per cent. The absorption of 50 per cent. seen in W.M. is therefore considerably above that found in patients with total gastrectomy. There was no anaemia, sideropaenia or evidence of recent bleeding to account for this finding. The patients studied were co-operative but still obviously drinking at the time of these studies (Table 10).

Chapter 10

THE EFFECT OF PANCREATIC EXTRACTS ON IRON ABSORPTION IN HAEMOCHROMATOSIS, CIRRHOSIS OF THE LIVER, CHRONIC ALCOHOLISM, ACUTE LIVER DISEASE AND IRON-DEFICIENCY ANAEMIA

The importance of the pancreas in relation to iron absorption was first demonstrated by Taylor, Stiven and Reid (1931). In studying the tissues of a cat that had survived in good health for a considerable time after pancreatectomy, they were surprised to find a heavy deposit of iron in the liver. Because spontaneous haemosiderosis may occur in town-dwelling cats they repeated the experiment with more carefully controlled conditions but this time instead of removing the pancreas they ligated the pancreatic duct. Tissue iron again increased, (Taylor, Stiven and Reid, 1935). In studying the small-intestinal mucosa of these animals they noted villous atrophy. They thought that because the mucosal cells at the tips of the villi were atrophied they could no longer prevent the absorption of iron. As this was seen in animals with a deficiency in vitamin A, it was postulated that the pancreas was responsible for the development of avitaminosis A which then produced mucosal atrophy. When the cellular 'barrier' to absorption was removed unrestricted absorption of iron occurred.

These findings were confirmed by Gillman, Gillman, Mandlestam and Gilbert (1947). The problem was restudied in dogs by Kinney, Finch, Kaufman, Hegsted and Partington in 1950. Successful ligation of the pancreatic duct with resultant atrophy of the acinar portion

of the pancreas was associated with a five-fold increase of hepatic iron. In one dog studied with radioactive iron following duct ligation the absorption was found to be greater than in normal control dogs. When dogs who had been subjected to pancreatic duct ligation were fed raw pancreas the increase in hepatic iron was not seen. These experiments would suggest that there is a factor present in the secretion of the pancreas which reduces the absorption of iron. These studies do not support the hypothesis that vitamin A deficiency is the reason for the increased absorption seen in the absence of pancreatic secretion.

However there are some conflicting results, for in studies on two dogs who had been subjected to pancreatic ligation Wheby, Conrad, Hedberg and Crosby (1961) found decreased absorption of iron.

Damage can be produced in the pancreatic tissue of rats by feeding the methionine antagonist d,l-ethionine. Kinney, Kaufman and Klavins (1955) found that the livers of rats who had been given ethionine contained more iron than normal controls. This could be exaggerated by feeding the rats on a diet with an increased iron content. It was shown that in addition to an increase of iron in the

liver there was an increase in the total body iron, hence the increased amount of iron in the liver could not be explained by redistribution. A criticism of this experiment is that ethionine not only damages the pancreas and makes the animals ill but also produces marked changes in the intestinal epithelium and the liver (Biggs, 1964, personal communication).

Brown (1964) has attempted to repeat some of these observations using smaller doses of ethionine coupled with a methionine-deficient diet. She hoped thereby to reduce the damage to other tissues to a minimum and to get a more clearcut disturbance of pancreatic function. The effectsof ethionine and methionine-deficient diets were studied in normal rats and rats who had been rendered iron-deficient by rearing them on a diet low in iron.

In the normal group the animals were divided so that one-half had ethionine and the other half acted as controls. At the end of the first week the animals were all given labelled ferrous sulphate and the iron absorption of the two groups was compared. The rats given ethionine absorbed significantly more iron than the control animals.

The experiment was repeated in an exactly similar way except that iron-deficient rats were used. Brown was surprised to find that the iron-deficient rats given ethionine absorbed significantly less iron than the iron-deficient controls.

TABLE 11

Studies on Absorption in Pancreatic Disease and Haemochromatosis

Condition studied	Authors	Method of studying iron absorption	Dose of iron	Percentage of iron absorbed	Percentage of iron absorbed in controls
Chronic pancreatitis	Davis and Badenoch (1962)	Labelled meal; radio-isotope balance technique	3 mg. in standard meal	23,24,24,25,31,39	0-13 (8 controls) Mean 6%
Chronic pancreatitis	Saunders, Banks, Arch and Williams (1963)	Labelled $FeSO_4$; radio-isotope balance technique	Not given	6 showed increased absorption	16 controls
Fibrocystic disease in children	Sephton Smith (1964)	Labelled Hb; radio-isotope balance technique	2 or 5 mg. of iron as Hb	19 and 30.5 (untreated) 4.0 to 12.5 (mean 7.5) in 11 patients treated for over 1 year with pancreatin	11-38 (8 controls) Mean 22.4%
Haemo-chromatosis	Biggs and Davis (1963)	Labelled $FeSO_4$; whole-body counting technique	5 mg.	22-96 (mean 51). 0-86 (mean 35) in 10 patients given pancreatin	Not stated

In discussing these results she considered that the gastro-intestinal tract must be altered in iron deficiency so that pancreatic secretion actually promotes absorption. In a further experiment it was demonstrated that when ethionine-treated iron-deficient rats were given pancreatin the absorption of iron was increased to within normal limits.

In order to avoid the severe disturbance produced by metabolic poisons Biggs and Davis (1964) studied the effect of pancreatic enzymes in isolated loops of rat small intestine. They have used the rate of transference of radioactive iron out of these loops as a measure of absorption and have shown it is reduced by pancreatin. This inhibition of absorption is not affected by altering the pH of the contents of the lumen.

Iron metabolism in human pancreatic disease was first noticed to be abnormal by Andersen (1938). In post-mortem studies on 49 children who died from fibrocystic disease of the pancreas, 14 were noted to have haemosiderosis of the liver.

Davis and Badenoch (1962) showed that increased absorption of iron occurred in six out of 14 patients with chronic pancreatitis (Table 11). They found that only one of their seven patients with severe chronic pancreatitis and pancreatic calcification had normal iron absorption and this patient had been treated with pancreatin for

four years. The increase in absorption was not seen in patients with less severe degrees of chronic pancreatitis or patients with acute pancreatitis. They noted that iron absorption was reduced to normal in one patient given pancreatin at the time of the iron absorption test. Similar findings were reported in South African patients with chronic pancreatitis by Saunders, Bank, Airth and Williams (1963) but details of their studies are not given.

Sephton Smith (1964), studying children with fibrocystic disease of the pancreas, has noted a high absorption of iron in untreated children which has gradually fallen during continuous treatment with pancreatin. Decreased iron absorption occurred after as little as three months on pancreatin. Low absorption was found when the test was carried out five days after stopping pancreatin.

From the studies by Badenoch and Davis in adults and Sephton Smith in children, increased iron absorption can be seen in patients with primary pancreatic disease. Pancreatic fibrosis is known to occur in haemochromatosis and Biggs and Davis showed that the absorption of ferrous iron was increased in this condition and could be reduced to normal levels by giving pancreatin.

Woldman, Fishman and Segal (1959) and MacDonald and Mallory (1960) have reported a high incidence of pancreatic fibrosis in cirrhosis of the liver. It was of particular interest to see whether

Fig. 15: The effect of pancreatin on oral iron absorption in patients with haemochromatosis.

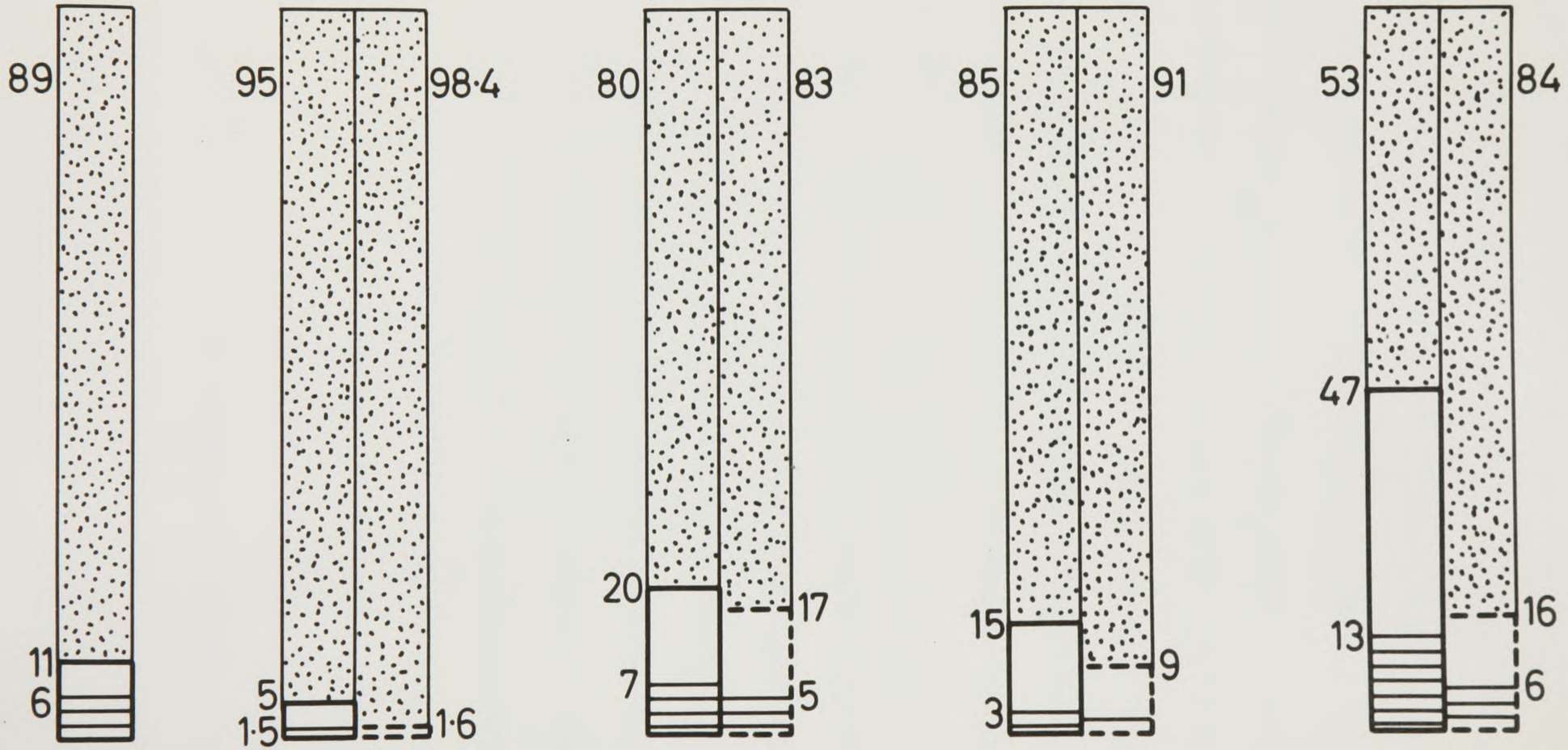
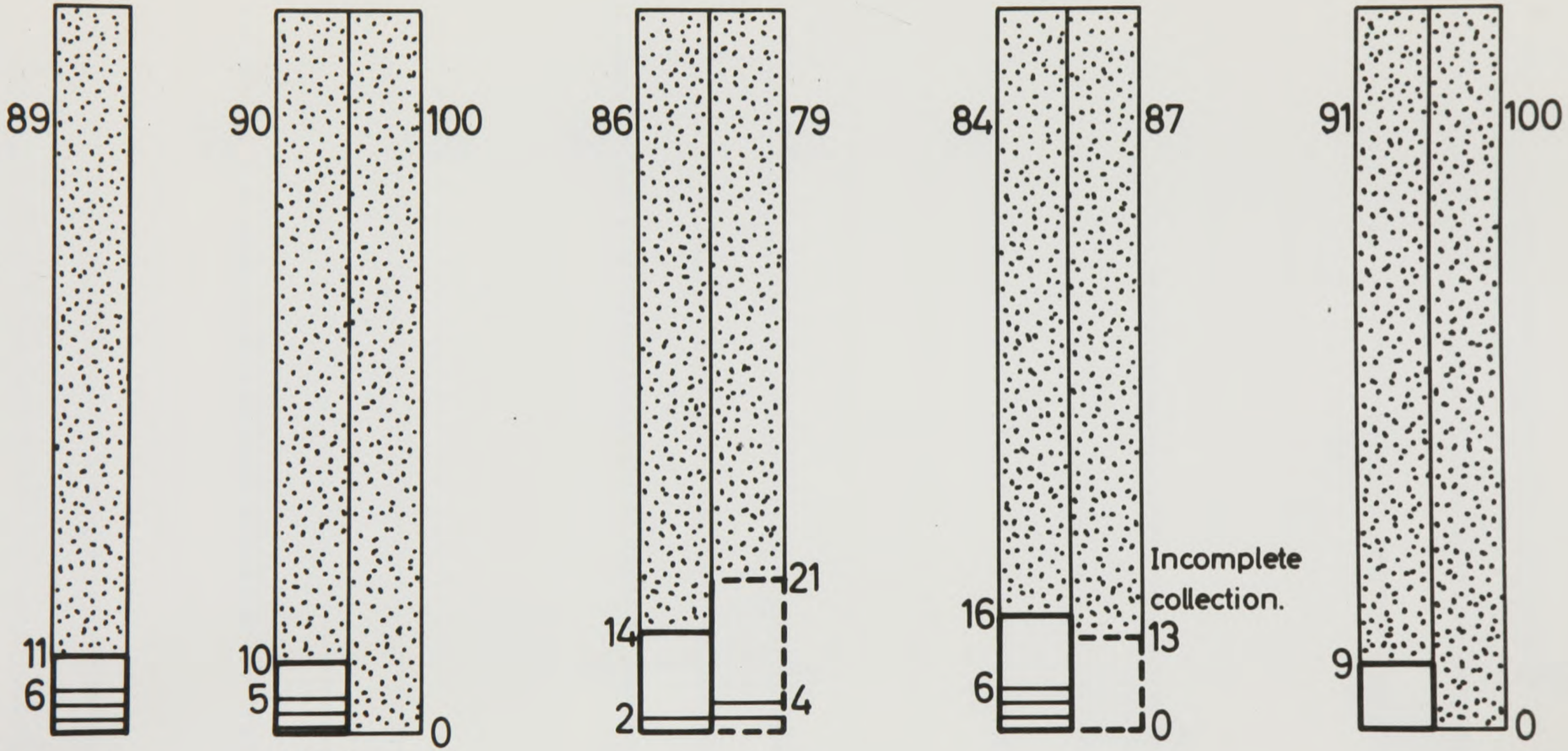


Fig. 16: The effect of pancreatin on oral iron absorption in patients with haemochromatosis.



Normal control.

Patient. RF

ET

BJ

LD

Mean absorption of ^{59}FeHb in 24 normal subjects.

% absorbed given with pancreatin.

the increased absorption noted in some patients with cirrhosis of the liver could be affected by pancreatin. The present study is an extension of the investigation first reported by Callender and Malpas (1963). When it became evident that pancreatin had an effect on absorption in chronic liver disease the possibility that it might affect iron absorption in acute liver damage was investigated and in addition a few observations were made on the activity of one constituent of pancreatin, the enzyme trypsin.

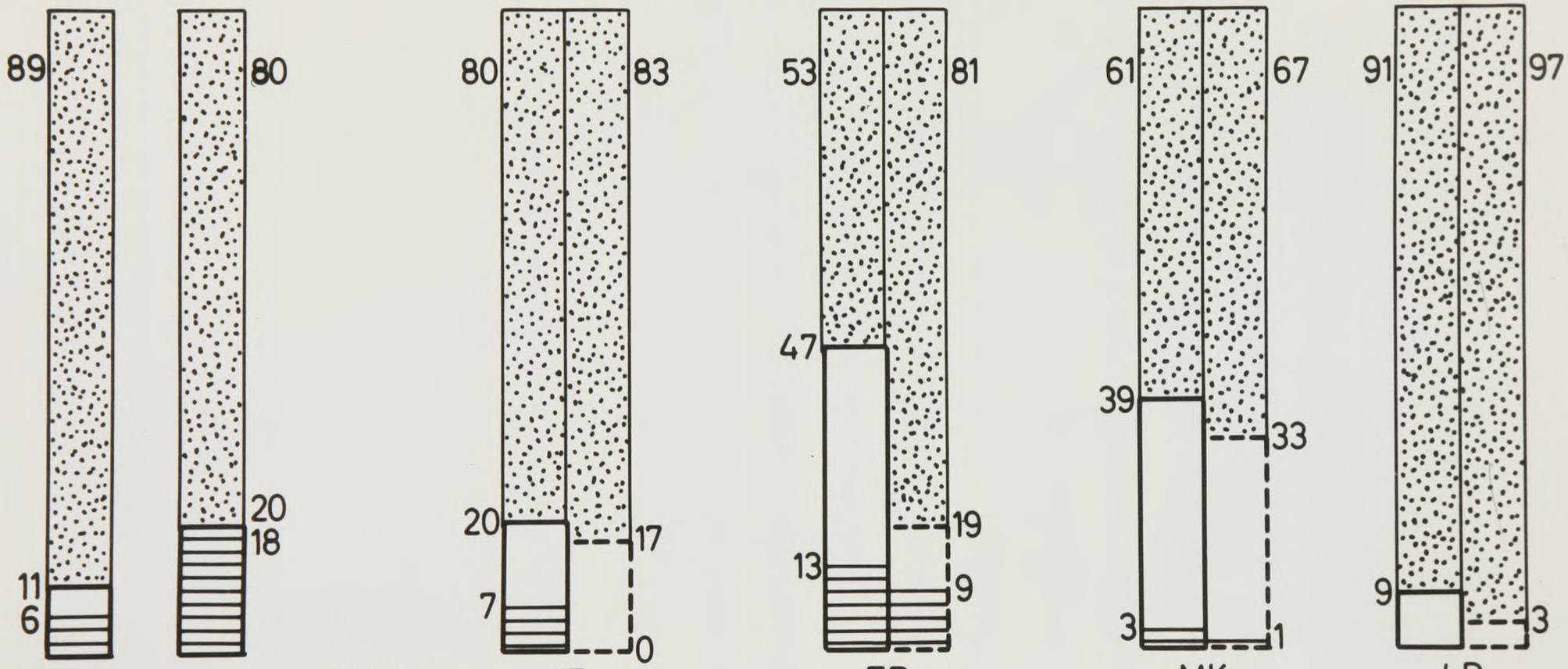
The contradictory results obtained by Brown in iron absorption studies on normal and iron-deficient rats with pancreatic damage suggested that human subjects with iron-deficiency anaemia should be investigated. These patients represent a relatively simple model for study since they have an avidity for iron but no pancreatic damage.

Results in the Present Study

The effect of pancreatin on iron absorption in patients with haemochromatosis: The effect of 2.5 g. of pancreatin given as Cotazym (Organon) was studied in eight patients with haemochromatosis (Figures 15 and 16). In every instance the iron was given in the form of labelled haemoglobin. The pancreatin was dissolved in water immediately before administration. It was given to the patient about five minutes before the dose of labelled haemoglobin. The absorption of haemoglobin iron ranged from 5 to 47 per cent. with a mean of 17

Fig. 17:

The effect of trypsin on the absorption of labelled haemoglobin in patients with cirrhosis and haemochromatosis.



Mean absorption in 24 normal controls and 27 iron deficient patients given ^{59}Fe labelled haemoglobin.

□ % absorbed given with trypsin.

per cent. when given unaccompanied, to 0 to 21 per cent. with a mean of 9.7 per cent. when given with pancreatin. Whilst pancreatin produces a definite trend towards decreased absorption, it is not statistically significant (see Statistical Appendix III).

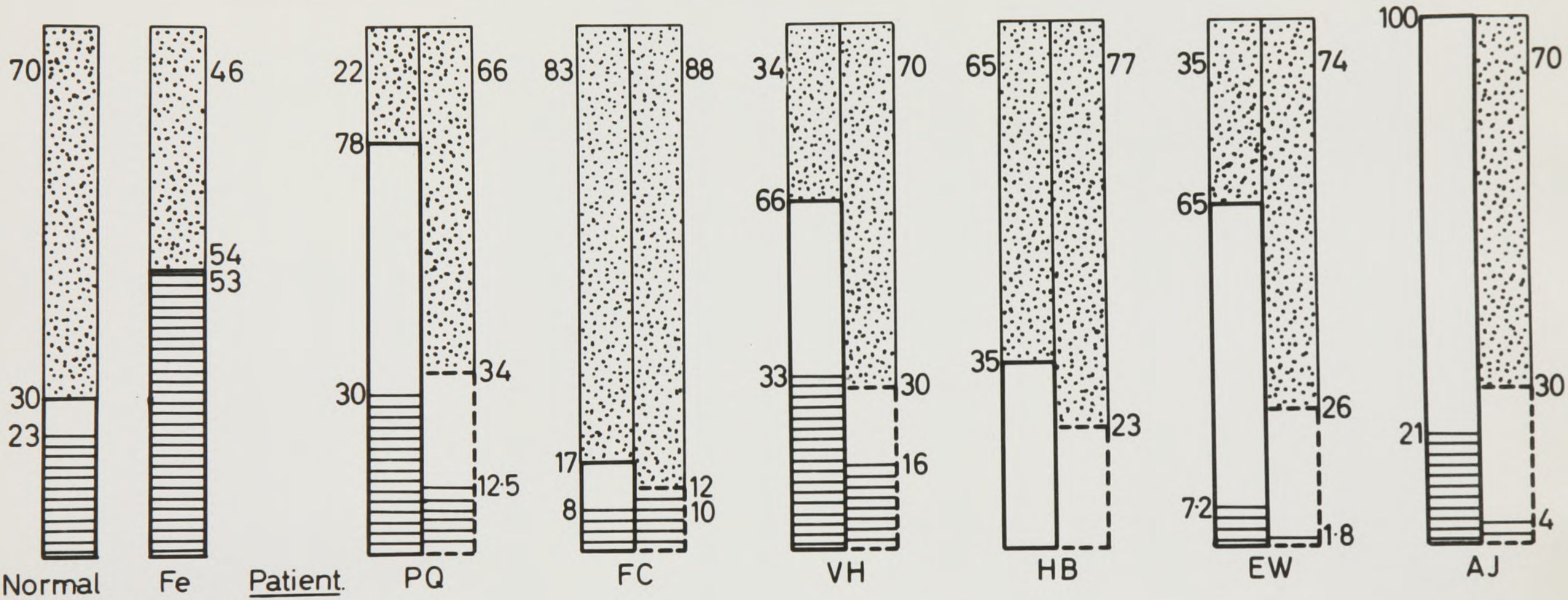
The most definite trend was seen in the patient (E.P.) who had completed a course of venesections and had the greatest increase in iron absorption, vide supra. On analogy with this patient it might well be that further studies in patients with haemochromatosis who have been successfully treated by venesection could be more conclusive in demonstrating the effect of pancreatin.

The effect of trypsin on iron absorption in haemochromatosis is shown in Figure 17. Three patients with haemochromatosis were studied and the absorption of labelled haemoglobin was reduced from 20 per cent. to 17 per cent., from 47 to 19 per cent. and from 9 to 3 per cent., respectively. This shows a trend towards decreased absorption when trypsin is given but the number of studies is, of course, small.

In conclusion, therefore, pancreatin produces a reduction in the amount of iron absorbed in the form of labelled haemoglobin but the effect is not so dramatic as that which was noted by Biggs and Davis when the dose was given as an inorganic iron salt.

Fig. 18:

The effect of pancreatin on the absorption of labelled ferrous sulphate given by mouth.

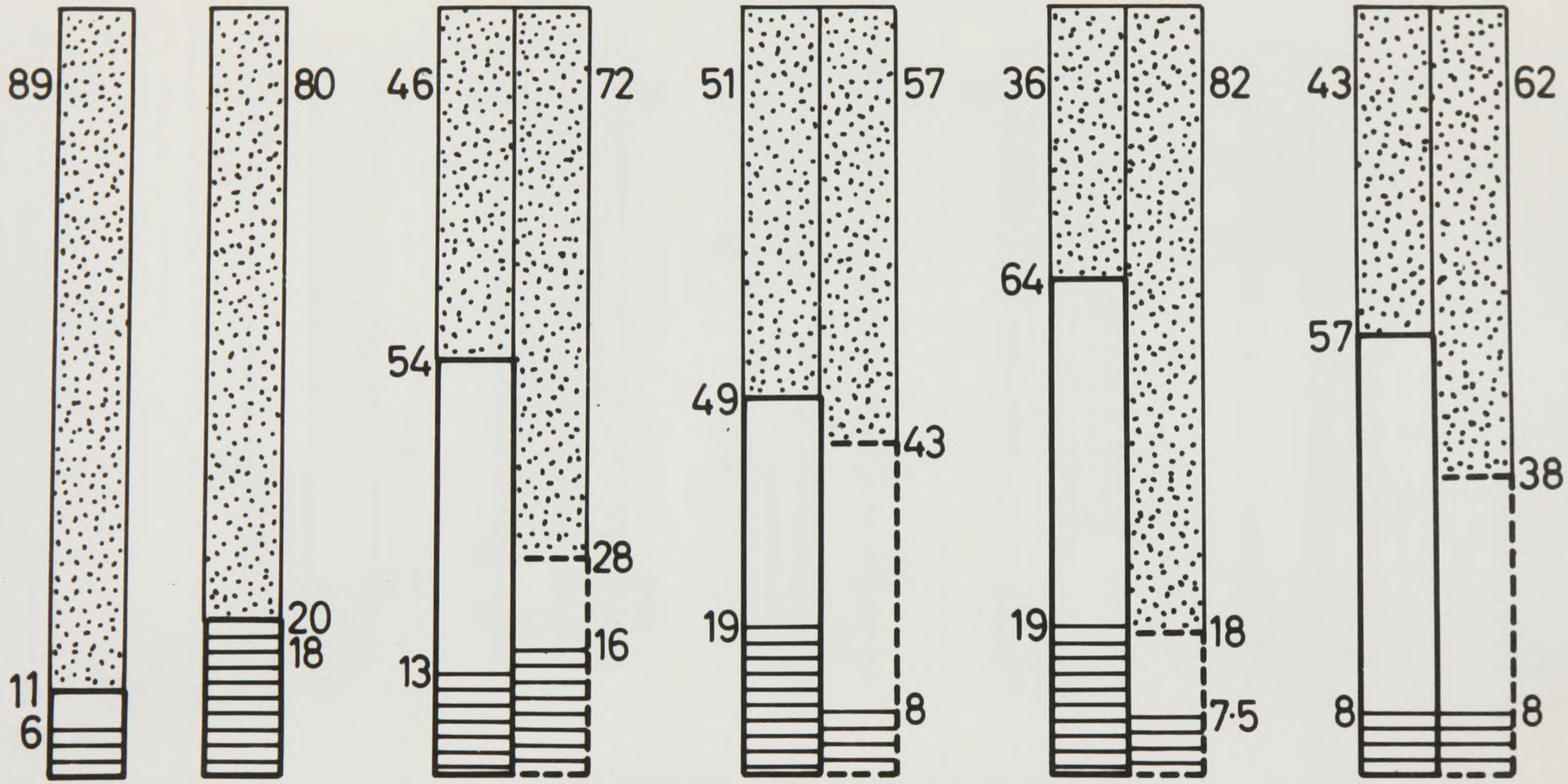


Mean absorption in 37 normal controls and 19 iron deficient patients given ^{59}Fe labelled ferrous sulphate.

All patients had cirrhosis of the liver.

% absorbed given with pancreatin.

Fig. 19: **The effect of pancreatin on the absorption of labelled haemoglobin given by mouth.**



Mean absorption in 24 normal controls and 27 iron deficient patients given ^{59}Fe labelled haemoglobin.

All patients had cirrhosis of the liver.

□ % absorbed given with pancreatin.

The effect of pancreatin on iron absorption in patients with cirrhosis of the liver: Studies have been carried out on iron absorption in 10 patients who had alcoholic or non-alcoholic cirrhosis (Tables 5 and 6). The effect of 2.5 g. of pancreatin on the absorption of radioactive iron was studied in paired absorption tests. The order in which the pancreatin was administered was randomised. Six patients were given labelled ferrous sulphate and four were given iron in the form of haemoglobin (Figures 18 and 19).

The absorption of iron was reduced in each of the six patients when pancreatin was given with a dose of labelled ferrous sulphate. The absorption of labelled ferrous sulphate alone ranged from 17 to 100 per cent. with a mean of 60 per cent. Absorption was reduced to 12 to 34 per cent. with a mean of 26 per cent. when the dose was given with pancreatin. In four patients who had markedly increased absorption (P.Q., V.H., E.W. and A.J.) pancreatin reduced the level of absorption to about the mean value found in normal control subjects. This reduction was statistically significant (see Statistical Appendix III).

In patients H.B., P.Q., E.W. and P.J. the same decreased absorption of inorganic iron when pancreatin accompanied the dose could be demonstrated by the double isotope technique of Brise and Hallberg (Figure 20). The poor utilisation of the isotopic iron in patients with cirrhosis makes the method less suitable than the balance technique.

Fig. 20: **THE EFFECT OF PANCREATIN ON THE ABSORPTION OF IRON DEMONSTRATED BY THE DOUBLE ISOTOPE TECHNIQUE.**

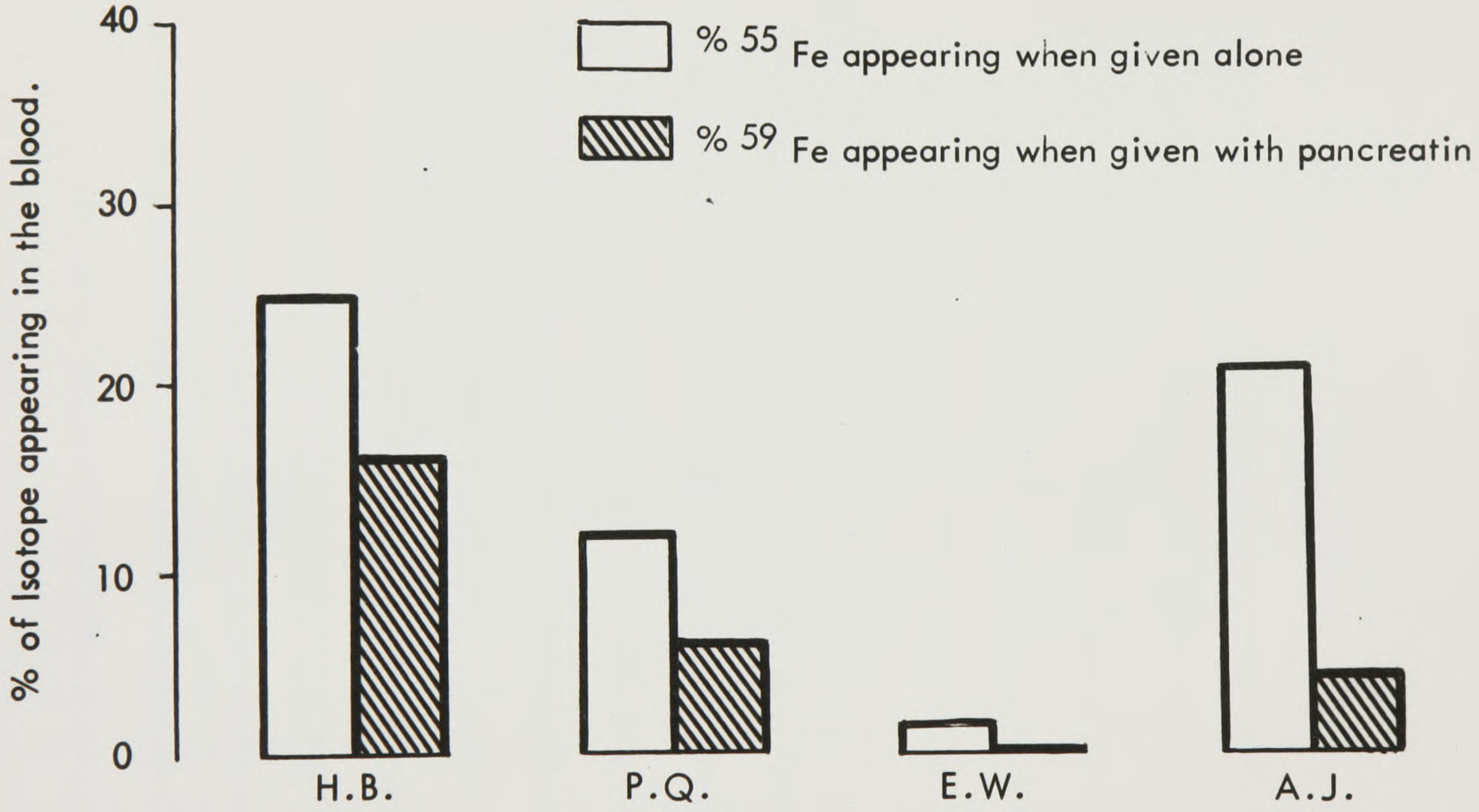
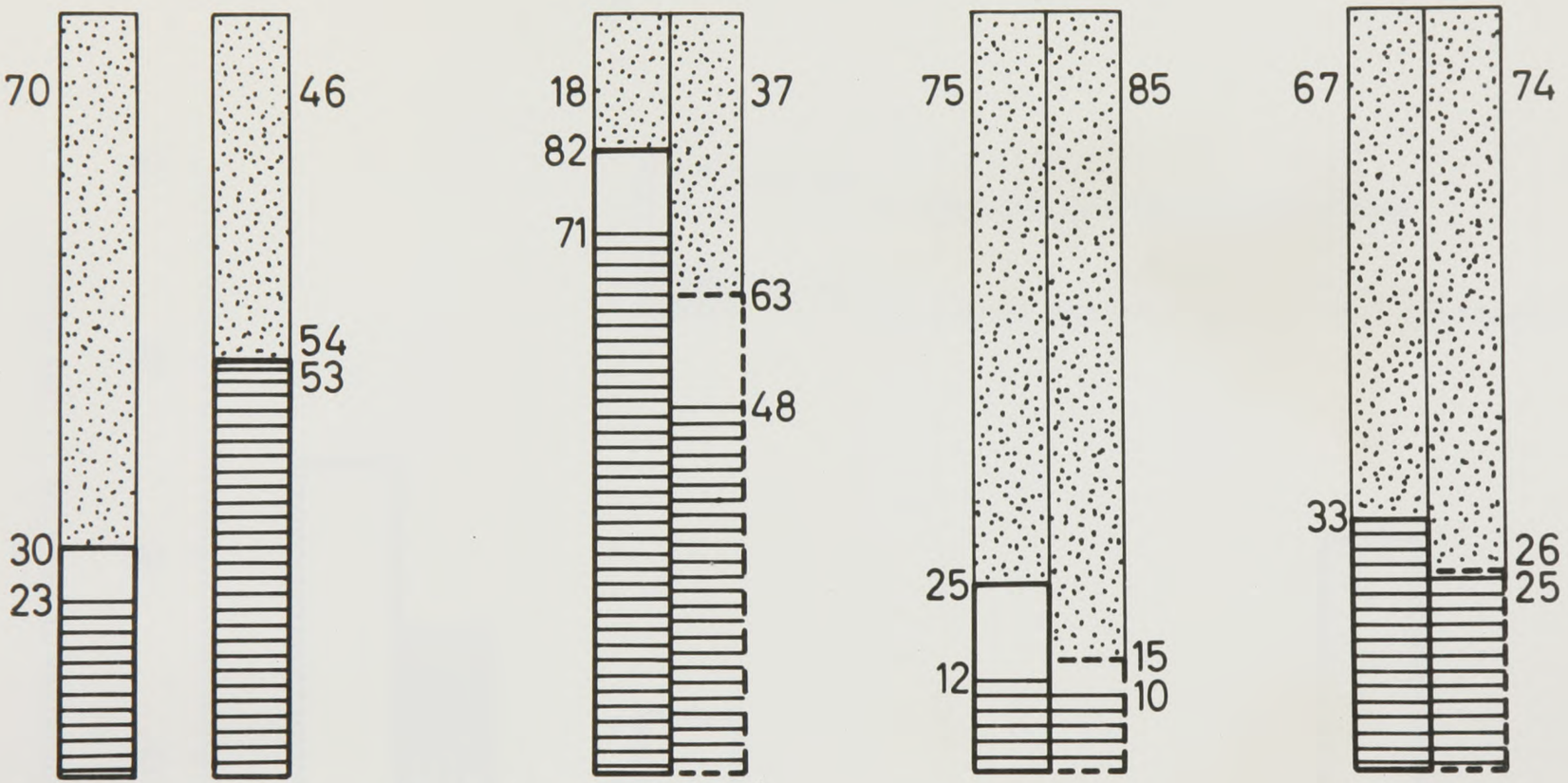


Fig. 21: The effect of trypsin on the absorption of labelled ferrous sulphate in patients with cirrhosis.



Mean absorption in 37 normal controls and 19 iron deficient patients given ^{59}Fe labelled ferrous sulphate.

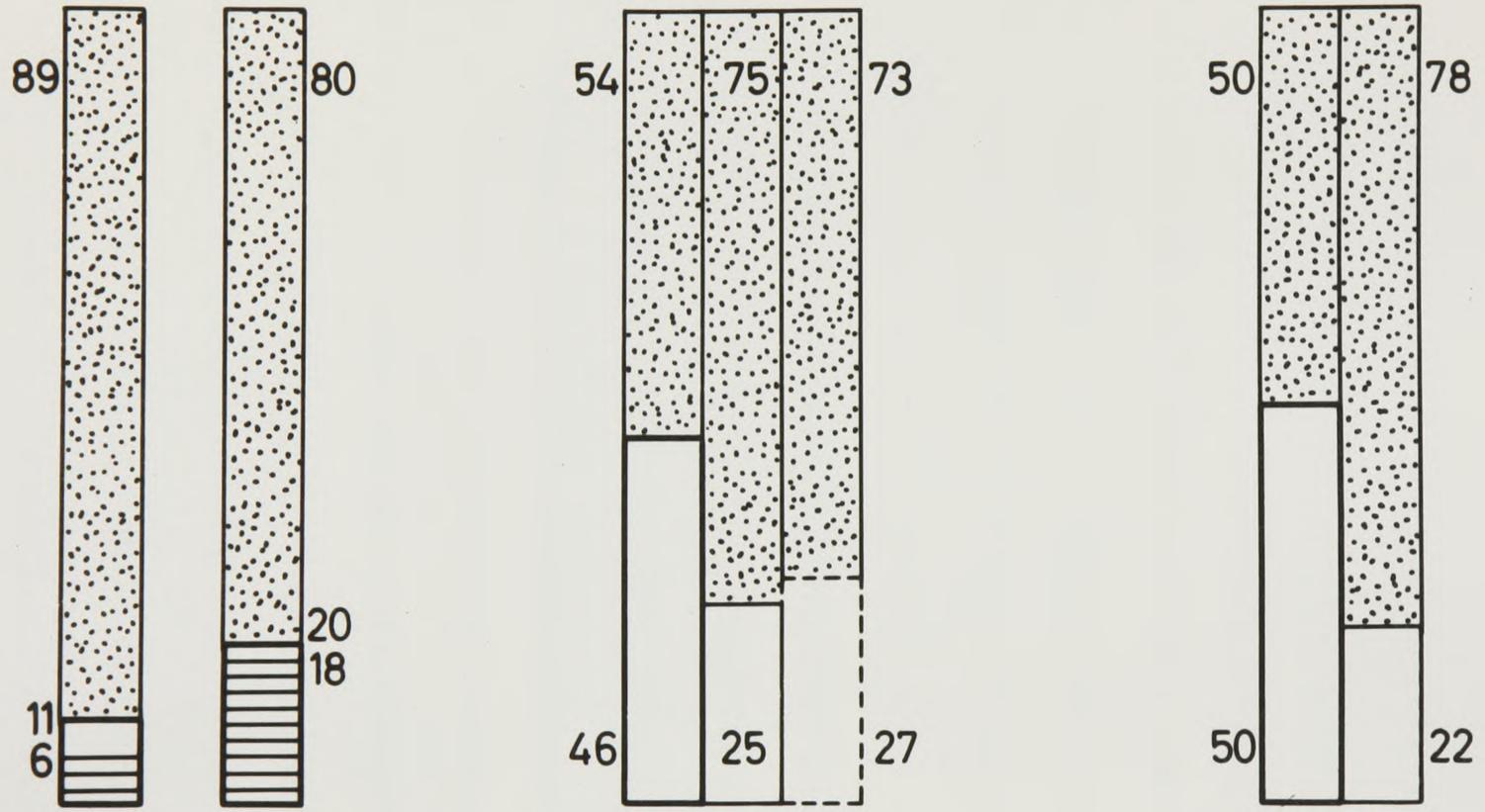
Absorption of iron was decreased when 2.5 g. of pancreatin was added to the dose of labelled haemoglobin given to four subjects. The percentage absorption when haemoglobin was given alone was 49, 54, 57 and 69 per cent. with a mean absorption of 56 per cent., which was reduced to 28, 43, 18 and 38 per cent. with a mean absorption of 31 per cent. when given with pancreatin.

In four patients with cirrhosis in whom the effect of trypsin was studied a decrease in absorption was also noted (Figures 17-21). Three were given labelled ferrous sulphate and absorption was reduced from 82 to 63, 25 to 15 and 33 to 26 per cent. respectively. Absorption was thus diminished by trypsin in each case. In one patient given labelled haemoglobin the absorption was reduced from 39 to 33 per cent. by trypsin, which is clearly inconclusive.

Preliminary studies on the nature of the inhibitory factor: It was evident from the effect of pancreatin in reducing absorption both of inorganic and haemoglobin iron, that the effect of pancreatin was not simply due to precipitation of the iron. When pancreatin was boiled and then given to a patient with cirrhosis of the liver it had no significant effect on the absorption of labelled haemoglobin; the inhibitory effect was abolished. In the patient studied absorption was 12 per cent. of the dose given alone and 16 per cent. when accompanied by boiled pancreatin.

When pancreatin was acidified to pH 2.5 and allowed to stand

Fig. 22: **The effect of pancreatin and trypsin on the absorption of labelled haemoglobin in patients with chronic alcoholism.**



Mean absorption in 24 normal controls and 27 iron deficient patients given ^{59}Fe labelled haemoglobin.

Percentage absorbed given alone.

Percentage absorbed given with pancreatin.

Percentage absorbed given with trypsin.

for an hour and then neutralised it retained much of its capacity to reduce iron absorption. The results of the studies in two cirrhotic patients are shown in Table 12.

TABLE 12

Patient	% absorption of inorganic iron given alone	% absorption with 2.5 g. treated pancreatin	% absorption with 2.5 g. untreated pancreatin
1	100	60	30
2	60	29	30

The treatment of pancreatin with acid in this way destroys lipase and amylase but preserves the activity of trypsin.

These preliminary studies, together with the effect observed with pure trypsin, suggest that enzymes and possibly trypsin are concerned at some stage of the process of preventing iron absorption.

The effect of pancreatin on iron absorption in patients with chronic alcoholism: In two patients with chronic alcoholism but with little evidence of liver damage both pancreatin and trypsin reduced the absorption of labelled haemoglobin (Figure 22).

In the above experiments the attempt was made to give equivalent amounts of Cotazym and trypsin. Little information was available on the relative activities of the two preparations. It is of interest to note that in two patients (L.K., the chronic alcoholic and E.P.: Figure 22) there was quite similar effect on the inhibition

Fig. 23:

The effect of pancreatin on the absorption of labelled ferrous sulphate and labelled haemoglobin given by mouth in patients with acute liver disease.

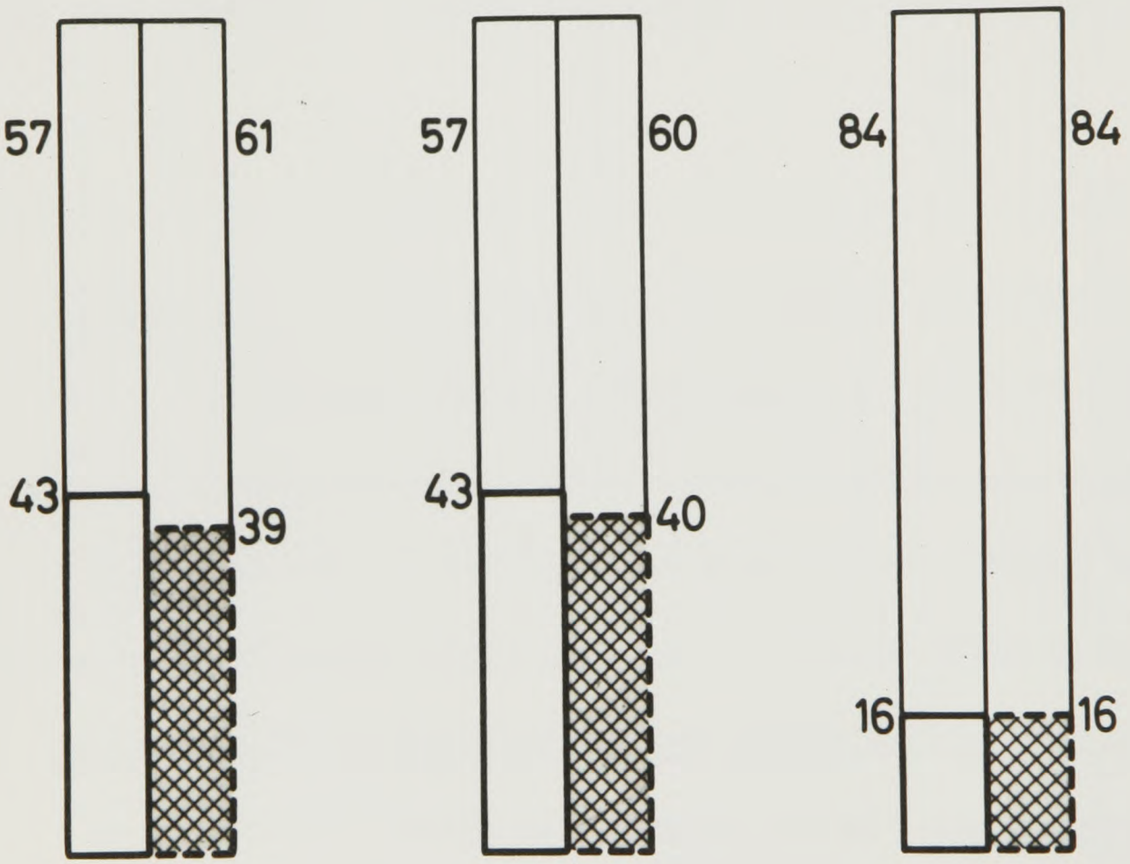


TABLE 12a

Effect of pancreatin on oral iron absorption in patients with iron-deficiency anaemia

Subject	Absorption of $^{59}FeSO_4$ given alone %	Absorption of $^{59}FeSO_4$ when given with pancreatin, %
1	7	28
2	71	20
3	56	34
4	51	44
5	19	20
6	61.5	64
7	44	66
8	84.5	55
9	62	74
10	37	13
11	93	82

	Absorption of $^{59}FeHb$ given alone	Absorption of $^{59}FeHb$ when given with pancreatin
12	4.4	0
13	0	13
14	3	27
15	16	53
16	56	22

Fig. 24:

The effect of pancreatin on oral iron absorption when given to patients with iron deficiency anaemia.

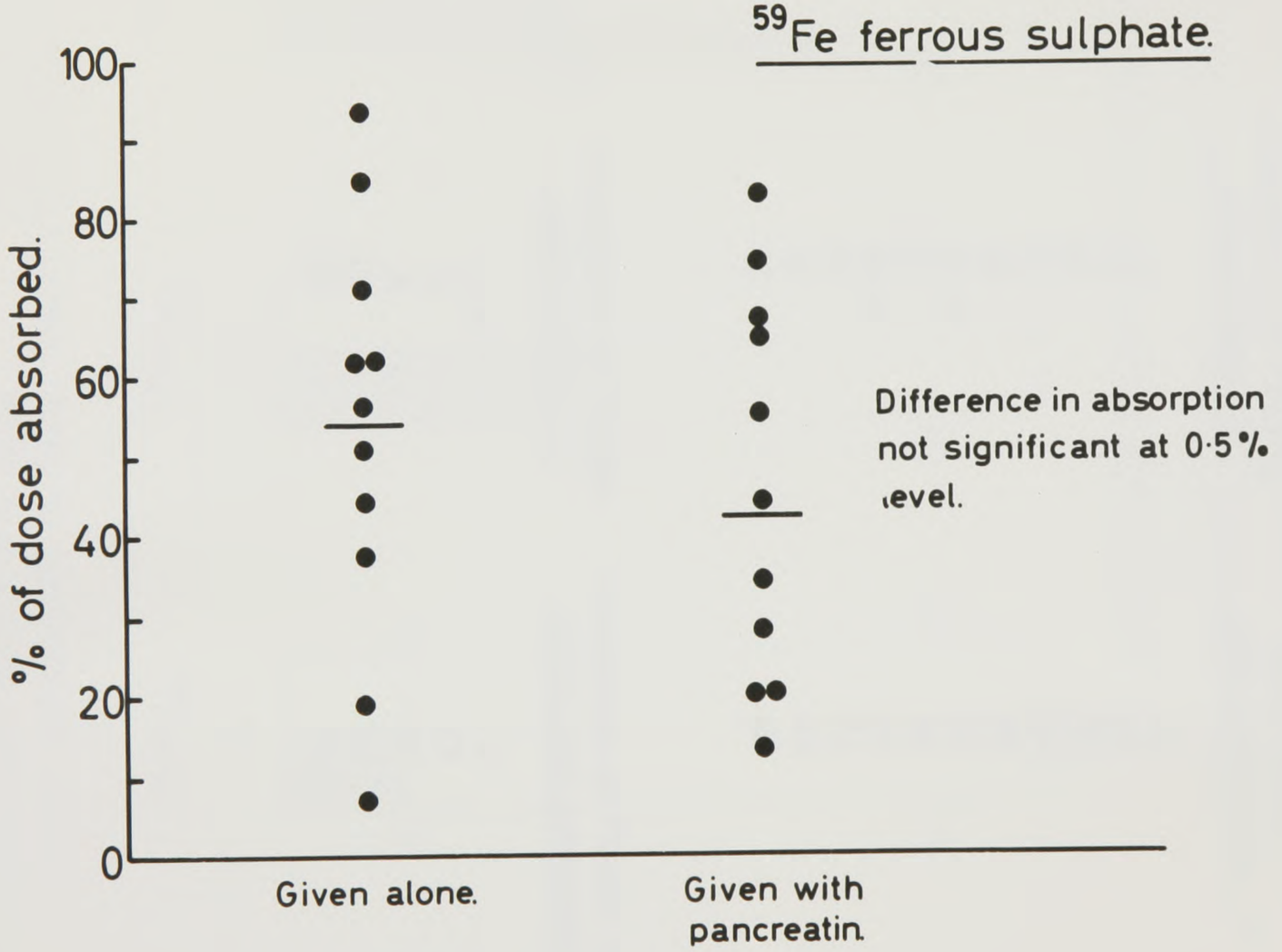
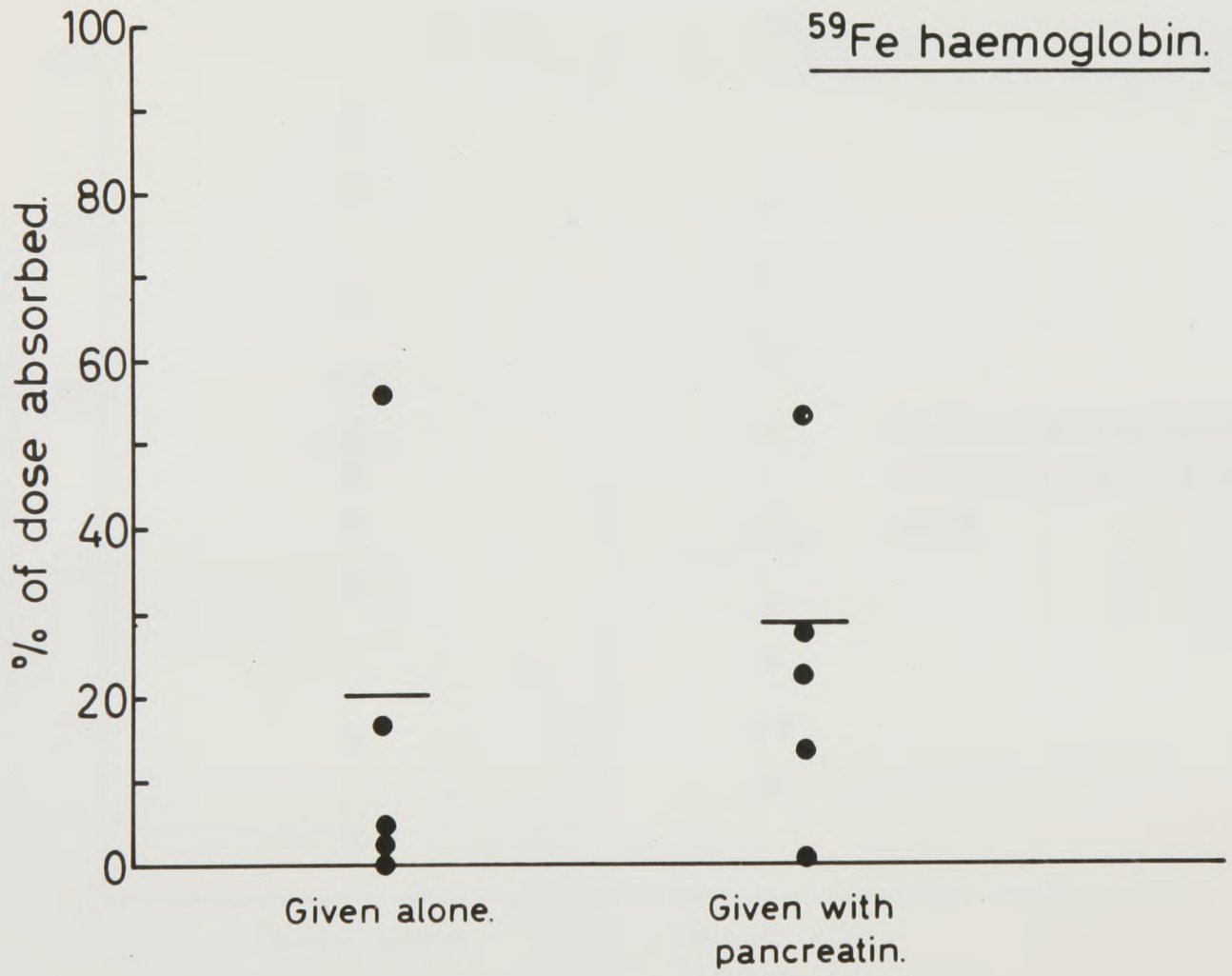


Fig. 25:

The effect of pancreatin on oral iron absorption when given to patients with iron deficiency anaemia.



of iron absorption of the 2.5 g. of pancreatin and 6-g. dose of trypsin.

The effect of pancreatin on iron absorption in acute liver disease: No effect on either labelled ferrous sulphate absorption or the absorption of haemoglobin iron was seen in three patients with acute liver disease (Figure 23). Two patients with quite high absorption for this group were deliberately chosen for further study (G.P. and M.D.). The former had iron-deficiency anaemia and the latter had untreated lupoid hepatitis. Even so no decrease in absorption was seen with a dose of 2.5 g. of pancreatin.

The effect of pancreatin on iron absorption in iron-deficiency anaemia: Patients with iron deficiency form a relatively simple model of a state in which there is an increased avidity for iron. The effect of pancreatin on the absorption of labelled ferrous sulphate or labelled haemoglobin was studied because it was considered that these patients would not have pancreatic fibrosis and the effect of pancreatin in a condition unlikely to be accompanied by diminished pancreatic function would be of interest. Eleven iron-deficient patients had paired absorption studies using labelled ferrous sulphate and five were given haemoglobin iron. The results are shown in Figures 24 and 25 and in Table 12a.

The effect of pancreatin on the absorption of labelled iron:

Pancreatin reduced absorption of ferrous sulphate in six patients and absorption of labelled haemoglobin in one patient. It appeared to enhance absorption in other patients. The variability of the results in iron-deficient subjects contrasts with the consistent reduction in absorption seen in patients with cirrhosis of the liver.

The increase in absorption seen in some patients with iron deficiency given pancreatin recalls the results that Brown observed in her iron-deficient rats. At the moment these observations are not satisfactorily explained.

The remarkable variation in the effect of pancreatin did not seem to be associated with any particular feature of the patients' anaemia, for example, the severity of the anaemia or its aetiology. Not enough studies on gastric acid secretion were available in the group to justify any conclusions about the presence of acid.

This factor of acidity might well be important in explaining some of the results obtained. The secretions of the pancreas are stimulated by the presence of acid in the duodenum and this acid is secreted from the gastric mucosa in response to a meal. The decrease in absorption of iron which has been noted to occur when a meal is given together with labelled iron has been explained largely on a

mechanical basis. If, as seems likely, the pancreas plays a part in inhibiting absorption of iron, a humoral explanation might be more likely. The presence of a meal may be a better stimulus to pancreatic secretion than a fluid test dose of iron and therefore a larger amount of inhibitor may be produced, reducing the absorption of iron from the meal.

Further work on the effect of pancreatin on iron absorption in normal subjects, iron-deficient subjects and patients who are achlorhydric would be necessary to justify this hypothesis.

Conclusion

Pancreatin reduces the absorption of both ferrous sulphate and haemoglobin iron in patients with cirrhosis of the liver, haemochromatosis and chronic alcoholism. It does not affect absorption in acute liver disease. The effect on iron absorption in patients with iron-deficiency anaemia is extremely variable and at the moment inexplicable.

Chapter II

DISCUSSION

The present studies on the absorption of iron in cirrhosis of the liver have demonstrated an increase not only in the absorption of inorganic iron but also food iron as represented by labelled haemoglobin. In some patients in this study the presence of anaemia or recent bleeding would account for the increase in iron absorption. However, in a proportion of those studied the cause for the increase is not obvious.

Bothwell, Pirzio-Biroli and Finch (1958), Pirzio-Biroli and Finch,(1960) Cantrill and Walsh (1962) and Cantrill, Walsh, Brading and George (1962) have shown that the two main factors influencing the absorption of iron are the rate of erythropoiesis and the state of the body iron stores. It would be expected that patients who had recent episodes of gastrointestinal bleeding would show an increase of erythropoietic activity. When bone marrow smears from four patients with cirrhosis in the present study who had no evidence of recent bleeding were examined, a moderate increase in erythropoietic activity was seen in three of the patients. Increase in erythropoiesis may also be due to haemolysis which may be present in patients with chronic liver disease.

In studying a large number of patients with all types of liver disease Pitcher (1962) has demonstrated by using red cells labelled

with chromium^{51} that many of these patients have some shortening of red-cell survival times and hence a mild haemolytic state. Conrad, Berman and Crosby (1962), studying one of their patients with cirrhosis of the liver who showed increased iron absorption and increased tissue iron stores, found that chromium^{51}-labelled red cells survived a normal length of time. Greenberg, Strohmeyer, Hine, Keene, Curtis and Chalmers (1964) examined bone marrow biopsies from a large series of cirrhotic patients showing increased absorption of iron. They found no increase in the relationship of red-cell precursors to the white cells and concluded that the erythropoietic activity of the marrow was not increased. The balance of evidence, therefore, does not suggest that the increase in iron absorption can be explained satisfactorily on increased erythropoietic activity.

The second major influence on the absorption of iron is the state of the body's iron stores. An estimate of the tissue stores may be made from the determination of the iron in the serum, from determination of the saturation of the iron-binding protein transferrin, and from bone marrow biopsies or liver biopsies stained for iron.

In studies on the patients' sera the saturation of the iron-binding capacity gives a better indication of the tissue iron stores than the serum iron alone. Both were estimated in patients in the

TABLE 13

Serum iron, total iron-binding capacity and percentage saturation in patients with cirrhosis of the liver

Patient	Diagnosis	Serum iron µg./100ml.	Total Iron-Binding Capacity: Peters' method µg./100 ml.	Total Iron-Binding Capacity: Radio-isotope method µg./100ml.	Total Iron-Binding Capacity: Immuno-diffusion method µg./100ml.	TIBC: % saturation (from Peters' and radio-isotope method	TIBC: % saturation (from the immunodiffusion method*
V.H.	Non-alcoholic cirrhosis	292	370	--	--	79	--
E.B.W.	Non-alcoholic cirrhosis	144	--	294	275	49	--
E.W.	Non-alcoholic cirrhosis	184	--	322	350	57	--
E.R.	Non-alcoholic cirrhosis	96	291	284	300	33	--
	(In liver failure)	212	270	278	200	78	67
P.Q.	Alcoholic cirrhosis	252	277	281	200	91	87

*This value for the percentage saturation was calculated when there was a great discrepancy between the colorimetric and radioisotope methods and the immunodiffusion method.

Percentage saturation based on immunodiffusion method

$$= \frac{\text{Serum iron} - (\text{TIBC colorimetric method} - \text{TIBC immunodiffusion})}{\text{TIBC immunodiffusion method}}$$

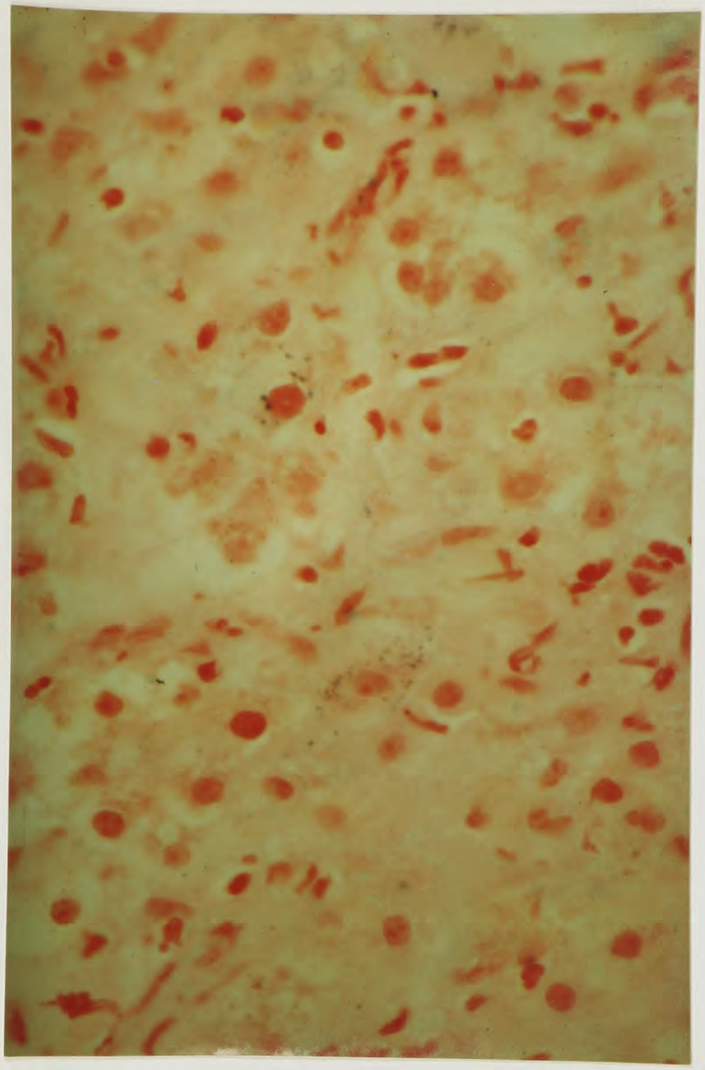


PLATE 1: Liver biopsy stained for iron by Perl's method. Magnification x 40. Patient A.J., with cirrhosis of the liver.

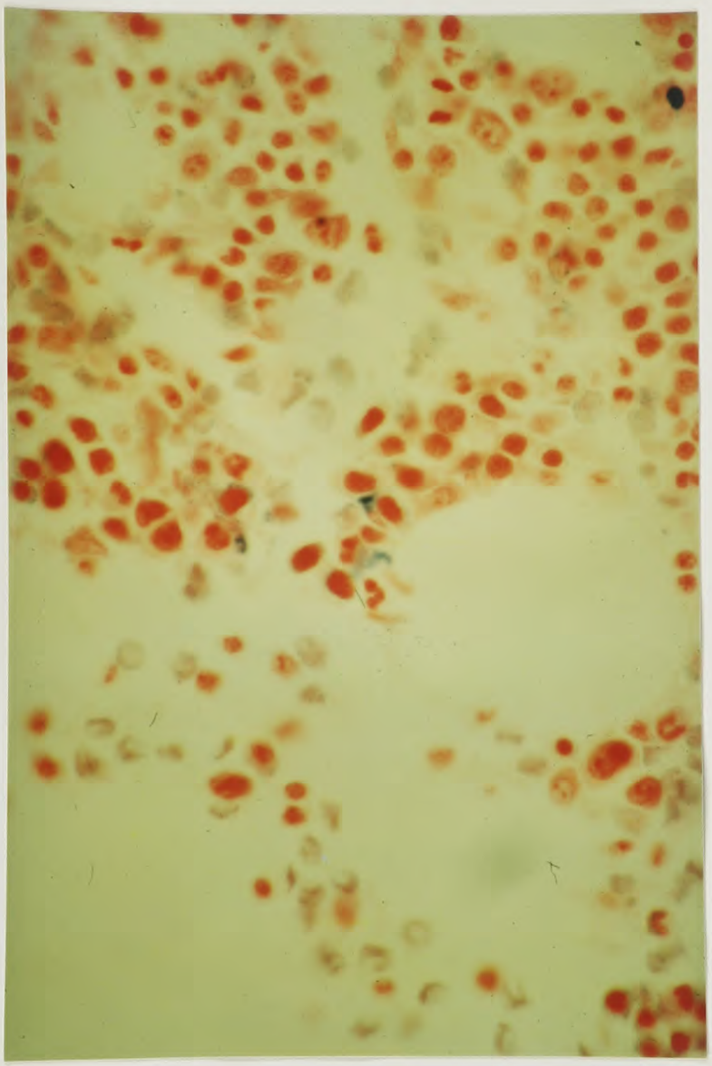


PLATE 2: Bone-marrow section stained for iron by Perl's method. Magnification x 40. Patient V.H., with cirrhosis of the liver.

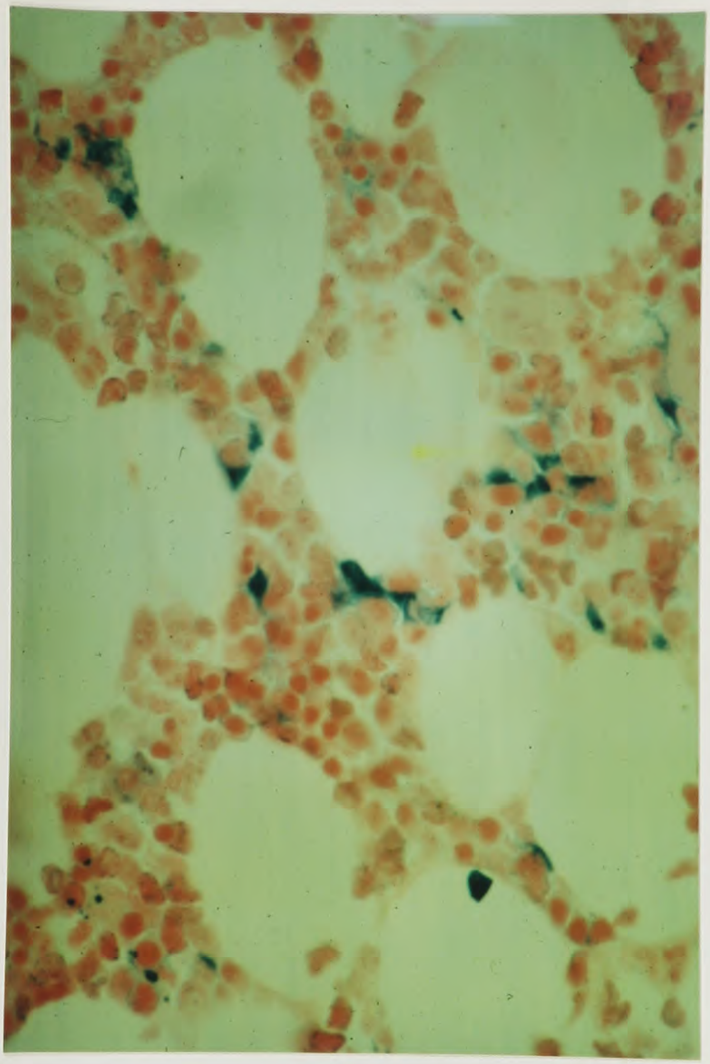


PLATE 3: Bone marrow section stained for iron by Perl's method. Magnification x 40. Patient P.Q., with cirrhosis of the liver.

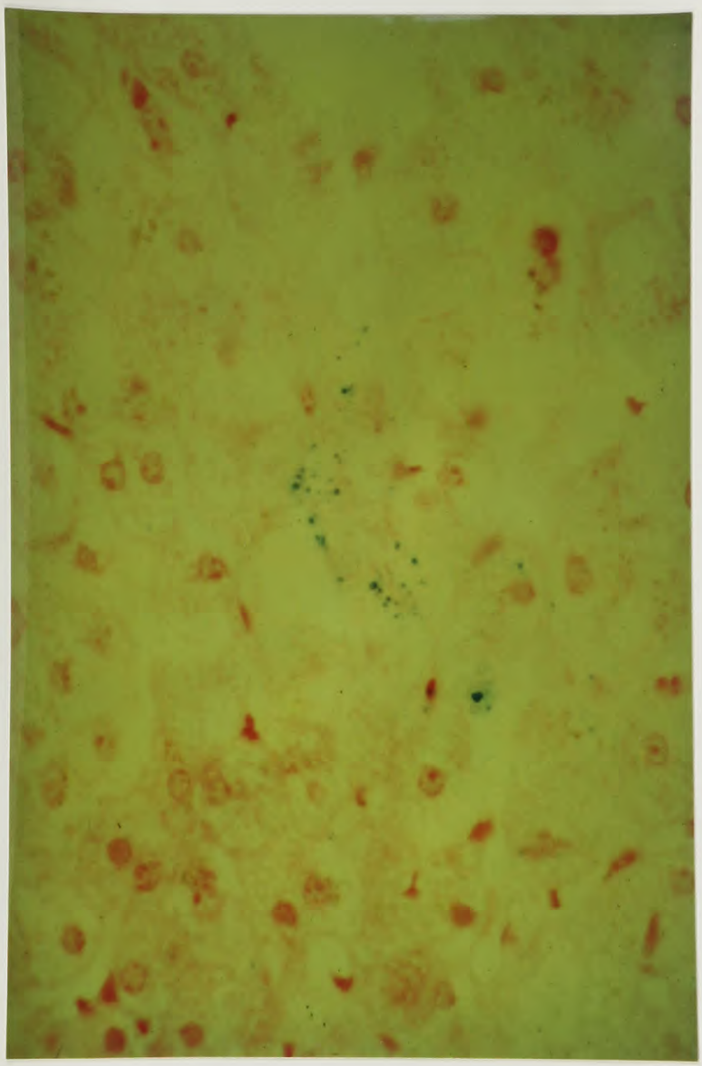


PLATE 4: Liver biopsy stained for iron by Perl's method. Magnification x 40. Patient E.B.W., with cirrhosis of the liver.

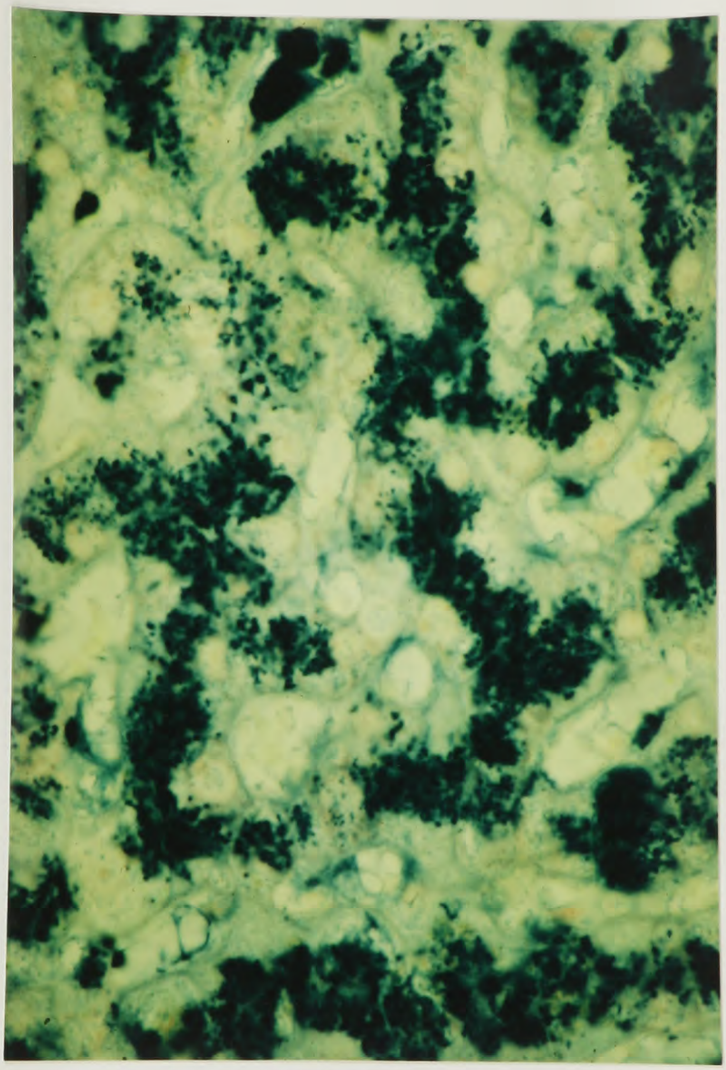


PLATE 5: Liver biopsy stained for iron by Perl's method. Magnification x 40. Patient A.C., with haemochromatosis.

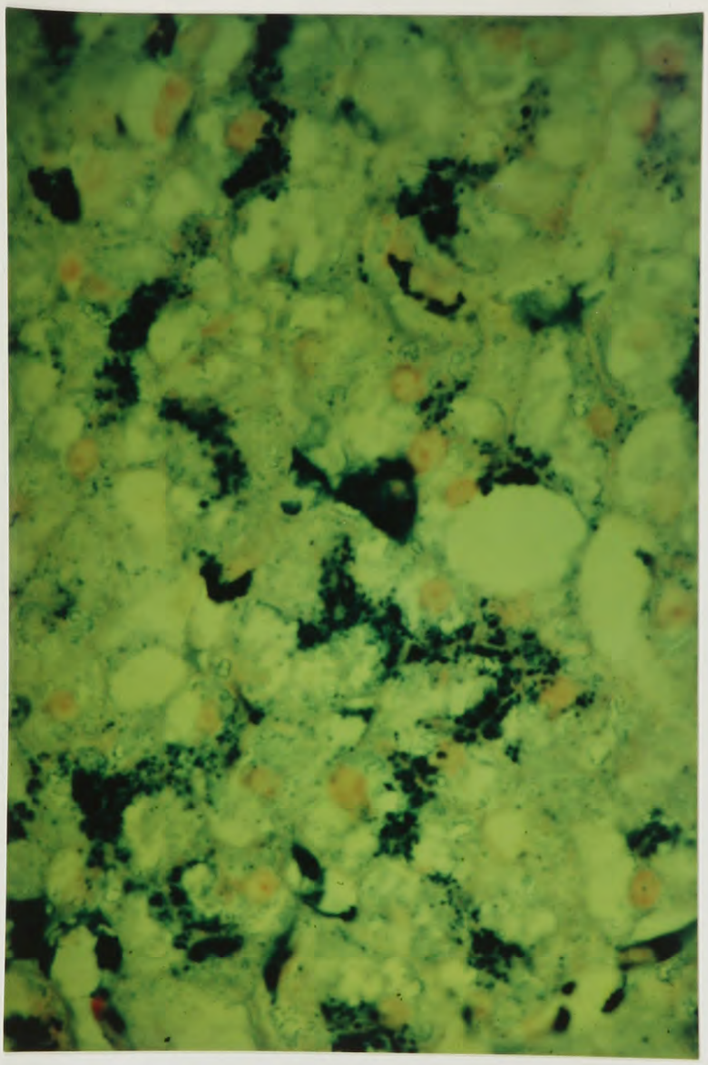


PLATE 6: Liver biopsy stained for iron by Perl's method. Magnification x 40. Patient F.A., with transfusion haemosiderosis.

present study (Table 13). In many of the cirrhotics the total iron-binding capacity was measured by a colorimetric method and an immunodiffusion method. Stojceski, Malpas and Witts (1964) have pointed out that a discrepancy exists in some patients with cirrhosis between the total iron-binding capacity estimated by a colorimetric method and an immunodiffusion method. This is due to the presence of iron which is not bound to transferrin, presumably ferritin, the tissue saturation being appreciably lower in patients in whom the discrepancy is allowed for (Table 13). In the patients studied even when this allowance is made the saturation of the total iron-binding capacity is still above normal and this is never reduced to a level where the observed markedly increased absorption of iron would be expected.

In this study the increased saturation of the iron-binding capacity is accompanied by either no iron in the liver (Plate 1) or a slight excess of iron in the bone marrow (Plates 2 and 3) or liver biopsy (Plate 4). This result is similar to the findings of Bothwell, Cohen, Abrahams and Perold (1959). In contrast, Williams, Scheuer and Sherlock (1962) have noted that normal levels of serum iron may accompany deposits of iron in the liver. The findings of Bothwell et al. and those of the present study seem easier to explain in that

TABLE 14

The presence of iron in bone marrow and liver biopsies in patients with cirrhosis of the liver, transfusion haemosiderosis and haemochromatosis

Patient	Diagnosis	Bone-marrow biopsy	Liver biopsy
H.B.	Cirrhosis of the liver	+	-
V.H.	Ditto	++	-
A.J.	Ditto	-	0
E.W.	Ditto	+	-
E.B.W.	Ditto	+	+
W.W.	Ditto	0	-
F.A.	Transfusion haemosiderosis	++	++++
A.C	Primary haemochromatosis	0	++++

0 = none
+ = minimal
++ = present in moderate amounts
+++ = marked
++++ = heavy
- = not done

TABLE 15

Comparison of the clinical findings in patients with cirrhosis with secondary haemosiderosis and patients with cirrhosis and increased iron absorption

Clinical features	Cirrhosis of the liver with increased iron absorption	Cirrhosis of the liver with haemosiderosis
Number of patients	6	20
Sex: Male	50%	80%
Female	50%	20%
Melanodermia	16%	100%
Size of liver: Enlarged	33%	70%
Shrunken	33%	5%
Normal	33%	25%
Portal hypertension : Splenomegaly	82%	40%
Diabetes	16%	30%
Endocrine changes	100% of 3 males	50%
Alcoholism	33%	100%

Results in cirrhosis of the liver with haemosiderosis after Caroli and André (1964).

a high serum iron level would, presumably, precede a deposition of iron in the tissues.

The deposition of iron in the tissues of the patients with cirrhosis would appear to be small (Plate 4) compared with the appearance of iron in a typical case of untreated idiopathic haemochromatosis (Plate 5) or transfusion haemosiderosis (Plate 6).

It is not possible to say whether the patients in this study who showed increased absorption of iron in the absence of an obvious stimulus represent those patients who will eventually show histological evidence of marked deposition of iron in the tissues. The liver and bone marrow biopsies show little evidence of increased amounts of iron at the present time (Table 14). It seems unlikely that the increased absorption preceded the cirrhosis and therefore a relatively short time for the production of siderosis has elapsed.

It is of interest to compare the patients in this study who had increased iron absorption with a group of 20 patients reported by Caroli and André (1964) with cirrhosis of the liver and secondary haemosiderosis (Table 15). There is a close similarity between the two groups in the sex incidence, the estimation of liver size, the incidence of portal hypertension and diabetes. Melanodermia was more frequent in Caroli and André's patients but this is probably dependent on time for its development. The haematological findings

TABLE 16

Comparison of the mean level of serum iron and percentage saturation of the iron-binding capacity in patients with secondary haemosiderosis and patients with cirrhosis and increased iron absorption

	Cirrhosis of the liver with increased iron absorption	Cirrhosis of the liver with haemosiderosis
Number of patients	6	20
Serum iron (mean value)	225 µg %	192 µg %
Saturation of the iron-binding capacity (mean value)	71.0%	68.7%

Results in cirrhosis of the liver with haemosiderosis after Caroli and André (1964).

are also comparable (Table 16).

The incidence of secondary haemosiderosis in cirrhosis of the liver is impossible to estimate. Studies reported so far are too small and too highly selected to give an answer. Two out of nine patients (Van Hoek, 1961), three out of 10 (Conrad, Berman and Crosby, 1962) and one patient out ot five (Sherlock, 1964) showed evidence of secondary haemosiderosis. In the present study if the six surviving patients with high iron absorption remain alive they may eventually develop secondary haemosiderosis. This incidence, however, may well be reduced by the onset of gastrointestinal haemorrhage secondary to portal hypertension.

A better assessment of the incidence may be obtained from post-mortem studies. Herbut and Tamaki (1946) reported on 115 patients with cirrhosis of the liver coming to post-mortem; 33 had evidence of some degree of haemosiderosis. Bell (1955) studied 932 autopsies of patients with portal cirrhosis diagnosed in life. Microscopic sections of the liver were stained for iron in 733. Some degree of haemosiderosis of the liver was found in 34.2 per cent. of 506 males and 23 per cent. of 226 females. A lower incidence of haemosiderosis complicating cirrhosis of the liver was given by Cachin, Pergola, Debesse and Levillain (1958) who estimated the incidence at about 8.6 per cent.

In the few studies on patients with cirrhosis from the larger post-mortem series it would seem that the incidence of haemosiderosis complicating cirrhosis of the liver is of the order of 10 to 30 per cent.

In discussion on the cause of haemosiderosis of the liver, it was stated earlier that the aetiology was obscure. There are indications from the present investigation that deficiencies in the pancreatic secretions may play some part in the observed increase in iron absorption.

In post-mortem examinations in patients with cirrhosis of the liver Herbut and Tamaki (1946) noted pancreatic fibrosis in 51 out of 60 patients. Woldman, Fishman and Segal (1959) found pancreatic fibrosis in 70 per cent. of 222 patients with cirrhosis. Sobel and Waye (1963) suggested from a comparison of patients with alcoholic cirrhosis and non-alcoholic cirrhosis that the incidence of pancreatic fibrosis is twice as high in patients with portal cirrhosis compared with post-necrotic cirrhosis. There is some controversy on this because studies by Stinson, Baggenstoss and Morlock (1952) found no difference in the incidence of inflammatory change and fibrosis in alcoholic and non-alcoholic cirrhotics.

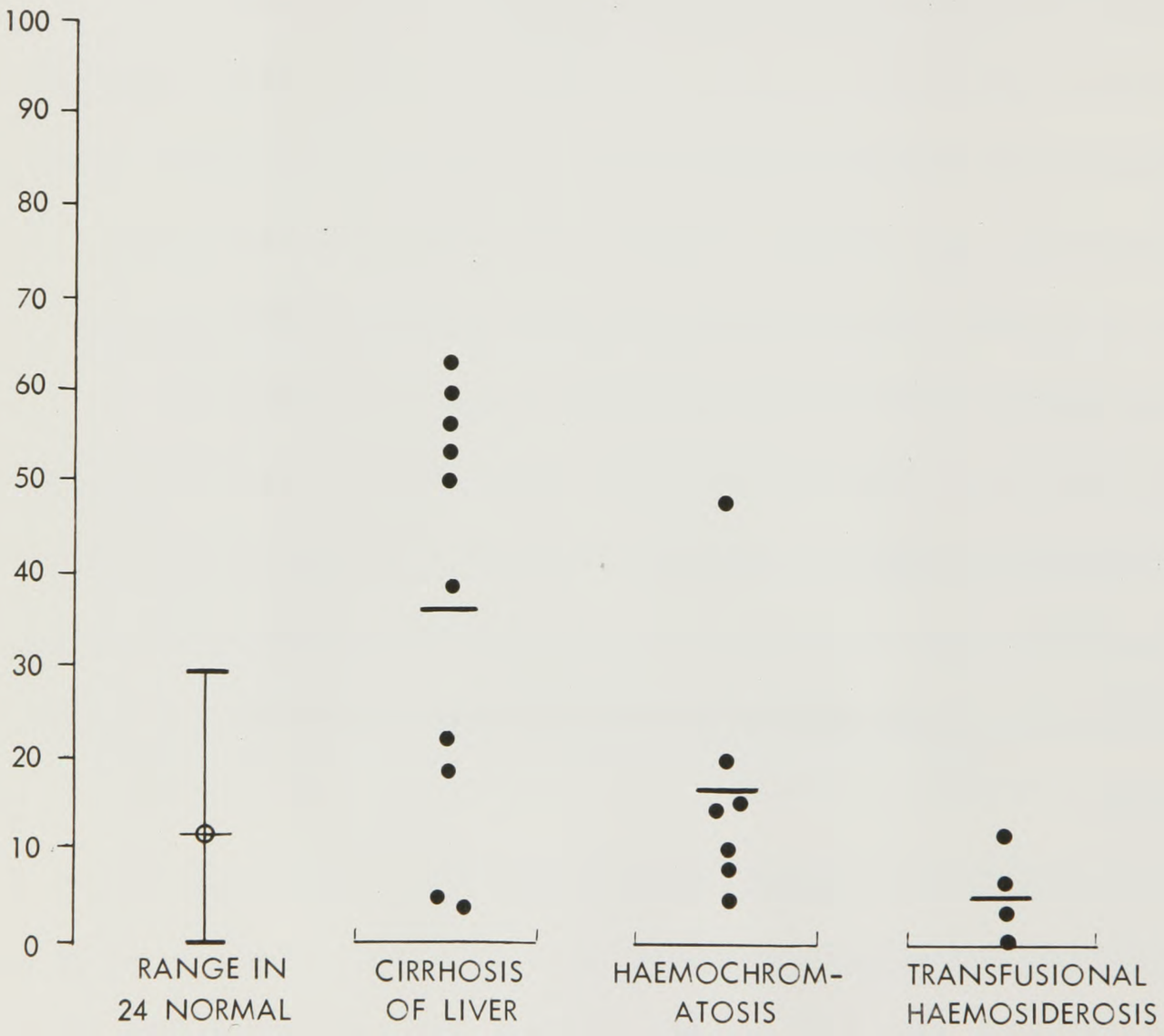
Whilst post-mortem studies would strongly suggest that the pancreas is involved, actual studies of pancreatic function are more

equivocal. Gross, Comfort, Wollaeger and Power (1950) could find no difference in patients with liver disease with regard to the volume of pancreatic juice secreted and its content of bicarbonate when compared with normal control subjects. Haverback, Dyce, Gutentag and Montgomery (1963) found no evidence of a low faecal excretion of trypsin and chymotrypsin in patients with Laennec's cirrhosis. Linscheer, Greenberg, Moore and Chalmers (1964) found no abnormal features in the duodenal aspirate of 10 patients with cirrhosis. Tests on osmolarity, pH, volume, amylase, lipase and bilirubin content were all normal. Trypsin levels were not estimated. Van Goidsenhoven, Henke, Valla and Knight (1963), using a secretin test in conjunction with duodenal intubation, showed in 11 out of 19 patients with cirrhosis of the liver definite abnormality with regard to volume of pancreatic juice which was increased and bicarbonate content which was low when compared with the findings in a group of normal control subjects.

Howatt (1964, personal communication) has also found that in patients with cirrhosis there is a greater volume of aspirate in response to secretin and pancreozymin given parenterally. In all other respects the aspirate appeared normal. On this evidence it could be that either the volume of secretion is a most important factor or that absorption is influenced by some factor in pancreatic juice which is not, at present, being subjected to examination.

Fig. 26:

ABSORPTION OF LABELLED HAEMOGLOBIN IN NORMAL CONTROLS, PATIENTS WITH CIRRHOSIS OF THE LIVER, HAEMOCHROMATOSIS & HAEMOSIDEROSIS.



In patients with primary haemochromatosis pancreatic abnormality is an accepted part of the syndrome. Sheldon (1935) gives an incidence for pancreatic fibrosis of 90 per cent. in a large series of autopsies and MacDonald and Mallory (1960) give an incidence of 100 per cent. Few studies on pancreatic function are available but Howatt (1964, personal communication) has found that as in cirrhosis the volume of pancreatic secretion is increased but its constituents appear to be normal.

In marked contrast with cirrhosis of the liver and haemochromatosis, studies on the pancreatic histology in transfusion siderosis show no evidence of fibrosis. In five autopsies Cappell, Hutchison and Jowett (1957) were unable to demonstrate any pancreatic fibrosis.

However in the present study one patient who had received over 300 units of blood died and at post-mortem she had a heavy deposition of iron in her pancreas and well-marked fibrosis. Iron absorption studies during life had shown a low level of absorption similar to other patients with transfusion haemosiderosis (Figure 26). On the basis of the histology which showed pancreatic damage, this patient might have been expected to show some increase of iron absorption, but this was not seen.

The Manner in which Pancreatin Obtains its Effect

As Sherlock has pointed out, there is a marked effect on iron absorption by a relatively small amount of pancreatin (Fig. 28). A dose of 2.5 g. represents a fraction of the amount of pancreatic extract required for maintenance therapy in the patients with chronic pancreatitis. The small amount of pancreatin required to achieve the effect would be a further argument against any simple precipitation of the iron to form an insoluble complex. It has been suggested in Chapter 10 that pancreatin could be effective due to its enzyme content and in particular the trypsin that it contains. The action of trypsin is to convert protein into peptides and amino acids. It has been shown in rats by Libre and McFarland (1964) that hydrolysed casein and iron compete for the transport mechanisms across the intestinal mucosa. The production of amino acids in the upper intestine might therefore profoundly affect iron absorption. Sørenson (1964), studying human subjects, has shown that when iron was given with either carbohydrate or fat or protein the only constituent which produced a significant decrease in absorption was protein.

In order to examine this hypothesis further, the effect of protein on the absorption of iron in patients with primary pancreatic disease and with cirrhosis of the liver and haemochromatosis would have to be investigated. Failure to digest protein can be observed clinically

TABLE 17

Liver biopsy stained for iron in 436 patients with hepatic disease compared with 122 normal subjects. Iron in parenchyma labelled P and in Kupffer cells K.

	P	K	Total number of subjects
With hepatic disease	119	76	195
No hepatic disease	16	16	32

Differences are significant at 5% level in each group.

After Zimmerman, H.J., Chomet, B., Kulesh, M.H. and McWhorter, C.A. (1961).

in severe chronic pancreatitis, whether this occurs in cirrhosis of the liver or haemochromatosis would seem unlikely. The temporary increase in amino-acid concentration in the upper intestine would not account for the delayed effect noted by Sephton Smith.

Taylor, Stiven and Reid (1935) noted the changes which occurred in the intestinal mucosa in cats following pancreatectomy or ligation of the pancreatic duct. It is possible that the mucosa in patients with either primary pancreatic disease or cirrhosis of the liver might be abnormal but a limited number of small-intestinal biopsies so far obtained in this condition have been normal.

The Rôle of the Liver in the Absorption of Iron

The studies on the effect of pancreatin are strongly in favour of the pancreas playing an important part in influencing the absorption of iron in chronic liver disease but the possibility of the liver per se having an effect on absorption should be considered.

Smith and Crandall (1942) showed that dogs with biliary fistulae became anaemic when they were maintained without bile being allowed to enter their small intestine. Tests on iron absorption using plasma iron curves suggested no abnormality to be present. Wheby, Conrad, Hedberg and Crosby (1962), using a cross-circulation

technique in which bile from an anaemic dog poured into the small intestine of a normal dog, showed that absorption was not increased when a whole-body counting technique was used. These short-term animal experiments would suggest that the liver does not affect iron absorption.

However some clinical and experimental observations in human liver disease are not in accord with these findings. Zimmerman, Chomet, Kulesh and McWhorter (1961) have studied liver biopsies stained for iron in 436 patients with hepatic disease and 122 normal controls (Table 17). They found a highly significant increase in the iron content in patients with all forms of liver disease. This increase in stainable iron could have been due to a redistribution of iron rather than an increase in total body iron. A compensated haemolytic anaemia would be a possible mechanism. Pitcher has shown that this occurs in all forms of liver disease. Zimmerman, Chomet, Kulesh and McWhorter considered this possibility in the patients with acute hepatitis. They compared the haematological findings in patients with acute liver disease with those who in addition showed an increase of iron in the tissues. There was no significant difference with regard to the haematological indices in the two groups.

A clinical condition which suggests that the liver plays a

more active rôle with regard to iron absorption is that of haemosiderosis of the Bantu.

Strachan (1929) first drew attention to a condition which simulated haemochromatosis and which was found in the Bantu population of South Africa. Wainwright (1957), in an extensive study, showed that these people lived on a diet with a high iron content and low protein and absorbed a considerable excess of iron. This is stored both in the reticuloendothelial system and in the liver cells and eventually hypertrophic cirrhosis develops. Bradlow, Dunn and Heggeman (1961) showed that in Bantu who had not yet developed cirrhosis deposits of iron were found only in the liver. As soon as cirrhosis developed not only was there a greatly increased total body iron store but other parenchymatous organs were involved in a similar manner to haemochromatosis of idiopathic type. It is possible, of course, that chronic pancreatitis develops concurrently under the influence of the abnormal diet. These authors did find an increasing amount of pancreatic iron associated with cirrhosis, but they did not comment on the incidence of pancreatic fibrosis in their study. However, Marks and Bank (1963) have shown the increased prevalence of chronic pancreatitis in South Africa in a similar population.

Implications in the Management of Anaemia in Patients with Cirrhosis of the Liver

Many cirrhotic patients develop anaemia and a proportion of these have an iron-deficiency anaemia. This requires oral iron therapy for its correction. In the absence of any haematological indication, however, the long-continued administration of oral doses of inorganic iron salts would seem to be inadvisable, since there is a possibility that secondary haemosiderosis may result.

The development of haemochromatosis by patients with portacaval anastomosis has been shown by Tuttle, Figueroa and Crossman (1959), Tisdale (1961) and Schaeffer, Arrick, Oikawa and Schiff (1962). It is difficult to say whether the cessation of gastrointestinal blood loss is responsible for the increase in tissue iron or whether it is the portacaval anastomosis per se which leads to a rapid increase in absorption. Greenberg, Strohmeyer, Hine, Keene, Curtis and Chalmers (1964) found increased absorption of iron in two out of three patients with portacaval anastomosis but the levels of absorption were no greater in fact than that seen in other cirrhotics.

There are no other studies available on human subjects with portal systemic anastomoses. Noyes and Jordan (1964), studying iron absorption in dogs with portacaval transplantation, and Gervertz,

Rubin, Wasserman and Jacobson (1964), studying rats with side-to-side portacaval anastomosis, have demonstrated that increased absorption of iron occurs. Both groups of workers found increased deposition of iron in the livers.

Insufficient information is available as yet to determine the severity of the effect on iron metabolism or the frequency of haemochromatosis in patients having portal systemic anastomoses. When employed as an emergency measure for the arrest of bleeding, such considerations would not be very important.

Recently, it has been suggested that in patients with cirrhosis of the liver and oesophageal varices portacaval anastomoses should be performed as a prophylactic measure. The results of a control trial reported by Conn and Lindenmuth(1962) are not encouraging but if such treatment became more widely used it would be of considerable interest to know what the risk of tissue iron overload is in patients following portacaval and other forms of anastomosis. A possible approach would be to carry out sequential iron absorption studies before and after portacaval anastomosis to determine what effect the shunt has on absorption.

Fig. 27:

ABSORPTION OF LABELLED FERROUS SULPHATE IN NORMAL CONTROLS, PATIENTS WITH CIRRHOSIS OF THE LIVER AND ACUTE LIVER DISEASE.

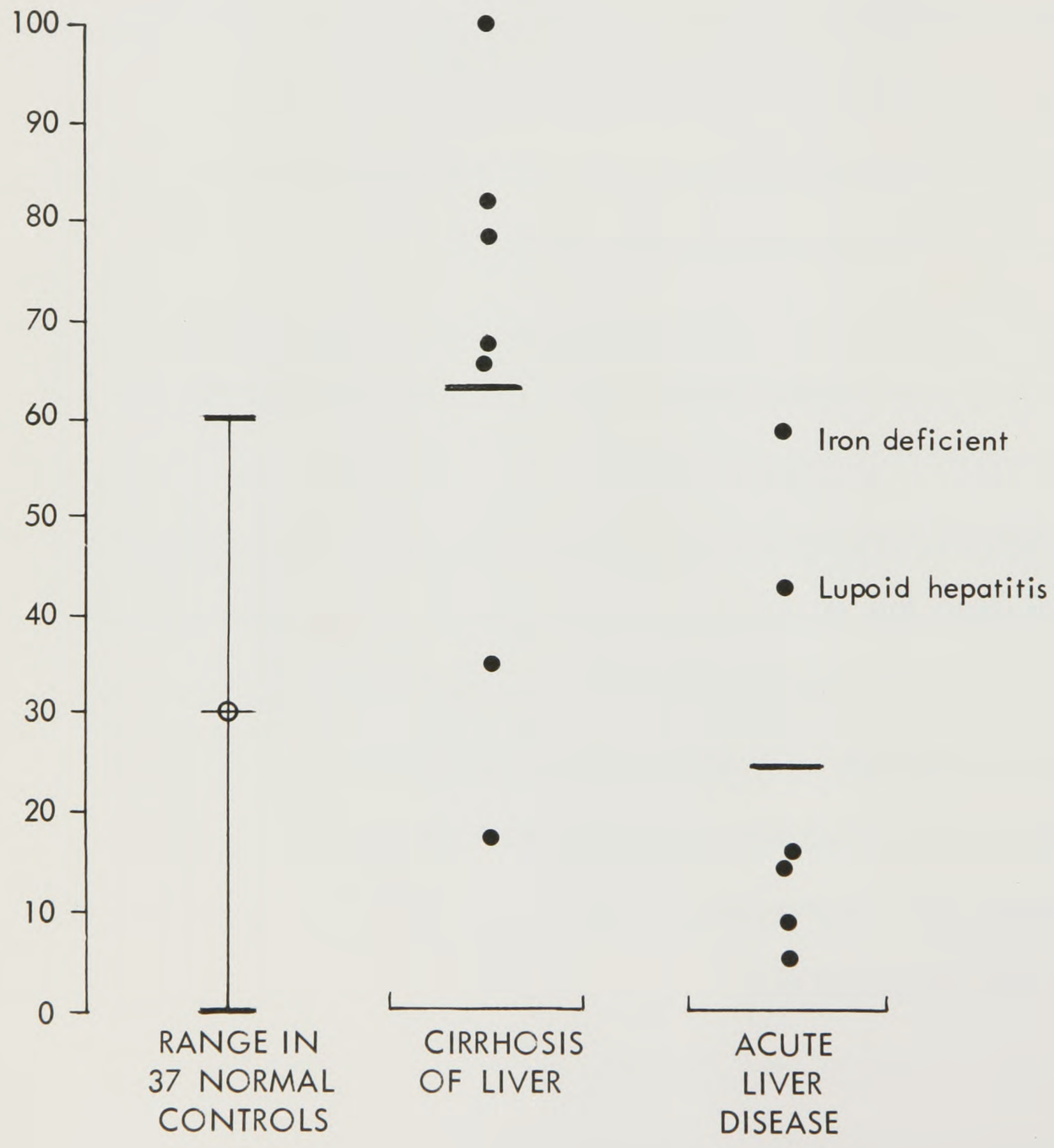


Fig. 28:

EFFECT OF PANCREATIN ON THE ABSORPTION OF IRON IN PATIENTS WITH CIRRHOSIS OF THE LIVER.

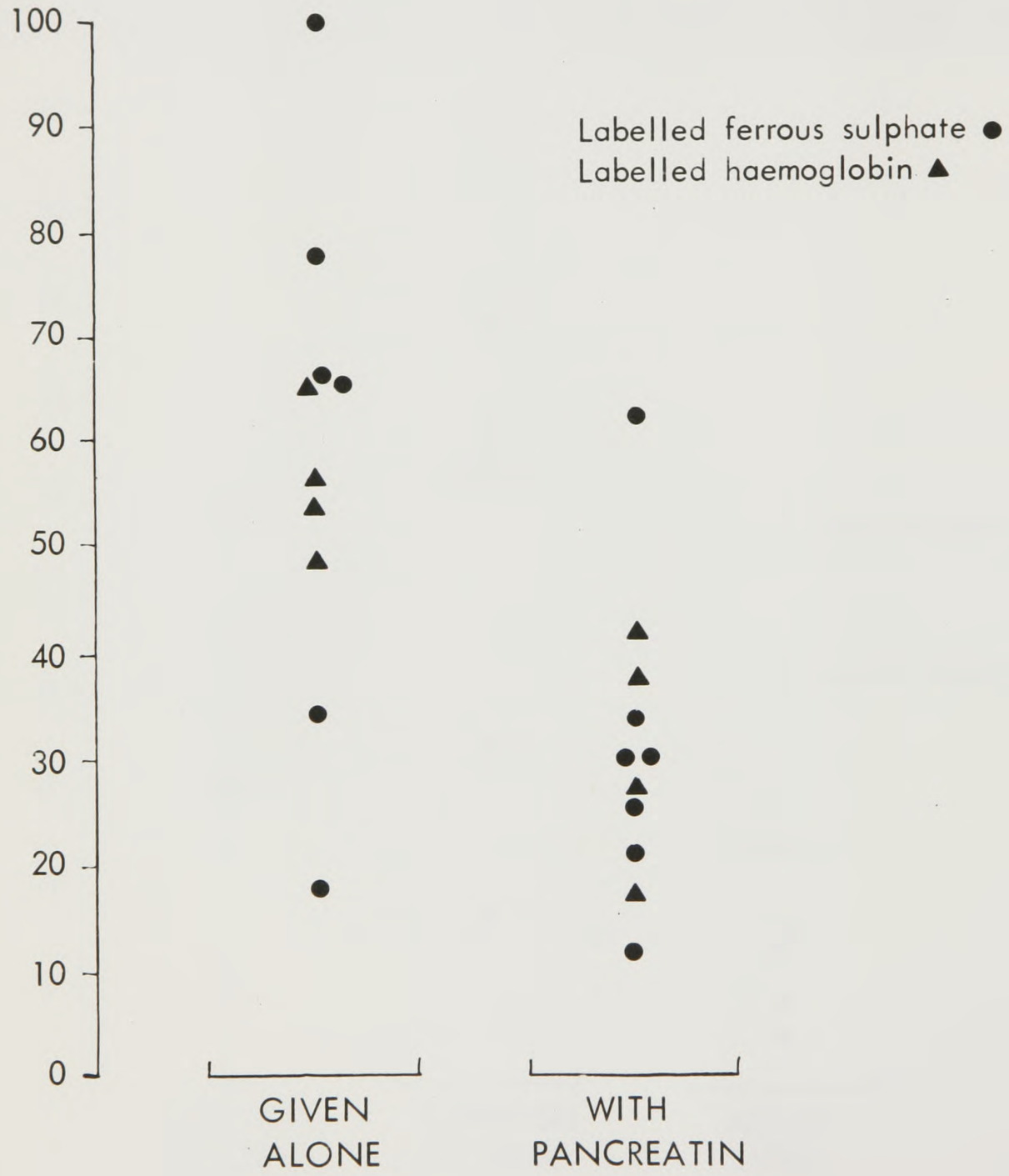
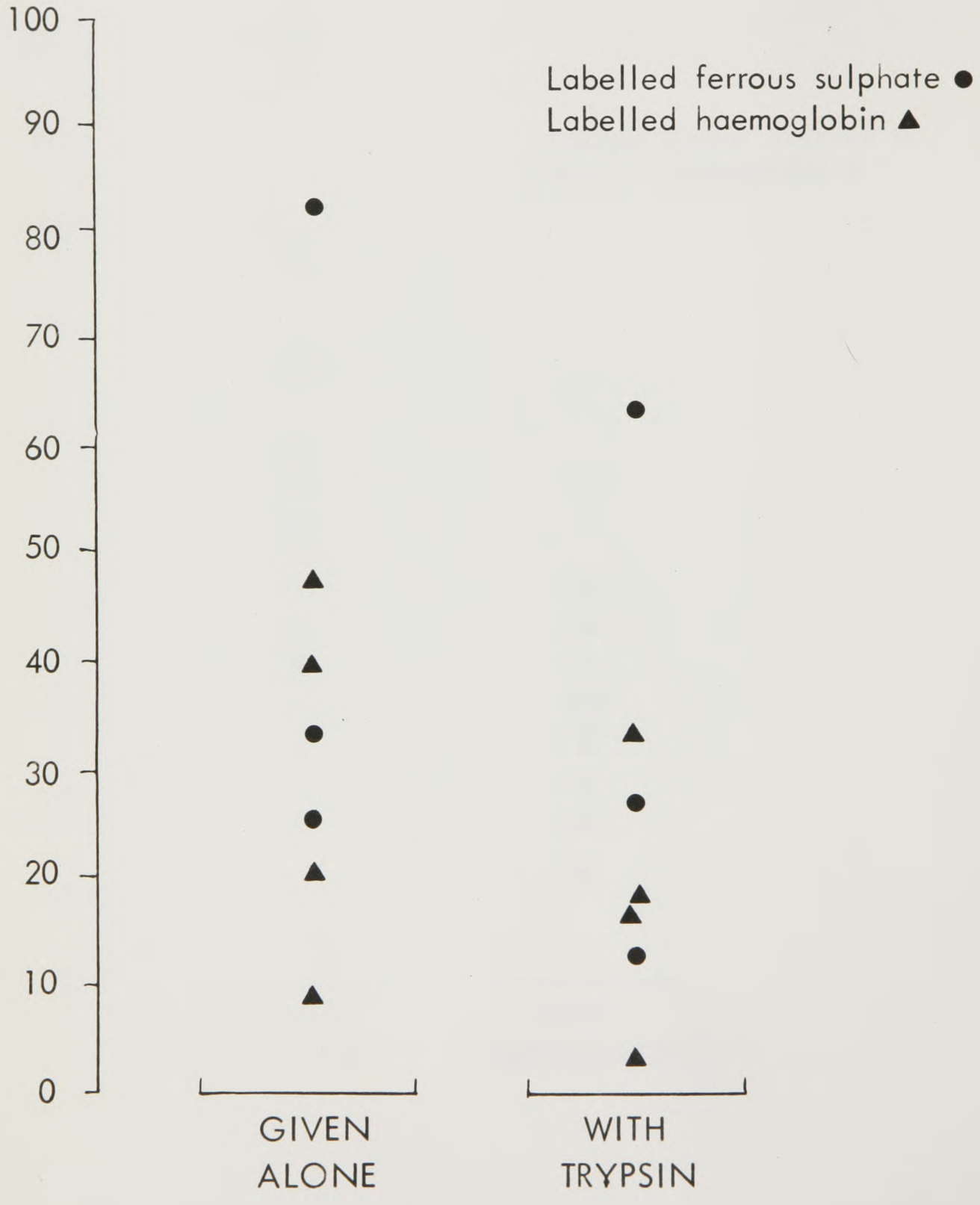


Fig. 29:

EFFECT OF TRYPSIN ON THE ABSORPTION OF IRON IN PATIENTS WITH CIRRHOSIS AND HAEMOCHROMATOSIS.



Conclusion

The absorption of both inorganic and haemoglobin iron has been investigated in a number of patients with liver disease. Patients with cirrhosis of the liver have shown increased absorption. Normal levels of absorption in the presence of markedly increased tissue iron stores were seen in patients with haemochromatosis. A low level of absorption was seen, in general, in patients with acute liver disease and very low levels occurred in patients with transfusion haemosiderosis (Figures 26 and 27).

In those conditions in which pancreatic fibrosis might coexist, namely cirrhosis of the liver, haemochromatosis and chronic alcoholism, pancreatic extract and trypsin proved effective in reducing the absorption of iron (Figs. 26 and 29). It did not reduce absorption of iron in acute liver disease. In patients with iron deficiency the effect was extremely variable.

The increase in iron absorption seen in patients with cirrhosis provides an explanation for the secondary haemosiderosis sometimes observed in these patients. Attention has been drawn to the possible ill effects of oral iron therapy in patients with cirrhosis who are not sideropaenic or who have had episodes of bleeding.

The more general implication of the present study has been to emphasise the importance of influences within the intestinal lumen

and more particularly the secretions of the pancreas in the control of iron absorption.

APPENDIX I

Results of Absorption Studies in Normal Subjects and Iron-Deficient Patients

Iron absorption and utilisation in normal subjects given ^{59}Fe labelled ferrous sulphate

Subject	Percentage in faeces	Percentage retained	Percentage in blood
1	31	69	--
2	56	44	--
3	71	29	--
3	62	38	24
4	83	17	--
5	82	18	--
6	72	28	--
7	74	26	--
8	63	37	--
9	67	33	--
9	73	27	18
10	92	8	3
11	77	23	11
12	81	9	10
13	79	21	--
13	75	25	31
14	60	40	--
15	82	18	24
16	93	7	2
16	83	17	8
17	67	33	7
18	52	48	41
19	59	41	25
20	47	53	41
21	34	66	56
22	64	36	31
23	85	15	21
24	44	56	57
25	76	24	26
25	71	29	30
26	61	39	22
26	64	36	21

(Continued on next page)

Iron absorption and utilisation in normal subjects given ^{59}Fe-labelled ferrous sulphate (continued)

Subject	Percentage in faeces	Percentage retained	Percentage in blood
27	92	8	<1
28	81	19	16
29	85	15	9
29	72	28	15
30	68	32	33
31	71	29	20
32	83	17	7
32	58	42	26
33	65	35	33
34	86	14	11
35	73	27	21
36	30	70	68
37	80	20	--

Mean absorption (45 observations in 37 patients) 30%.

Mean blood levels 23%.

Iron absorption and tutilisation in hypochromic anaemia patients given ^{59}Fe-labelled ferrous sulphate

Subject	Percentage in faeces	Percentage retained	Percentage in blood
1	44	56	57
2	86	14	8
2	80	20	27
3	20	80	71
3	43	57	63
4	20	80	79
5	25	75	72
6	53	47	49
7	55	45	51
8	63	37	30
9	44	56	55
10	43	57	55
10	50	50	50
11	6	94	96
12	34	66	64
13	63	37	24
14	28	72	77
15	35	65	70
16	--	--	90
16	81	19	--
17	49	51	46
18	56	44	50
19	39	61	70

22 observations in 19 subjects; mean absorption 54%.

Mean blood levels 53%.

Iron absorption and utilisation in normal subjects given ^{59}Fe-labelled haemoglobin

Subject	Percentage in faeces	Percentage retained	Percentage in blood
1	81	9	1
2	78	22	17
3	68	32	21
4	75	25	11
5	83	17	14
6	92	8	5
7	85	15	16
8	99	1	8
9	85	15	3
10	92	8	5
11	78	22	2
12	100	0	2
13	94	6	3
14	91	9	4
15	96	4	5
16	>99	<1	5
17	90	10	6
18	98.5	1.5	1
19	100	0	0
20	76	24	1
21	95	5	4
22	87	13	5
23	100	0	7
24	84	16	14

In 24 subjects mean absorption = 11%.

Mean percentage in blood = 6%.

Iron absorption and utilisation in hypochromic anaemia subjects given ^{59}Fe-labelled haemoglobin

Subjects	Percentage in faeces	Percentage retained	Percentage in blood
1	72	28	29
2	81	9	0
3	76	24	24
4	60	40	40
5	99	1	11
6	88	12	10
7	79	21	18
18	58	42	36
22	--	--	29
9	94	6	7
10	93	7	9
11	94	6	7
12	72	28	--
13	63	37	33
14	78	22	17
15	69	31	31
16	81	19	23
17	91	9	10
18	82	18	18
19	90	10	--
20	94	16	12
21	91	9	4
22	69	31	17
23	89	11	9
24	96	4	5
25	83	17	15
26	44	56	--
27	80	20	--

In 27 subjects mean percentage absorbed = 20%.

Mean percentage appearing in the blood = 18%.

APPENDIX II

CASE HISTORIES

ABBREVIATIONS

ANF:	Anti nuclear factor.
B.P.:	Blood pressure systolic and diastolic reading.
BSP:	Bromsulphthalein retention.
C.I.:	Colour index.
ESR:	Erythrocyte sedimentation rate (Westergren).
Hb:	Haemoglobin.
Haemat.:	Haematocrit.
LE:	Lupus erythematosus cell.
MCHC:	Mean corpuscular haemoglobin concentration.
n:	Number in sample.
P:	'Probability'.
Retics.:	Reticulocyte count.
R.I.no.:	Radcliffe Infirmary registration number.
S.D.:	Standard deviation.
SGOT:	Serum glutamic oxaloacetic transaminase.
SGPT:	Serum glutamic pyruvate transaminase.
t:	Student's t test.
TIBC:	Total iron-binding capacity.
WBC:	White blood cell count.

PATIENTS WITH

CIRRHOSIS OF THE LIVER

Mrs. R.A. R.I. No. 201969. Age 61. Born 1901

Diagnosis: Cirrhosis of the liver with hypersplenism.

1955 - Episode of jaundice with dark urine and pale stools. Ill for 7 weeks, then recovered.

1958 - Occasional swelling of left leg. She had had a white leg following pregnancy some 34 years before.

1961 - Developed some shallow ulcers in the left popliteal fossa which would not heal. She had been tired and unwell for last few months. She had been taking Anadin tablets. No haematemesis or melaena. No history of alcoholism.

Examination: Pale little lady. Not jaundiced. No skin rash but three indolent ulcers in left popliteal fossa. No tremor. No spider naevi or liver palms. Cardiovascular system normal. B.P. 120/80. Spleen enlarged to the umbilicus. Bilateral ankle oedema.

Investigations at time of absorption studies

Haematological findings: Hb 10 g./100 ml. MCHC 31%. Retics. 0.8%. WBC 1400/c.mm. Coombs test negative. Platelets 140,000/ c.mm. ESR 14 mm. in 1 hr. Bone-marrow; active myelopoiesis. Serum iron 48 µg./100 ml. Saturation of TIBC 13%.

Liver function tests: Serum bilirubin 0.8-1.8 mg./100 ml. Total proteins 7.3 g./100 ml., albumin 3.1 g./100 ml. Alkaline phosphatase 8 units. Prothrombin time 68 sec.

BSP; 25% retained at 45 min. ANF negative. LE cells negative. Occult blood negative.

Barium swallow showed oesophageal varices. Barium meal showed a large spleen. Pneumoperitoneum showed a small irregular liver.

Results of absorption studies

Percentage of dose of ^{59}FeHb absorbed when given alone; 57%.
Percentage of dose of ^{59}FeHb absorbed when given with Cotazym ; 38%.

Mr. H.B. R.I. No. 338877. Age 46. Born 1916.

Diagnosis: Cirrhosis of the liver. Chronic alcoholism.

1942 - Jaundiced whilst on active service. Jaundice lasted 7 months.

1959 - Had been very considerable drinker of alcohol for many years when he suffered an episode of unconsciousness after a particularly severe bout of drinking. He was found to have impaired liver function and was considered to have alcoholic cirrhosis.

He continued to drink and had several admissions to hospital in 1960. At the end of the year he started to have flapping tremor and disorientation.

1961 - Some bleeding from haemorrhoids and continued to have frequent admissions in 1961 and 1962 with alcoholic intoxication.

1963 - No further bleeding but considerable mental disturbance. Continued to drink. No treatment with iron medicines was noted.

Examination: No evidence of intoxication on admission. No flapping tremor and quite well orientated. Many spider naevi on face and trunk. Marked gynaecomastia. Cardiovascular system normal. B.P. 175/80. No ankle or sacral oedema. Ascites present in the abdomen. Liver enlarged and soft some 9 cm. below costal margin. Testes atrophic.

Investigations at time of absorption studies

Haematological fiddings: Hb 12.6 g./100 ml. MCHC 34%. Retics. 2.0%. WBC 4500/c.mm. Platelets 382,000/c.mm. Serum iron 200 µg./100 ml.

Liver function tests: Plasma bilirubin 1.8mg./100 ml. Alkaline phosphatase 9 units. Total proteins 6.3 g./100 ml., albumin 2.7 g./100 ml. Pseudocholinesterase 13 units.

BSP 33% retained at 45 min.
SGPT 26 units. Occult blood in stools; negative.

Sternal marrow; cellular smear with a macronormoblastic marrow. Moderate amount of iron present.

Barium swallow; oesophageal varices present.

Results of absorption studies

Percentage of dose of $^{59}FeSO_4$ absorbed when given alone; 35%.
Percentage of dose of $^{59}FeSO_4$ absorbed when given with Cotazym : 23%.

Mrs. F C. R.I No. 346828 Age 65 Born 1898.

Diagnosis: Cryptogenic cirrhosis. Syphilis.

1949 - Onset of diarrhoea. Was admitted to Hillingdon Hospital where she was found to have an enlarged liver. Liver biopsy confirmed the diagnosis of cirrhosis. She then had many episodes of major gastrointestinal bleeding, and varices and splenomegaly were noted on subsequent admissions. She contracted syphilis in 1919. There was no past history of jaundice and she denied ever having taken much alcohol.

Examination: Pale lady, not icteric. No spider naevi noted. No palmar erythema. No abnormality in the cardiovascular system. B.P. 140/90. In the abdomen there was no ascites and no organs were palpable.

Investigations at time of absorption studies

Haematological findings: Hb 10.3 g./100 ml. Haemat. 31. Retics. 1.6%. WBC 1,600/c.mm. Platelets 204,000/c.mm. ESR 8 mm. in 1 hr. Serum iron 160 µg./100 ml.

Liver function tests: Bilirubin 0.8 mg./100 ml. Alkaline phosphatase 7 units. Total serum proteins 5.9 g./100 ml., albumin 2.4 g./100 ml. SGPT 18 units/100 ml. Serum pseudocholinesterase 19 units.

Flocculation tests negative. Prothrombin time 29% of normal on two-stage test. BSP; 46% remained after 45 min. Glucose tolerance test; diabetic in type.

Occult blood in faeces positive on 3 occasions. Wassermann and Kahn; positive.

Plain X-ray of abdomen shows a small liver with no evidence of pancreatic calcification.

Results of iron absorption studies

Absorption of ^{59}Fe-labelled ferrous sulphate given alone; 17%.
Absorption of ^{59}Fe-labelled ferrous sulphate given with Cotazym ; 12%.

Mrs. A.D. R.I. No. 334312. Age 47. Born 1917.

Diagnosis: Cirrhosis of the liver. Chronic alcoholism.

1964 - Admitted following a haematemesis of about half a pint of bright blood. She gave no previous history of having bled. She had been a heavy drinker for many years and as a hotel manager she had been unable to give up alcohol. She had been given a high-protein diet but had had no iron therapy.

Examination: Markedly jaundiced, euphoric woman. Both parotid glands swollen. Some oedema of both legs. Spider naevi present on chest. Cardiovascular system normal. B.P. 105/65. Liver palpable 6 cm. below costal margin; hard. Spleen not palpable.

Progress: Patient had one further bleed on day of admission. She was then transfused. When absorption studies were done some 3 weeks later she had had no further bleeding. Stools were negative for occult blood and she was not anaemic.

Investigations at time of absorption study

Haematological findings: Hb 12.0 g./100 ml. WBC 3,900/c.mm. Platelets 51,000/c.mm. Serum iron 212 µg./100 ml. Saturation of TIBC; 72%.

Liver function tests: Plasma bilirubin 1.2 mg./100 ml. Alkaline phosphatase 22 units. Plasma proteins 7 g./100 ml., albumin 3.9 g./100 ml. Transaminase SGPT 8 units. Pseudocholinesterase 28 units.

Barium meal showed oesophageal varices.

Occult blood in stools negative repeatedly before test.

Results of absorption studies

Percentage of ^{59}Hb absorbed when given alone; 60%.

Mrs. C.H. R.I. No. 96651. Age 43. Born 1920.

Diagnosis: Cirrhosis of the liver. Chronic alcoholism.

1964 - Developed deterioration in vision, tiredness, nausea, vomiting and jaundice. She was known to be a severe alcoholic for many years. Recently because of family worries she had been drinking very heavily. In the few weeks before admission she had noted ankle oedema and had found difficulty with her eyesight.

Examination: Co-operative jaundiced lady. Spider naevi seen on arms. Not anaemic. No abnormality in cardiovascular system. B.P. 110/65. Abdomen distended. Liver enlarged. Spleen not palpable. Marked ankle oedema.

Investigations at time of absorption studies

Haematological findings: Hb 9.3 g./100 ml. Serum iron 116 µg./100 ml. Percentage saturation of TIBC; 39%.

Liver function tests: Plasma bilirubin 0.2 mg./100 ml. Alkaline phosphatase 7 units. Proteins; total 7.1 g./100 ml., albumin 3.0 g./100 ml. Pseudocholinesterase 24 units. Transaminase SGPT 18 units. Serum electrophoresis showed a raised γ-globulin. BSP; 22% of dose retained after 30 min.

Barium meal showed no evidence of varices or an enlarged spleen. Liver appeared enlarged.

Results of absorption studies

Percentage absorption of ^{59}Fe-labelled haemoglobin given alone; 18%.

Mr. V.H. R.I. No. 310561. Aged 58. Born 1902.

Diagnosis: Cirrhosis of the liver. ? Post-necrotic cirrhosis.

1940 - Episode of jaundice which lasted for 6 weeks. He recovered spontaneously. He had had no medication. He had been a moderate drinker of alcohol.

1961 (August) - After injuring his ankle he bled for several days. No previous history of bleeding or bruising.

Examination: Healthy-looking man. Not jaundiced. No cutaneous manifestations of liver disease. Cardiovascular system normal. B.P. 130/70. Spleen palpable 12 cm. below left costal margin. Liver not palpable. Widespread petechiae on legs. Right leg swollen and tender.

Investigations at time of diagnosis

Hb 15.4 g./100 ml. Retics. 1.8%. WBC 2,900/c.mm. Neutrophils 40%. Platelets 31,000/c.mm.

Liver function tests: Plasma bilirubin 1.6 mg./100 ml. Alkaline phosphatase 11 units. Total protein 6.8 g./100 ml., albumin 3.6 g./100 ml. Electrophoresis showed a raised γ-globulin. Prothrombin time 34%. BSP; 39% retained at 45 min.

Barium meal showed no evidence of varices; liver small; spleen enlarged.

Splenectomy was carried out and after a stormy post-operative course he made a good recovery. The liver was small and nodular at laparotomy. There were gross posterior wall varices and the spleen showed the changes of portal hypertension. No biopsy of the liver was taken at operation.

Investigations at time of absorption studies

Haematological findings: Hb 14.9 g./100 ml. Retics. 1.6%. MCHC 33%. Haemat. 47%. WBC 4,900/c.mm. Platelets 441,000/c.mm. Serum iron 292 μg./100 ml. Saturation of TIBC 79%.

Liver function tests: Plasma bilirubin 0.5 mg./100 ml. Alkaline phosphatase 10 units. Total proteins 6.3 g./100 ml., albumin 3.6 g./100 ml. Pseudocholinesterase 41 units. BSP; 46% of dose retained at 45 min.

Mr. V.H. (continued)

Plain X-ray of abdomen showed no pancreatic calcification.

Bone-marrow biopsy showed a cellular marrow with a little increase of stainable iron.

Results of absorption studies

Percentage of dose of ^{59}Fe-labelled $FeSO_4$ absorbed; 66%.
Percentage of dose of ^{59}Fe-labelled $FeSO_4$ absorbed when given with Cotazym ; 30%.

Mr. A.J. R.I. No. 343144. Age 76. Born 1887

Diagnosis: Cryptogenic cirrhosis of the liver.

1962 (Dec.) - Developed marked indigestion, with regurgitation and abdominal distension. He started to vomit after meals and could not tolerate fatty foods. He lost 2 stone in weight. He was treated with antacids but no iron medicines.

1963 - Developed jaundice and ascites and was treated at Cheltenham Hospital with rest, diuretics and a high-protein diet, with considerable improvement.

When readmitted for study was still faintly jaundiced. Although in the Indian Army for many years, he denied having been a regular drinker.

Examination: Slight jaundice. Not pigmented. Palmar erythema and bilateral Dupuytren's contracture. Fingernails pale. Cardiovascular system normal. B.P. 110/60. Slight gynaecomastia but normal distribution of hair. Some ascites still present. Liver and spleen impalpable.

Investigations at time of absorption study

Haematological findings: Hb 17.4 g./100 ml. C.I. 1.1. Retics. 1.2%. MCHC 33%. WBC 8,800/c.mm. Serum iron 264 µg./100 ml. Saturation of TIBC 79%.

Liver function tests: Plasma bilirubin 1.8 mg./100 ml. Alkaline phosphatase 21 units. Proteins 8.2 g./100 ml., albumin 4.2 g./100 ml. BSP; 63% retained. Pseudocholinesterase 16 units. Diabetic glucose tolerance curve.

Liver biopsy showed advanced fatty and albuminous degeneration of parenchyma with fibrosis. There was hyperplasia of the bile ducts with appearance of Laennec's cirrhosis.

X-ray of abdomen showed no pancreatic calcification. Barium meal showed no evidence of oesophageal varices.

Faecal fat: 24-hour excretion of fat over a 4-day period was 20 g. Occult blood in stools persistently negative.

Results of absorption studies

Percentage of dose of $^{59}FeSO_4$ absorbed when given alone; 100%. Percentage of dose of $^{59}FeSO_4$ absorbed when given with acidified Cotazym ; 61%. Percentage of dose of $^{59}FeSO_4$ absorbed when given with Cotazym ; 30%.

Mrs. M.K. R.I. No. 353916. Age 49. Born 1915.

Diagnosis: Cirrhosis of the liver. Chronic alcoholism.

1963 (Dec.) - General ill-health for 4 years before admission. She had been depressed and had been drinking heavily. During the few months before the haematemesis which caused her admission, she had noted marked bruising and a swollen abdomen. She gave no history of jaundice.

Examination: Pleasant woman with a spider naevus at the bridge of her nose and on her left wrist. She was slightly jaundiced. Her liver was markedly enlarged and her spleen was palpable. She had haemorrhoids.

Investigations at time of absorption studies

Haematological findings: Initial Hb was 14 g./100 ml., falling to 7 g./100 ml. within first few days of admission. WBC 4,500/c.mm. Platelets 40,000, rising to 221,000/c.mm. Serum iron 202 µg./100 ml.

Liver function tests: Bilirubin on admission 3 mg./100 ml.; rose to 10 mg./100 ml., then fell to 1.8 mg./100 ml. on discharge. Alkaline phosphatase 10 units. SGPT 51 units on admission; rose to 120 units and then fell to 28 units on discharge. Serum albumin 2.8 g./100 ml.; rose to 3.9 g./100 ml. at time of discharge.

Faecal fat excretion 10 g./day.

During the absorption studies this lady's clinical condition was variable. A steady improvement occurred. Although no occult blood was present when absorption studies started, the investigations occurred probably within 2 weeks of the last haemorrhage. It was thought, therefore, that trypsin should be given during the first absorption test. This test was then followed by a control absorption test.

Results of absorption studies

Percentage of dose of ^{59}FeHb absorbed when given with trypsin; 81%. Percentage of dose of ^{59}FeHb absorbed when given alone; 39%. Percentage of dose of ^{59}FeHb absorbed when given with trypsin, when Hb and iron levels stabilised; 33%.

Mrs. S.M. R.I. No. 211315. Age 39. Born 1924.

Diagnosis: Cryptogenic cirrhosis of the liver.

1959 - Developed thyrotoxicosis with loss of weight, tachycardia and an enlarged thyroid. Treated with neomercazole and then radioactive iodine. Then quite well until -

1964 (March) - Readmitted with symptoms of anaemia and a return of her thyrotoxic symptoms.

Examination: A thin, very pale lady. No stigmata of liver disease. She had a diffuse goitre with a bruit over both poles. Cardiovascular system was normal. B.P. 190/70; slight tachycardia. Spleen palpable 4 cm. below costal margin. Liver felt as a hard irregular organ some 2 cm. below costal margin. Rectal examination was normal.

Investigations at time of absorption studies

Haematological findings: Hb 10 g./100 ml. Retics. 1%. WBC 2500/c.mm. Serum iron 78 µg./100 ml. Saturation of TIBC 30%. Folic acid 6.2 µg/ml. Serum B_{12} 186 µg./ml.

Liver function tests: Serum bilirubin 0.2 mg./100 ml. Total protein 6.6 g./100 ml., albumin 4.2 g./100 ml. Serum transaminase SGOT <4. SGPT 4. Flocculation tests negative. Pseudocholinesterase 36 units. BSP; 22% retained at 45 min.

Barium swallow showed no varices. Barium meal confirmed enlarged spleen.

Occult blood tests negative on 4 occasions.

Results of absorption studies

Percentage of dose of ^{59}Fe absorbed when given alone; 22%.

Mr. P.O. R.I. No. 300408. Age 53. Born 1909.

Diagnosis: Cirrhosis of the liver. Alcoholism.

1950 - Illness in which he was jaundiced for some weeks. Features of obstructive jaundice.

1956 - Recurrence of all previous symptoms, but did not become jaundiced.

1960 - Onset of nausea and loss of weight. Urine was noted to be dark for a few days, but stools always a normal colour. Nausea was relieved by an alcoholic drink. Has always had a very heavy intake of both beer and spirits. Would drink as much as 5 to 10 bottles of whisky a week. Usually eats well with a satisfactory intake of protein.

Examination: Cheerful Irishman. Sclera slightly icteric and has 4 spider naevi on trunk and right elbow. Liver greatly enlarged with nodular surface. Spleen palpable. No ascites or ankle oedema. Cardiovascular system normal. B.P. 115/75.

Investigations Dec. 1960 - Jan. 1961

Haematological findings: Hb 16.1 g./100 ml. WBC 6200/c.mm. ESR 30 mm./hr.

Liver function tests: Plasma bilirubin 0.6mg./100 ml. Alkaline phosphatase 13 units. Proteins 7.8 g./100 ml., albumin 3.4 g./100 ml. Flocculation tests: Thymol turbidity strongly positive. One-stage prothrombin time 51% of normal. Two-stage prothrombin time 21% of normal. Plasma amylase 7 units.

Barium meal showed a very large liver. Pneumoperitoneum confirmed this and also revealed a large spleen.

The low prothrombin level persisted after vitamin K. A liver biopsy was not taken.

At the time of the investigation he was well. There was no evidence of recent bleeding. He was not nauseated and was not clinically jaundiced. Two studies were carried out in March, 1963 and November, 1963.

Mr. P.O. (continued)

Investigations at time of absorption studies; March, 1963

Haematological findings: Hb 16.6 g./100 ml. Retics. 3.4%. Serum iron 274 µg./100 ml.

Liver function tests: Serum bilirubin 0.2 mg./100 ml. Alkaline phosphatase 10 units. Serum proteins 9.0 g./100 ml., albumin 3.6 g./100 ml. Flocculation tests strongly positive. BSP; 39% retained 45 min. after injection.

Investigations at time of absorption studies; Nov., 1963

Haematological findings: Hb 14.2 g./100 ml. Retics. 5.0%. Haemat. 32. MCHC 33%. Serum iron 252 µg./100 ml. Saturation of TIBC 90%.

Liver function tests: Serum bilirubin 2.7 mg./100 ml. Alkaline phosphatase 12 units. Proteins 8.9 g./100 ml., albumin 3.5 g./100 ml. Flocculation tests strongly positive. Serum pseudocholinesterase 16 units.

Faecal fat 4.9 g./day and 5.6 g./day on two separate 4-day collections.

Bone-marrow biopsy showed cellular marrow with increased amounts of iron present.

Results of absorption studies; March, 1963

Percentage absorption of $^{59}FeSO_4$ given alone; 78%.
Percentage absorption of $^{59}FeSO_4$ given with Cotazym ; 33.75%.

Nov., 1963

Percentage absorption of $^{59}FeSO_4$ given alone; 48.2%.
Percentage absorption of $^{59}FeSO_4$ given with trypsin; 44.5%.

Mrs. E.R. R.I. No. 358828. Age 52. Born 1911.

Diagnosis: Cryptogenic cirrhosis in liver failure.

1963 - Tiredness and lassitude developing progressively.

1964 (Jan.) - Noticed to be jaundiced; passed pale stools and dark urine for a few days. Then noticed abdominal distension.

She had never been previously jaundiced. There was no history of an excessive intake of alcohol. No previous medication with iron.

Examination: She was icteric with scattered spider naevi. She had well-marked clubbing of her fingers together with a flapping tremor. The cardiovascular system showed the presence of a hyperdynamic circulatory state. There was marked ascites and the spleen and liver could not be felt. Some pitting oedema of the legs up to the thighs was present.

She was treated with diuretics and made a good recovery. No studies were carried out until she had no oedema and there was no evidence of gastrointestinal bleeding.

Investigations at time of absorption studies

Haematological investigations: Hb 15.4 g./100 ml. MCHC 34%. ESR 35 mm./hr. ANF negative. LE cells negative. Serum iron on admission 96 µg./100 ml.; at time of absorption studies 212 µg./100 ml. Saturation of TIBC 76%.

Liver function tests: Plasma bilirubin 9.5 mg./100 ml. Plasma proteins 8.2 g./100 ml., albumin 2.6 g./100 ml. Alkaline phosphatase 30 units. Transaminase SGOT 80 units. Pseudocholinesterase 9 units.

Faecal fat excretion 12.7 g./day.

Barium meal showed some enlargement of the spleen, a large gastric ulcer and a small liver shadow.

Results of absorption studies

Percentage of ^{59}FeHb absorbed when given fasting; 3%.

Mr. A.S. R.I. No. 292701. Age 43. Born 1919.

Diagnosis: Cirrhosis of the liver. Chronic alcoholism.

Many years' history of excessive intake of alcohol. As much as a bottle of spirits a day was taken quite regularly up to his first admission with hepatic precoma.

1964 (April) - Depression and drowsiness resulted in his admission. His liver failure was treated successfully and he was discharged only to return to a heavy intake of alcohol.

Readmitted later in the month feeling weak and with a tremor. He had developed severe nausea and vomiting.

Examination: Tall man with severe jaundice. Large numbers of spider naevi over neck and chest. Cardiovascular system normal. B.P. 130/65. Liver palpable 5 cm. below costal margin. Spleen not palpable. No ankle or sacral oedema. He had a fine tremor.

Investigations at time of absorption studies

Haematological findings: Hb 10.2 g./100 ml. MCHC 33%. WBC 6,100/c.mm. Serum iron 72 µg./100 ml. Saturation of TIBC 30%.

Liver function tests: Plasma bilirubin 3.5 mg./100 ml. Alkaline phosphatase 11 units. Plasma proteins 7.5 g./100 ml., albumin 3.0 g./100 ml. Pseudocholinesterase 24 units. Transaminase SGOT 70 units; SGPT 28 units.

Faecal fat excretion 9.1 g./day.

Results of absorption studies

Percentage of dose of ^{59}FeHb absorbed; 4%.

Mrs. V.S. R.I. No. 326218. Age 49. Born 1913.

Diagnosis: Cryptogenic cirrhosis.

1962 - Developed an abdominal swelling and also attacks of intermittent diarrhoea. She was found to have ascites. Investigation confirmed the diagnosis of hepatic cirrhosis, and she was found to have oesophageal varices.

1963 (Jan.) - Admitted following her first haematemesis. An unsuccessful attempt to inject the oesophageal varices resulted in further bleeding, and after this she lapsed into coma.

1963 (May) - A second severe haematemesis resulted in her admission. The bleeding was controlled by Pitressin and a Sengstaken tube. She again lapsed into coma.

1963 (June) - Admitted to the Royal Free Hospital under the care of Professor Sheila Sherlock. She was noted to be lethargic but not icteric, and had an enlarged liver and spleen. Liver biopsy showed a coarse cirrhosis with areas of intralobular liver cell necrosis. Splenic venogram showed a patent portal vein. Large gastric collaterals were shown and the intrasplenic pressure was 25 mm. of mercury (raised).

She was discharged on diuretics and potassium supplements, and has since been in quite good health.

Examination at the time of diagnosis

No evidence of liver disease on inspection. Not anaemic and not jaundiced. No palpable liver or spleen, but marked ascites. Bilateral ankle oedema.

Investigations at time of absorption studies

Haematological findings: Hb 12.8 g./100 ml. Haemat. 40%. MCHC 32%. Serum iron 60µg./100 ml. Saturation of TIBC 21%.

Liver function tests: Serum bilirubin 1.0 mg./100 ml. Alkaline phosphatase 16 units. Total proteins 7.1 g./100 ml., albumin 3.2 g./100 ml. Flocculation tests negative. Serum pseudocholinesterase 16 units.

Barium swallow showed oesophageal varices and a large spleen.

Faecal fat excretion was 6 g./day on a 4-day collection.

Mrs. V.S. (continued)

Results of absorption studies

Absorption of a dose of ^{59}FeHb given alone; 64%. Absorption of a dose of ^{59}FeHb with 2.5 g. of Cotazym ; 18%.

Mrs. E.W. R.I. No. 321114. Aged 73. Born 1888.

Diagnosis: Cryptogenic cirrhosis.

1962 (Feb.) - Developed bronchopneumonia. Shortly afterwards had a melaena. On admission she was anaemic, had hepatomegaly and splenomegaly and was found to be diabetic. She was considered to have portal hypertension as a barium swallow showed oesophageal varices.

1962 (April) - Successful mastectomy for carcinoma of the left breast. Followed by radiotherapy.

1962 (Sept.) - Admitted following a second melaena.

1963 (March) - Admitted for her first series of iron studies and was admitted again in Nov. 1963 for a second group. She was well during both studies with no evidence of further bleeding.

Examination at time of absorption studies

Cheerful, obese lady. Several spider naevi on face. Slight foetor hepaticus. Cardiovascular system - diastolic murmur at apex of heart. B.P. 170/80. Liver and spleen just palpable.

Investigations at time of absorption studies (March, 1963)

Haematological findings: Hb 13.4 g./100 ml. MCHC 36%. Haemat. 37. Retics. 1.8%. WBC 2400/c.mm. Normal differential. ESR 28 mm./hr. Serum iron 184 µg./100 ml. Saturation of TIBC 57%.

Liver function tests: Plasma bilirubin 0.8 mg./100 ml. Alkaline phosphatase 16 units. Serum proteins 6.9 g./100 ml., albumin 3.6 g./100 ml. Plasma transaminase SGPT 44 units. Flocculation tests negative. BSP; 23% of dye retained at 45 min.

Faecal fat excretion 4.7 g./day on a 4-day collection.

(Nov., 1963)

Haematological findings: Hb 12.6 g./100 ml. Retics. 1.8 to 3.8%. WBC 3300/c.mm. ESR 17 mm./hr.

Liver function tests: Plasma bilirubin 0.8 mg./100 ml. Alkaline phosphatase 8 units. Serum proteins 6.7 g./100 ml., albumin 3.9 g./100 ml. Serum electrophoresis showed a raised γ-globulin. BSP showed 4% of dye remained in blood 45 min. after injection. Plasma transaminase SGOT 28 units.

Bone-marrow biopsy showed hypoplastic marrow with no increase in stainable iron.

Mrs. E.W. (continued)

Results of iron absorption studies (March, 1963)

Percentage absorption of a dose of $^{59}FeSO_4$ given alone; 65%.
Percentage absorption of a dose of $^{59}FeSO_4$ given with 2.5 g. of Cotazym : 26%.

(Nov. 1963)

Percentage absorption of a dose of $^{59}FeSO_4$ given alone; 25%.
Percentage absorption of a dose of $^{59}FeSO_4$ given with 6 g. of trypsin; 15.7%.

Mrs. E.B.W. R.I. No. 291112. Aged 59. Born 1902.

Diagnosis: Cryptogenic cirrhosis of the liver.

1960 (May) - Onset of left-sided abdominal pain. This occurred at mealtimes and was followed by diarrhoea.

1962 (April) - Developed post-menopausal bleeding. Was admitted for gynaecological investigations. Splenomegaly, neutropaenia and thrombocytopenia were noted. Transferred to Nuffield Medical Unit for investigations.

No past history of jaundice. No excessive intake of alcohol. Dietary history normal.

Examination: Not anaemic. Spider naevi present over face and chest. Not jaundiced. Palmar erythema. Spleen palpable. Cardiovascular system normal. B.P. 110/85.

Investigations at time of diagnosis

Haematological findings: Hb 14.5 g./100 ml. PCV 41%. MCHC 35%. Retics. 1.6%. WBC 3500/c.mm. Platelets 210,000/c.mm. Film showed a mild neutropenia.

Liver function tests: Serum bilirubin 0.4 mg./100 ml. Serum proteins 6.7 g./100 ml. Raised globulin. Prothrombin activity normal. BSP; 22% remained at 45 min. Coombs test negative. LE cells negative.

Barium meal confirmed the splenomegaly and oesophageal varices were noted.

Liver biopsy; no marked changes were seen in the small liver specimen obtained, but specimen very small. Small amounts of stainable iron present.

Investigations at time of absorption studies (Nov. - Dec., 1962)

Haematological findings: Hb 14.5 g./100 ml. PCV 44%. MCHC 33%. WBC 4000/c.mm. Platelets 240,000/c.mm. Serum iron 144 µg./100 ml. Saturation of TIBC 49%.

Liver function tests: Plasma bilirubin 1.6 mg./100 ml. Alkaline phosphatase 5 units. Plasma proteins 6.9 g./100 ml., albumin 4.2 g./100 ml. Flocculation tests: pseudocholinesterase 30 units.

Faecal fat excretion 2.5 g./day.

Mrs.E.B.W. (continued)

Investigations (Dec. 1963)

Hb 14.6 g./100 ml. WBC 5,900/c.mm. ESR 3 mm./hr. Platelets 300,000/c.mm.

Liver function tests: Plasma bilirubin 1.0 mg./100 ml. Alkaline phosphatase 5 units. Total proteins 6.8 g./100 ml., albumin 4.4 g./100 ml. Pseudocholinesterase 35 units. Serum iron 104 µg./100 ml.

Faecal fat excretion 1.8 g./day and 4.5 g./day.

Results of absorption studies (Nov. - Dec., 1962)

Percentage of dose of ^{59}FeHb absorbed when given alone; 54%. Percentage of dose of ^{59}FeHb absorbed when given with 2.5 g. of Cotazym : 26%.

(Dec., 1963)

Percentage of dose of $^{59}FeSO_4$ absorbed when given alone; 33%. Percentage of dose of $^{59}FeSO_4$ absorbed when given with 6 g. of pure trypsin; 26%.

Mrs. K.W. R.I. No. 163711. Age 74. Born 1888.

Diagnosis: Cryptogenic cirrhosis.

1962 (Dec.) - Nausea, flatulence and some loss of weight for about a year. More recently her stools were pale and her urine dark, and she had become jaundiced. There was no history of drugs which might have induced jaundice. There was no evidence of excessive alcohol intake in the past.

Examination: She was a mildly icteric woman with no cutaneous evidence of cirrhosis hepatis. She was a little disorientated and at times had a flapping tremor. Cardiovascular system normal. B.P. 160/95. Liver edge 4 cm. below costal margin, irregular and hard.

Investigations at time of diagnosis and absorption studies

Haematological findings: Hb 10.9 g./100 ml. MCHC 32%. WBC 7,100/c.mm. Platelets 350,000/c.mm. Serum iron 130 μg./100 ml. Saturation of TIBC 50%.

Liver function tests: Plasma bilirubin 3.0 mg./100 ml. Alkaline phosphatase 80 units. Proteins 7.4 g./100 ml., albumin 2.6 g./100 ml. Electrophoresis showed a raised γ-globulin. Plasma transaminase SGPT 80 units.

Faecal fat excretion 4.40 g./day.

Results of iron absorption studies

Percentage of dose of ^{59}FeHb absorbed when given alone; 49%. Percentage of dose of ^{59}FeHb absorbed when given with 2.8 g. of Cotazym : 43%.

Mr. W.W. R.I. No. 149628. Age 74. Born 1889.

Diagnosis: Cryptogenic cirrhosis of the liver. Tertiary syphilis.

1951 - This patient, who had been a known tabetic since 1931, developed transient jaundice and was noted to have an enlarged liver and spleen. The jaundice spontaneously disappeared.

1955 - He was noted to be anaemic. The anaemia was due to chronic blood loss and responded well to iron therapy. No abnormalities were noted on a barium meal and enema. Started on iron.

1963 - A further episode of bleeding caused anaemia and he was admitted. There was no evidence of iron deficiency.

On examination at time of investigations

Not anaemic. Not jaundiced. No evidence of cutaneous signs of liver disease. Spleen just palpable.

Investigations at time of absorption studies

Haematological findings: Hb 13.7 g./100 ml. Haemat. 45%. MCHC 30%. WBC 4,400/c.mm. Platelets 697,000/c.mm. Serum iron 384 µg./100 ml. Saturation of TIBC 80%.

Liver function tests: Plasma bilirubin 0.6 mg./100 ml. Proteins 7.5 g./100 ml. Serum amylase 7 units. Pseudocholinesterase 43.5 units.

Barium swallow showed oesophageal varices, a small liver and an enlarged spleen.

Faecal fat 7 g./day. Occult blood positive on one occasion.

Results of absorption studies

Percentage absorption of $^{59}FeSO_4$ given alone; 82%. Percentage absorption of $^{59}FeSO_4$ given with 250,000 units of trypsin; 63%.

PATIENTS WITH

ACUTE LIVER DISEASE

Mrs. N.C. R.I. No. 351005. Aged 63. Born 1900.

Diagnosis: Infective hepatitis.

1963 (May) - Became unwell. Then developed jaundice with pale stools and dark urine. Jaundice fluctuated in severity and after clearing temporarily returned with increased severity in September. Was accompanied this time by severe anorexia and weight loss.

Examination: Marked jaundice. Apyrexial. Some finger clubbing. Cardiovascular system, auricular fibrillation and mitral incompetence. B.P. 145/65. Liver not palpable. Spleen just palpable.

Investigations at time of diagnosis and absorption studies

Haematological findings: Hb 12.3 g./100 ml. Haemat. 39%. MCHC 32%. WBC 7,200/c.mm. ESR 15 mm./hr. LE cells negative on three occasions. Antinuclear factor negative.

Liver function tests and serum iron (9.10.63): Plasma bilirubin 11.6 g./100 ml. Alkaline phosphatase 18 units. Proteins 7.5 g./100 ml., albumin 3.5 g./100 ml. Pseudocholinesterase 39 units. Transaminase levels - SGOT >2000. SGPT 1500. Thymol turbidity positive. Serum iron 256 µg./100 ml.

Faecal fat excretion 5.3 g./day.

Chest X-ray showed cardiac enlargement compatible with mitral incompetence. ECG confirmed atrial fibrillation. Barium meal confirmed splenomegaly.

Liver function tests and serum iron (16.10.63, after starting steroids): Plasma bilirubin 9 mg./100 ml. Alkaline phosphatase 13 units. Transaminase - SGOT 300 units. SGPT 130 units. Serum iron 140 µg./100 ml.

Faecal fat 1.7 g./day.

Liver function tests (on 15.11.63, when jaundice almost gone): Bilirubin 2 mg./100 ml. Alkaline phosphatase 8 units. Proteins 6.3 g./100 ml., albumin 4 g./100 ml. Flocculation tests negative. Transaminase - SGPT 10 units. Serum iron 100 µg./100 ml.

Faecal fat excretion 1.0 g./day.

Mrs. N.C. (continued)

Results of absorption studies

Percentage of dose of $^{59}FeSO_4$ absorbed during acute phase with both obstructive and cellular damage evident; 9%.
Percentage of dose of $^{59}FeSO_4$ with obstruction marked but evidence of hepatocellular damage less; 20%.
Percentage of dose of $^{59}FeSO_4$ with liver function tests nearly normal; 2%.

Mrs. M.D. R.I. No. 222632. Age 43. Born 1920.

Diagnosis: Lupoid hepatitis.

She has had a symmetrical polyarthritis for a number of years which required salicylates for the relief of pain.

1963 (July) ; She developed nausea, vomiting, anorexia and drowsiness, and this was attributed at first to the effect of salicylates. In August she developed jaundice and then remained persistently and severely jaundiced.

Examination: Thin, jaundiced woman with swelling and deformity of the small joints of her hands. She had some slight lid retraction and a palpable thyroid gland. Cardiovascular system normal. B.P. 120/80. The liver and spleen were not palpable.

Investigations at time of absorption studies

Initial investigations: Haematological findings: Hb 14.9 g./100 ml. MCHC 34%. Haemat. 44%. WBC 5700/c.mm. ESR 93 mm./hr.

Liver function tests: Plasma bilirubin 8 mg./100 ml. Alkaline phosphatase 18 units. SGOT 460 units. SGPT 400 units. Proteins 8.1 g./100 ml., albumin 3.2 g./100 ml. Electrophoresis showed increased γ-globulin. Prothrombin time, two-stage, 24% (normal >50%). Flocculation tests; thymol turbidity positive. Colloidal gold negative. Pseudocholinesterase 19 units.

Serum iron 164 μg./100 ml. Serum antinuclear factor strongly positive. LE cell phenomenon strongly positive.

Barium meal showed a slightly enlarged liver. No splenomegaly.

Faecal fat excretion 13.6 g./day.

Investigations following administration of prednisolone (i.e., during third absorption study)

Liver function tests: Plasma bilirubin 2 mg./100 ml. Serum alkaline phosphatase 13 units. SGOT 32 units. SGPT 20 units.

Serum iron 60 μg./100 ml.

Faecal fat 4.6 g./day.

Mrs. M.D. (continued)

Results of iron absorption studies

Before the administration of steroids, whilst showing evidence of severe jaundice and hepatocellular damage -
Percentage absorption of a dose of $^{59}FeSO_4$ given alone; 43%.
Percentage absorption of a dose of $^{59}FeSO_4$ given with 2.5 g. of Cotazym; 40%.

Following administration of prednisolone when clinically much better and when biochemical tests had improved -
Percentage absorption of a dose of $^{59}FeSO_4$ given alone; 29%.

Mrs. K.M. R.I. No. 257860. Age 29. Born 1934.

Diagnosis: Infective hepatitis. ? Chlorpromazine jaundice.

1963 (July) - Started to feel unwell. She became agitated, developed anorexia and a vague upper abdominal discomfort. She was given largactil a month later.

1963 (Sept.) - Two weeks after starting largactil she developed jaundice with pale motions and dark urine. The largactil was stopped but the jaundice continued and increased in intensity.

Examination: She was a thin woman obviously jaundiced. There were a few superficial skin excoriations as a result of pruritis. The cardiovascular system was normal. Her liver was just palpable and tender.

Investigations carried out at time of absorption studies

Sept., 1963. Haematological findings: Hb 12.5 g./100 ml. WBC 5200/c.mm. ESR 32 mm./hr. Serum iron 472 µg./100 ml.

Liver function tests: Plasma bilirubin 19.5 g./100 ml. Alkaline phosphatase 28 units. Plasma proteins 5.9 g./100 ml. Serum albumin 2.9 g./100 ml. Plasma transaminase SGOT 22 units. SGPT 22 units. Serum pseudocholinesterase 37 units. LE cells negative.

Faecal fat excretion 47 g./day.

May., 1964 Liver function tests: Plasma bilirubin 0.9 g./100 ml. Alkaline phosphatase 29 units. Total proteins 6.9 g./100 ml. Normal electrophoresis. Pseudocholinesterase 66 units. Transaminase SGPT 82 units.

Results of iron absorption tests

Percentage absorption of a dose of $^{59}FeSO_4$ given alone when ill; 15.7%. Percentage absorption of a dose of $^{59}FeSO_4$ given alone during recovery; 13.0%.

Miss G.P. R.I. No. 332446. Age 56. Born 1905.

Diagnosis: Infective hepatitis.

1962 (Sept.) - Onset of malaise, pale motions and dark urine followed by jaundice. Admitted under surgical firm when a laparotomy was done for possible obstructive jaundice. A liver biopsy was compatible with this diagnosis or drug-induced cholestatic jaundice. No evidence of obstruction was found at operation, and there was no history of drug-taking.

Examination: A jaundiced, mildly demented woman who had no other cutaneous signs of liver disease. Cardiovascular system was normal. B.P. 145/80. Liver and spleen were not palpable. No neurological signs of hepatic failure.

Investigations at time of absorption studies

Haematological findings: Hb 14.8 g./100 ml. Haemat. 45%. MCHC 33%. Retics. 2%. WBC 7,800/c.mm. ESR 13 mm./hr.

Liver function tests: Plasma bilirubin 0.3 mg./100 ml. Alkaline phosphatase 28 units. Proteins 7.6 g./100 ml., albumin 5.1 g./100 ml. Flocculation tests negative. Serum iron 52 μg./100 ml. Plasma pseudocholinesterase 52.5 units.

Faecal fat 3 g./day.

The patient was not on steroid therapy at time of investigation.

Results of absorption studies

Percentage absorption of ^{59}FeHb given alone; 39%.
Percentage absorption of ^{59}FeHb when given with Cotazym : 43%.

Mrs. I.P. R.I. No. 305111. Age 56. Born 1907.

Diagnosis: Lupoid hepatitis.

1961 (Feb.) - She developed bronchopneumonia. She was found to be in auricular fibrillation which was controlled by quinidine. On admission, examination showed lymphadenopathy, hepatomegaly and splenomegaly. A liver biopsy showed lymphocytic proliferation and plasma cells. A lymph node biopsy suggested disseminated lupus erythematosus.

1961 (May) - She was admitted with ascites and peripheral oedema. A pneumoperitoneum showed a small nodular liver. She improved on diuretic therapy and an increased dose of steroids.

1962 (Oct.) - Admitted in a relapse with pruritis, jaundice, dark urine and pale stools.

Examination: She was mildly icteric. She had hepatomegaly and splenomegaly. There was slight sacral oedema and some ascites.

Investigations at time of absorption studies

Haematological findings: Hb 13.9 g./100 ml. Haemat. 41%. MCHC 34%. WBC 7000/c.mm. ESR 58 mm./hr. Serum iron 120 µg./100 ml.

Liver function tests: Plasma bilirubin 3 mg./100 ml. Proteins 7.2 g./100 ml., albumin 2.6 g./100 ml. Plasma transminase SGPT 160 units. Prothrombin time 69 sec. ANF positive. LE cells positive.

Faecal fat excretion 7.8 g./day.

Results of absorption studies

All absorption studies carried out whilst on steroids. This was necessary for clinical reasons. The dosage was kept constant.

Percentage absorption of ^{59}FeHb given alone; 16.2%.
Percentage absorption of ^{59}FeHb given with Cotazym : 16.3%.

Mr. I.P. R.I. No. 351058. Age 36. Born 1927.

Diagnosis: Acute infective hepatitis.

1964 - Admitted for removal of ureteric calculus. Following removal of the stone, became anorexic, then mildly jaundiced. Liver became tender, stools pale and urine dark. No history of drugs likely to cause jaundice. Gradual recovery over 3 weeks.

Examination: Obese, cheerful man. Slightly icteric. No overt signs of liver disease. Cardiovascular system normal. B.P. 140/90. Spleen not palpable. Liver just palpable; quite tender. No oedema of ankles.

Investigations at time of absorption studies

Haematological investigations: Hb 14.4 g./100 ml. PCV 39%. Serum iron 172 µg./100 ml.

Liver function tests (when ill): Blood plasma bilirubin 1.8 mg./100 ml. Alkaline phosphatase 12 units. Plasma proteins 7 g./100 ml., albumin 3.9 g./100 ml. Pseudocholinesterase 30 units. Plasma transaminase SGPT 460 units. Faecal fat 6.4 g./100 ml.

Liver function tests (when recovered, May, 1964): Plasma bilirubin 0.7 mg./100 ml. Alkaline phosphatase 6 units. Plasma albumin 5.3 g./100 ml. Pseudocholinesterase 74 units.

Results of absorption studies

Percentage absorption of dose of $^{59}FeSO_4$ when ill; 15%.
Percentage absorption of dose of $^{59}FeSO_4$ when well; 32%.

Mr. I.T. R.I. No. 331726. Age 33. Born 1930.

Diagnosis: Infective hepatitis; iron-deficiency anaemia- partial gastrectomy.

1957 - Partial gastrectomy done following a severe haematemesis from a duodenal ulcer.

1962 (Sept.) - Noted soreness of lips and tongue together with dysphagia. Found to have an iron-deficiency anaemia. Treated with parenteral and oral iron.

1964 (March) - Whilst awaiting admission for investigation of a second episode of iron-deficiency anaemia, developed an acute febrile illness accompanied by jaundice, pale motions and dark urine. He became anorexic with extreme distaste for tobacco.

Examination: Pale, thin man who was moderately icteric. Tongue smooth. Cardiovascular system and B.P. 110/60. Liver not palpable but tender. Spleen not palpable.

Investigations at time of absorption studies

Haematological investigations: Hb 9 g./100 ml. MCHC 29%. Retics. 3.2%. Serum iron before jaundice 21 µg./100 ml.; after onset 101 µg./100 ml. Serum iron-binding capacity 321 µg./100 ml. (after onset of jaundice).

Liver function tests: Blood plasma bilirubin 2.5 mg./100 ml. Proteins 6.9 g./100 ml., albumin 3.5 g./100 ml. Alkaline phosphatase 48 units. Plasma transaminase SGPT 100 units. Pseudocholinesterase 27 units.

Faecal fat excretion 3.8 g./day.

Results of absorption studies

Percentage absorption of dose of $^{59}FeSO_4$; 49%.

Mrs. M.W. R.I. No. 271168. Age 35. Born 1927.

Diagnosis: Serum hepatitis.

1962 (Nov.) - Dental extraction with local anaesthetic and influenza virus vaccine.

1963 (March) - Onset of anorexia, abdominal pain and dark urine. Stools became pale and she became jaundiced. She lost about a stone in weight and could not eat very much. No history of jaundice in the neighbourhood. No history of alcohol or recent medication.

Examination: Looked fairly well but had lost weight. Slightly icteric with spider naevi on arms and hands and telangiectasia of the cheeks. Cardiovascular system was normal. B.P. 160/70. In the abdomen the spleen was not palpable. Liver just palpable and tender. No oedema of the legs.

Investigations at time of absorption studies

Haematological findings (May, 1963): Hb 10.9 g./100 ml. MCHC 32%. Retics. 1.6%. WBC 5,800/c.mm. ESR 92 mm./hr. Serum iron 200 μg./100 ml. ANF negative. LE cells negative.

Liver function tests: Plasma bilirubin 3.2 mg./100 ml. Alkaline phosphatase 10 units. Proteins 8 g./100 ml., albumin 2.2 g./100 ml. Flocculation tests positive. Transaminase SGPT 260 units. Pseudocholinesterase 14.5 units. Bromsulphthalein excretion; 65% remained after 45 min.

Faecal fat excretion 18.9 g./day on 4-day collection.

Radiographic examination: A barium meal showed no abnormality.

Haematological findings (June, 1963), when on prednisolone: Hb 11.7 g./100 ml. MCHC 32%. Retics. 3%. ESR 58 mm./hr. Serum iron 184 μg./100 ml. TIBC 322 μg./100 ml. Saturation of TIBC 57%.

Liver function tests: Plasma bilirubin 2.4 mg./100 ml. Alkaline phosphatase 10 units. Transaminase SGPT 106 units. BSP; 50% remained after 45 min.

Results of absorption studies

Percentage of dose of $^{59}FeSO_4$ absorbed in May, 1963; 5%. Percentage of dose of $^{59}FeSO_4$ absorbed when better and on steroids in June, 1963; 28%.

PATIENTS WITH HAEMOCHROMATOSIS

Mr. A.C. R.I. No. 300359. Age 53. Born 1910.

Diagnosis: Haemochromatosis.

1960 - Onset of diabetes mellitus. Started on insulin and was quite well until July, 1961. Sudden onset of diarrhoea and vomiting. Became dehydrated and drowsy. Persistent abdominal pain necessitated a laparotomy when an inflamed appendix was removed. Following this, his diabetes became well controlled. No history of excessive intake of alcohol.

Examination: Apparent weight loss. No apparent pigmentation. No signs of liver failure. Heart normal on examination. B.P. 145/80. Liver enlarged 4 fingerbreadths. No splenic enlargement.

Investigations at time of diagnosis (Aug. - Oct., 1961).

Haematological findings: Hb 14 g./100 ml. C.I. 0.9. Retics. 2.6%. WBC 15,700/c.mm. ESR 52 mm./hr. Sternal marrow normal. No excess iron. Serum iron 104 µg./100 ml.

Liver function tests: Serum bilirubin 0.4 mg./100 ml. Alkaline phosphatase 33 units. Proteins 6.1 g./100 ml., albumin 3.3 g./100 ml.

Liver biopsy (SH serial No. 6545): "The striking feature about the liver cells is the presence of a large amount of brown pigment granules present within the cytoplasm of the hepatic cells and within the cytoplasm of the cells lining the sinusoids. This material gives a strongly positive reaction for iron. The appearances are those of haemochromatosis with some degree of cirrhosis."

The patient's conditions was very satisfactory at the time of his investigation. His diabetes mellitus was under control.

Investigations at time of absorption studies

Haematological findings: Hb 17.2 g./100 ml. C.I. 1.0. Haemat. 49%. MCHC 38%. ESR 5 mm./hr. Platelets normal. Serum iron 296 µg./100 ml. Saturation of TIBC 94%.

Liver function tests: Serum bilirubin 0.9 mg./100 ml. Alkaline phosphatase 22 units. Proteins 6.3 g./100 ml., albumin 4.0 g./100 ml. Flocculation tests negative. Pseudocholinesterase 85 units.

Daily faecal fat excretion; 6.3 g. over a 4-day collection.

Results of iron absorption studies

Absorption of ^{59}FeHb given alone; 15%. Absorption of ^{59}FeHb given with 2.5 g. of Cotazym : 9%.

Mr. L.D. R.I. No. 340856. Age 70. Born 1893.

Diagnosis: Haemochromatosis.

1961 - Onset of diabetes. Was noted to be pigmented and to have a large liver. Liver biopsy at Stoke Mandeville Hospital confirmed the diagnosis. Wide variation in his insulin requirement during his course of 20 to 30 venesections.

1963 (Nov.) - Admitted for studies. Had been unwell, with loss of weight, fever, nausea and vomiting. He was considered to have developed a hepatoma but a pneumoperitoneum showed no abnormality. A positive blood culture for haemolytic streptococci responded well to penicillin and he made a good recovery. Iron absorption studies done at this time were unsatisfactory because of his general condition.

1964 (Jan.) - Admitted whilst well for absorption studies.

Examination: Typical pigmentation on face, forearms and trunk, less marked than on previous admission. Cardiovascular system normal. B.P. 140/80. Abdomen: liver enlarged 5 cm. below costal margin. Spleen not palpable. Testicular atrophy.

Investigations at time of absorption studies

Haematological findings: Hb 13.8 g./100 ml. C.I.·98. MCHC 32%. Retics. 1%. WBC 10,100/c.mm. Serum iron 172 µg./100 ml. TIBC 222 µg./100 ml. Saturation of TIBC 63%.

Liver function tests: Bilirubin 0.7 mg./100 ml. Alkaline phosphatase 21 units. Proteins 6.2 g./100 ml., albumin 3.6 g./100 ml.

Liver biopsy: Changes consistent with haemochromatosis.

Results of iron absorption studies

Percentage absorption of ^{59}FeHb absorbed when given alone; 9%. Percentage absorption of ^{59}FeHb absorbed when given with Cotazym : 0.7%. Percentage absorption of ^{59}FeHb absorbed when given with trypsin; 3.5%.

Mr. I.E. R.I. No. 210602. Age 61. Born 1902.

Diagnosis: Haemochromatosis.

1956 - Diagnosis of cirrhosis hepatis made at St. Thomas's Hospital, London.

1961 - Early in this year he started to feel unwell. Became tired and started to lose weight. In December he developed polyuria and thirst. Weight loss became more acute. Diagnosis of diabetes mellitus and possibly also haemochromatosis.

1961 (March) - Admitted to the Nuffield Department of Medicine for further investigation. No family history of haemochromatosis. Had been a very moderate drinker of alcohol.

Examination: Greyish-brown generalised pigmentation, accentuated on face, arms and lower legs. Sparse axillary and pubic hair. No gynaecomastia or gonadal atrophy. No stigmata of liver disease. Liver palpable 10 cm. below costal margin; spleen not palpable.

Investigations at time of diagnosis

Haematological findings: Hb 14.5 g./100 ml. Haemat. 42%. MCHC 34%. ESR 12 mm./hr. Platelets normal. Serum iron 274 g./100 ml. Saturation of TIBC 69%.

Liver function tests: Serum bilirubin 0.2 mg./100 ml. Proteins 6.8 g./100 ml., albumen 5.5 g./100 ml. Flocculation tests negative. Prothrombin time normal. Random blood sugar 170 mg. to 270 mg./100 ml.

Liver biopsy (serial No. 2307): Three fragments of tissue 0.1 x 0.1 x 0.6 cm. Histology: "The sections show fragments of liver parenchyma with a few periportal tracts. There are numerous haemosiderin granules in most of the hepatic cells. In Kupffer cells they form thick masses obscuring the nucleus. All these granules give positive Prussian blue reaction for iron. The periportal tracts are thickened and show proliferation of bile ducts and moderate round cell infiltration. There is very little iron pigment in the fibrous septa. The histological picture is that of a moderate fibrosis of the liver with marked deposition of iron in the liver parenchyma. It is consistent with the diagnosis of haemochromatosis."

Mr. I.E. (continued)

Investigations at time of absorption studies (July - Oct., 1963)

Haematological findings: Hb 14.5 g./100 ml. Haemat. 43. MCHC 34%. Serum iron 248 μg./100 ml. TIBC 300 μg./100 ml.

Alkaline phosphatase 12 units.

Faecal fat excretion 5.6 g./day on a 4-day collection.

Results of iron absorption studies

Absorption of ^{59}FeHb given alone; 20.2%.
Absorption of ^{59}FeHb given with 2.5 g. of Cotazym ; 17%.
Absorption of ^{59}FeHb given with 500,000 units of purified trypsin; 16.7%.

Mr. K.E. R.I. No. 347410. Age 33. Born 1930.

Diagnosis: Haemochromatosis.

1950 - Onset of impotence with loss of libido. Gradual onset of pigmentation and development of smooth skin and fair hair. Had felt increasingly tired over the last few years. Had a heavy consumption of alcohol at one time, but in the past year has almost completely abstained. No family history of diabetes or pigmentation.

Examination: Medium height but slightly built with a wizened face and smooth complexion. Face and limbs show a greyish-brown pigmentation. Almost no axillary or pubic hair. Marked palmar erythema. No abnormality in the cardiovascular system. B.P. 115/75. Respiratory system normal. In the abdomen the liver was enlarged, smooth in consistency, extending to 13.5 cm. below right costal margin. The spleen was just palpable. Testicular atrophy was present.

Investigations at time of diagnosis

Haematological findings: Hb 12.4 g./100 ml. WBC 6,400/c.mm. ESR 12 mm./hr. Serum iron 230 µg./100 ml. Iron-binding capacity 287 µg./100 ml.

Liver function tests normal.

Liver biopsy: "Portal cirrhosis with severe and diffuse infiltration with iron-containing pigment, indicative of haemochromatosis."

Urinary steroids, excretion of 17-ketosteroids and 17-hydroxycorticoids in two 24-hour collections was 4.4 mg. and 4.9 mg., and 7.1 mg. and 6.7 mg. No rise was seen following the injection of 750 mg. of Metoperine at 4-hourly intervals for 6 doses. This indicated pituitary dysfunction.

Blood urea 42 mg./100 ml. Normal glucose tolerance test.

Clinical condition at time of absorption studies

Had had two venesections some time prior to his admission. Was in good health. On this admission in July, 1963 was noted to have a spider naevus over right inner canthus and over his sternum.

Mr. K.E. (continued)

Investigations at time of absorption studies

Haematological findings: Hb 15.4 g./100 ml. C.I. 1.0. Retics. 0.6%. MCHC 35%. Haemat. 44. WBC 11,300/c.mm. ESR 61 mm./hr. Serum iron 228 g./100 ml. Saturation of TIBC 79%.

Liver function tests: Blood urea 28 mg./100 ml. Serum bilirubin 0.3 mg./100 ml. Alkaline phosphatase 12 units. Total proteins 6.7 g./100 ml. Flocculation tests negative. Pseudocholinesterase 51 units. Bromsulphthalein test; 14% retention after 45 min.

Barium meal showed moderate enlargement of the liver. Spleen probably enlarged also.

Results of investigations of iron absorption

Absorption of ^{59}FeHb given alone; 5% of dose.
Absorption of dose of ^{59}FeHb given with 2.5 g. of Cotazym ; 0% of dose.

Mr. R.F. R.I. No. 253000. Age 53. Born 1909.

Diagnosis: Haemochromatosis.

1955 - Noted gradual disappearance of axillary and pubic hair. Had always been pigmented.

1961 - Onset of loss of weight. Nearer to his admission he developed polydipsia and polyuria. On investigation the presence of pigmentation, hepatomegaly and diabetes suggested the diagnosis of haemochromatosis. Skin and marrow biopsy were compatible with the diagnosis. He was started on insulin and regular venesection.

1963 (June) - Admitted for an osteotomy. General condition much the same. Absorption studies carried out. Did not do well following operation. He was transferred to medical care in August, 1963. His liver started to enlarge rapidly and he died in September. Post-mortem showed a large hepatoma.

Patient had always been a heavy drinker - some 3 to 4 pints of beer daily and 2 to 3 brandies at night at least.

Examination: Thin man. Silver-grey pigmentation of the face. Bronze pigmentation of arms, legs and abdomen. Scanty axillary and pubic hair. Liver palpable 6.2 cm. below costal margin. Spleen not palpable. One small testis on left side. Cardiovascular system normal.

Investigations at time of absorption studies

Haematological findings: Hb 14.6 g./100 ml. Serum iron 152 µg./100 ml.

Liver functiont tests: Bilirubin 1.3 mg./100 ml. Serum proteins 5.6 g./1000 ml., albumin 2.9 g./100 ml. Flocculation tests negative. Alkalane phosphatase 13 units. Pseudocholinesterase 10 units.

Faecal fat excretion 2.2 g./day.

Results of absorption studies (June, 1963)

Percentage absorption of ^{59}FeHb when given alone; 9.3%.
Percentage absorption of ^{59}FeHb when given with 2.5 g. of Cotazym : 0%.

Mr. R.F. R.I. No. 253000. Age 53. Born 1909.

Post-mortem

Liver weighed 4,180 g.; light brown; showed a fine cirrhosis. In the upper part there was a primary carcinoma. Macroscopically and microscopically stainable iron was present in the liver.

The pancreas was brown, tough and heavily iron-laden. Other tissues such as heart and kidney showed little iron staining.

Mr. B.J. R.I. No. 354353. Age 46. Born 1917.

Diagnosis: Early haemochromatosis.

1954 - Duodenal ulcer proved on barium meal examination.

1955 - Perforation of duodenal ulcer and a few months later had a haematemesis and melaena. He had a polya type gastrectomy. Following this drank alcohol very heavily.

1962 - Admitted to St. John's Hospital with an organic dementia. Noted to have an enlarged liver but spleen was not palpable.

1963 - Admitted to Stoke Mandeville Hospital with depression, loss of weight and anorexia. Noted to have an enlarged liver. His haemoglobin was 40%. MCHC 29% but the marrow was heavily iron-laden. Serum iron 175 µg./100 ml. TIBC 233 µg./100 ml. Saturation of TIBC 75%. Liver biopsy showed increased fibrosis with excess of iron distributed like that of early haemochromatosis.

No family history of diabetes or pigmentation. (Had had a 4-pint blood transfusion and a short course of oral iron.)

Examination: Pigmented. Mucous membranes pale. Cardiovascular system normal. B.P. 130/70. Liver palpable 3 cm. below RCM. Spleen impalpable.

Investigations at time of absorption studies

Haematological findings: Hb 9.5 g./100 ml. C.I. 1.1. Retics. 4%. MCHC 32%. Serum iron 39 µg./100 ml. TIBC 228 µg./100 ml. Percentage saturation 17%.

Liver function tests: Bilirubin 0.7 mg./100 ml. Alkaline phosphatase 15 units. Total proteins 6.8 g./100 ml. Increased γ-globulin on electrophoresis. Pseudocholinesterase 41 units.

BSP; 6% of dye retained at 30 min. Occult blood negative.

Liver biopsy sections on further study compatible with haemochromatosis at an early stage.

Results of absorption studies

Percentage absorption of ^{59}FeHb when given alone; 16%.
Percentage absorption of ^{59}FeHb when given with Cotazym; 13%.
(Stool collected incomplete as patient discharged himself from the ward.)

Mr. E.P. R.I. No. 137420. Age 59. Born 1899.

Diagnosis: Haemochromatosis.

1953 - Onset of diabetes mellitus. Controlled satisfactorily on insulin.

1958 - Admitted to hospital because of a persistent cough. Noted to be pigmented, to have considerable hepatomegaly and to be poorly controlled as a diebetic.

Following this admission when a diagnosis of haemochromatosis was made, he was regularly venesected. His health improved and his insulin requirement diminished. No family history of diabetes. Occasional alcohol.

Examination: Tall, thin, pigmented man. Brown pigmentation chiefly on face and hands. Cardiovascular system normal. B.P. 140/80. Liver enlarged to the level of the iliac crest, hard and smooth. Spleen not palpable. No testicular atrophy.

Investigations: Haematological findings: Hb 14.2 g./100 ml. WBC 5,200/c.mm. Retics. 0. Plasma iron 221 µg./100 ml. TIBC 240 µg./100 ml.

Skin biopsy negative for iron.

Liver function tests: Fasting blood sugar 261 mg./100 ml. Plasma bilirubin 0.5 mg./100 ml. Alkaline phosphatase 5 units. Proteins 7.1 g./100 ml., albumin 3.6 g./100 ml. Flocculation tests negative.

Barium swallow showed no oesophageal varices.

Urine showed no iron granules in a centrifuged deposit.

Condition prior to investigation: Was very well and was under satisfactory control with regard to his diabetes. Vigorous venesection had reduced his serum iron level to low normal.

Investigations at time of absorption studies

Haematological findings: Hb 14.8 g./100 ml. Haemat. 45%. MCHC 33%. Serum iron 66 µg./100 ml. TIBC 279 µg./100 ml. Saturation of TIBC 24%.

Liver function tests: Serum bilirubin 0.2 mg./100 ml. Alkaline phosphatase 12 units. Proteins 6.1 g./100 ml., albumin 3.6 g./100 ml. Flocculation tests negative. Pseudocholinesterase 44 units.

Mr. E.P. (continued)

Results of iron absorption studies

Absorption of ^{59}FeHb when given alone; 47%.
Absorption of ^{59}FeHb when given with Cotazym ; 16.4%.
Absorption of ^{59}FeHb when given with trypsin; 19%.

Mr. E.T. R.I. No. 62073. Age 57. Born 1906.

Diagnosis: Haemochromatosis.

1963 (Aug.) - Onset of dyspnoea and pain in chest. Seen in out-patients when he was noted to have generalised pigmentation and hepatomegaly. Myocardial infarct confirmed by electrocardiograph.

1963 (Nov.) - Gradual disappearance of dyspnoea whilst on diuretics and digitalis. Admitted for confirmation of presumptive diagnosis of haemochromatosis.

There was a family history of father dying at 56 years of age with an enlarged liver. Mother died at 85, jaundiced, possibly pigmented. Three of four brothers dead. One elder brother survives with an enlarged liver. No family history of diabetes. Married; 2 children. Patient has had a moderate alcohol intake for last 30 years.

Examination: Silvery-brown pigmentation. No evidence of liver failure. Cardiovascular system normal. B.P. 120/70. Liver palpable 7 cm. below costal margin. Spleen impalpable. No testicular atrophy.

Investigations at time of absorption studies

Haematological findings: Hb 15.9 g./100 ml. C.I. 1.1. Retics. 5.4%. MCHC 34%. Haemat. 46. WBC 15,400/c.mm. Normal differential. ESR 18 mm./hr. Sternal marrow not diagnostic. Serum iron 200 μg./100 ml. Saturation of TIBC 83%.

Liver function tests: Plasma bilirubin 0.3 mg./100 ml. Alkaline phosphatase 10 units. Proteins 7.4 g./100 ml. Pseudocholinesterase 56 units. Glucose tolerance curve normal. Plasma electrophoresis showed a normal protein pattern. BSP excretion; 11% of dye retained 45 min. after injection.

Barium meal showed spleen enlarged to 3 or 4 times normal size.

Liver biopsy showed fragments of liver tissue with large deposits of golden brown pigment. There was an increase in portal fibrous tissue. The brown pigment was seen within the liver cells and stained strongly for iron. The appearances were those of the liver in haemo-chromatosis.

Results of absorption studies

Percentage absorption of dose of ^{59}FeHb when given alone; 13.6%.
Percentage absorption of dose of ^{59}FeHb when given with Cotazym: 21%.

PATIENTS WITH

TRANSFUSION HAEMOSIDEROSIS

Mr F.A. R.I. No. 324277. Age 54. Born 1910.

Diagnosis: Transfusion haemosiderosis. Aplastic anaemia.

1961 (July) - Patient had an attack of upper respiratory tract infection. He was noted to be pale and was given vitamins and antibiotics.

1961 (Nov.) - Started to have headaches and was anaemic. He was found to have occult blood loss in his stools and was given a course of iron. He improved temporarily.

1962 (Feb.) - Again became breathless and pale with a haemoglobin of only 7 g./100 ml. No cause was found and after transfusion he was admitted to the Nuffield Department of Medicine where a diagnosis of refractory anaemia with cellular bone-marrow was made.

Since that time he has required repeated transfusions and at the time of investigation had received 80 pints.

Examination: Rather quiet, co-operative man with a grey complexion. No lymphadenopathy. Cardiovascular system normal. B.P. 175/90. Abdomen: liver enlarged 2 cm. below costal margin. Spleen not palpable.

Investigations at time of absorption studies

Haematological findings: Hb 10.4 g./100 ml. MCHC 32%. Retics. 0.8%. ESR 7 mm./hr. Serum iron 244 µg./100 ml. TIBC 264 µg./100 ml. Saturation of TIBC 92%. Sternal marrow; very cellular marrow with an absence of red-cell precursors.

Liver function tests: Plasma bilirubin 0.7 mg./100 ml. Total proteins 66 g./100 ml., albumin 4.7 g./100 ml. Alkaline phosphatase 8 units.

Liver biopsy: Needle biopsy showed siderosis of the liver. There was no disturbance of hepatic architecture.

Results of absorption studies

Percentage of dose of ^{59}FeHb absorbed when fasting; 6%.

Mrs. F.D'A. R.I. No. 203782. Age 70. Born 1893.

Diagnosis: Transfusional haemosiderosis. Aplastic anaemia.

1954 - First noted to be anaemic. Treated with injections of liver without effect. Iron and folic acid treatment also not effective and she became much more anaemic.

1955 - Started on blood transfusions regularly. In good health apart from occasional symptoms of anaemia.

1963 - Started to have symptoms of congestive cardiac failure and this became so severe that she required diuretics.

1964 - Regular transfusions and diuretics kept her in good health. She has now had a total of more than 334 pints of blood.

Examination: Marked skin pigmentation. Cardiovascular system; hepato-jugular reflex present with ankle oedema. B.P. 90/60. Liver enlarged 15 cm. below costal margin. Spleen not palpable.

Investigations at time of absorption studies

Haematological findings: Hb 10.1 g./100 ml. MCHC 32%. Retics. 2.6%. WBC 2,600/c.mm. Serum iron 250 µg./100 ml. Saturation of TIBC 96%.

Liver function tests: Serum bilirubin 1 mg./100 ml. Alkaline phosphatase 5 units. Proteins 6.1 g./100 ml., albumin 3.5 g./100 ml. BSP retention of 9% at 45 min. Glucose tolerance tests normal.

Abdominal X-ray shows a large liver; no evidence of pancreatic calcification.

Results of absorption studies

Percentage absorption of a dose of ^{59}FeHb given alone; 3%.

Mr. A.Sa. R.I. No. 197740. Age 44. Born 1919.

Diagnosis: Transfusional haemosiderosis. Aplastic anaemia.

1960 (July) - Onset of lassitude and purpura with deterioration of vision one month later. Found to be very anaemic. Bone-marrow appearances those of aplasia or possibly a lymphatic leukaemia in an aplastic phase.

During the next years maintained by blood transfusion, receiving 130 units of blood to date.

Examination: Co-operative, intelligent man, pale with skin pigmentation. Petechiae over shins. Small firm lymph glands in both supraclavicular fossae. No other lymphadenopathy. Cardio-vascular system normal. B.P. 120/75. Spleen palpable 5 cm. below left costal margin. Liver not palpable.

Investigations at time of absorption studies

Haematological findings: Hb 4.5 g./100 ml. Was transfused to 10.6 g./100 ml. before absorption study. MCHC 34%. Retics. 0.4%. Platelets 102,000/c.mm. ESR 70 mm./hr. ANF negative. LE cells negative. Serum iron 358 µg./100 ml. Bone marrow; cellular marrow with areas of myeloid series. Excess of iron in the reticuloendothelial system.

Liver function tests: Plasma bilirubin 0.8 mg./100 ml. Alkaline phosphatase 9 units. Total proteins 7.4 g./100 ml. Raised γ-globulin on electrophoresis. Transaminase SGPT 160 units (?)

Results of absorption studies

Percentage absorption of a dose of ^{59}FeHb; 1%.

Mrs. A.W. R.I. No. 291832. Age 83. Born 1881.

Diagnosis: Transfusion haemosiderosis. Addisonian pernicious anaemia.

1950 - First diagnosed as having Addisonian pernicious anaemia. Satisfactory response to vitamin B_{12} ingestion and quite well until -

1960 - Onset of aplastic anaemia with hypoplastic bone-marrow. Started regular transfusions and has received to date nearly 80 pints of blood.

Examination: Co-operative, pigmented lady; a little anaemic. Heart sounds normal. B.P. 120/80. Liver and spleen not enlarged. No lymphadenopathy.

Investigations at time of absorption studies

Haematological findings: Hb 10.6 g./100 ml. Retics. 7.6%. MCHC 34%. Serum iron 184 µg./100 ml. (Possibly the aplastic anaemia is in process of remitting.)

Liver function tests: Serum bilirubin 0.5 mg./100 ml. Alkaline phosphatase 25 units. Plasma proteins 5.6 g./100 ml., albumin 3.5 g./100 ml. BSP retention 23% at 40 min.

Results of absorption studies

Percentage absorption of dose of ^{59}FeHb; 11%.

PATIENTS WITH
CHRONIC ALCOHOLISM

Mr. L.K. R.I. No. 277584. Age 56. Born 1907.

Diagnosis: Chronic alcoholic with minimal evidence of liver disease.

1963 (Aug.) - Onset of swelling of ankles.

1963 (Nov.) - Oedema persisted. No urinary symptoms. Had occasional sore throats.

Always had a considerable intake of alcohol; as much as 28 pints of beer a week. He had also been a blood donor and had given 28 pints of blood so far.

Examination: Rather pigmented man with no stigmata of liver disease. Pitting oedema of both ankles. Liver palpable 3 cm. below costal margin, smooth and firm. Oedema found to be due to local deep vein thrombosis.

Investigations at time of absorption studies

Haematological findings: Hb 14.1 g./100 ml. WBC 5700/c.mm. Normal differential. Serum iron 130 μg./100 ml. Saturation of TIBC 38%. ESR 3 mm./hr.

Liver function tests: Serum bilirubin 0.2 mg./100 ml. Alkaline phosphatase 11 units. Proteins 6.0 g./100 ml., albumin 4.1 g./100 ml. Thymol turbidity and colloidal gold; negative. Pseudocholinesterase 48 units. BSP retention of 17%. Serum electrophoresis showed a raised α_2- and β-globulin. γ-globulin decreased. LE cells and ANF negative.

Barium meal normal.

Liver biopsy normal.

Results of absorption studies

Percentage of ^{59}FeHb absorbed with no addition; 46%.
Percentage of ^{59}FeHb absorbed with Cotazym ; 25%.
Percentage of ^{59}FeHb absorbed with trypsin; 27%.

Mr. W.M. R.I. No. 350619. Age 58. Born 1905.

Diagnosis: Chronic alcoholism with minimal evidence of liver disease. Total gastrectomy. Roux-en-Y anastomosis with oesophago-jejunostomy.

1953 - Total gastrectomy with Roux-en-Y anastomosis for carcinoma of the stomach. Splenectomy and partial pancreatectomy at that time. He made an uneventful recovery but became more and more tired.

1962 (Oct.) - Admitted with jaundice to local hospital. Weight had fallen and he was anaemic. Improved on iron, folic acid and vitamin B_{12}. Was noted then to have marked alcoholism with a daily intake of up to 15 pints of beer.

1963 (June) - On examination not anaemic; not jaundiced; weight recovered to 7 st. 7 lb. No stigmata of liver disease. Liver palpable, smooth edge. Spleen not palpable. No ankle oedema.

Investigations at time of absorption studies

Haematological findings: Hb 16.6 g./100 ml. C.I. 1.1. Retics. 1.4%. MCHC 32%. Serum iron 124 µg./100 ml.

Liver function tests: Total proteins 6.8 g./100 ml., albumin 4.4 g./100 ml. Alkaline phosphatase 3 units. Plasma bilirubin 0.2 mg./100 ml. Prothrombin time normal. Pseudocholinesterase 63 units. BSP retention of 3.5% at end of 45 min. Faecal fat 3.2 g./24 hr.

Barium swallow and meal: No oesophageal varices seen. No abnormality seen at the oesophago-jejunal anastomosis.

Liver biopsy: The biopsy shows no excess of connective tissue. There is no disturbance of lobular architecture. Parenchymal cells showed small fat vacuoles.

Results of absorption studies

Percentage of ^{59}FeHb absorbed when given alone; 50%.
Percentage of ^{59}FeHb absorbed when given with Cotazym : 22%.

APPENDIX III

Statistical Analysis

STATISTICAL APPENDIX

Absorption of labelled Hb in patients with cirrhosis and normal controls

	Mean	SD	t	n	P
Absorption in control subjects	10.98	9.2	5.6	24	<0.001
Absorption in cirrhotic patients	37.1	23.8	--	10	--

The increase in absorption is significant at the 0.1% level.

Absorption of labelled $FeSO_4$ in all patients with cirrhosis studied

	Mean	SD	t	n	P
Absorption in control subjects	30.36	15.6	4.59	45	<0.001
Absorption in all cirrhotic patients	63.29	28.5	--	7	--

The increase in absorption is significant at the 0.1% level.

Absorption of labelled $FeSO_4$ in cirrhotic patients without anaemia, evidence of bleeding, etc.

	Mean	SD	t	n	P
Absorption in control subjects	30.36	15.6	4.813	45	<0.001
Absorption in cirrhotic patients	68.8	23.6	--	5	--

The increase in absorption is significant at the 0.1% level.

Comparison of effect of pancreatin on the absorption of labelled $FeSO_4$ in patients with cirrhosis.

	Mean	SD	t	n	P
Absorption without addition of pancreatin	60.17	29.9	--	6	--
Absorption with addition of pancreatin	25.83	7.8	--	6	--
Difference between paired observations	34.33	23.4	3.59	--	<0.02

The decrease in absorption is significant at the 2% level.

Comparison of effect of pancreatin on the absorption of labelled Hb in patients with cirrhosis

	Mean	SD	t	n	P
Absorption without addition of pancreatin	56	6.3	--	4	--
Absorption with addition of pancreatin	31.75	11.1	--	4	--
Difference between means	24.25	16.7	2.9	--	--*

*Not quite significant at the 5% level but very nearly so.

Absorption of labelled Hb in patients with haemochromatosis

	Mean	SD	t	n	P
Absorption without addition of pancreatin	17	13	1.9	8	--
Absorption with addition of pancreatin	9.7	8.3	--	--	--*

*Not significant at the 5% level.

Double isotope method for absorption of labelled $FeSO_4$ with and without pancreatin in patients with cirrhosis

	Mean	SD	t	n	P
Absorption without addition of pancreatin	14.9	10.44	--	4	--
Absorption with addition of pancreatin	6.5	6.81	--	4	--
Difference between means	8.38	6.52	2.58	4	--*

*Not significant at the 5% level.

Comparison of the effect of trypsin in patients with cirrhosis of the liver given labelled $FeSO_4$

	Mean	SD	t	n	P
Absorption without addition of trypsin	46.7	30.86	--	3	--
Absorption with addition of trypsin	34.17	25.15	--	3	--
Difference between means	12.0	6.2	3.35	--	--*

*Not significant but a trend apparent from the sign test.

Comparison of the effect of trypsin in patients with cirrhosis of the liver and haemochromatosis given labelled Hb

	Mean	SD	t	n	P
Absorption without addition of trypsin	28.8	17.37	--	4	--
Absorption with addition of trypsin	18.0	12.27	--	4	--
Difference between means	10.8	11.6	1.86	4	--*

*Not significant but a trend apparent from the sign test.

Comparison of effect of pancreatin in iron-deficient subjects given labelled $FeSO_4$

	Mean	SD	t	n	P
Absorption without addition of pancreatin	53.27	25.9	--	11	--
Absorption with addition of pancreatin	45.45	24.1	--	11	--
Difference between means	7.82	22.6	1.11	11	--*

*Not significant at the 5% level.

REFERENCES

Alper, T., Savage, D.V. and Bothwell, T.H. (1951) Radioiron studies in a case of haemochromatosis. J.Lab.clin.Med., 37, 665.

Andersen, D.H. (1938) Cystic fibrosis of the pancreas and its relation to celiac disease. Amer.J.Dis.Child., 56, 344.

Basset, S.H., Killip, T. and McCann, W.S. (1931) Mineral exchanges in man: III. Mineral metabolism during treatment of a case of polycythaemia vera. J.clin.Invest., 10, 771.

Bell, E.T. (1955) The relation of portal cirrhosis to hemochromatosis and to diabetes mellitus. Diabetes, 4, 435.

Biggs, J.C. (1964) Personal communication.

Biggs, J.C. and Davis, A.E. (1963) Relationship of diminished pancreatic secretion to haemochromatosis. Lancet, 2, 814.

Bothwell, T.H. (1964) Discussion on digestive absorption of iron in iron metabolism. Ciba Symposium. Springer-Verlag, Berlin, p. 198.

Bothwell, T.H., Cohen, I., Abrahams, O.L. and Perold, S.M. (1959) A familial study in idiopathic hemochromatosis. Amer.J.Med., 27, 730.

Bothwell, T.H., Jacobs, P. and Kamener, R. (1959). The determination of the unsaturated iron-binding capacity of serum using radioactive iron. S.Afr.J.med.Sci., 24, 93.

Bothwell, T.H. and Mallett, B.J. (1955). The determination of iron in plasma or serum. Biochem.J., 59, 599.

Bothwell, T.H., Pirzio-Biroli, G. and Finch, C.A. (1958) Iron absorption: I. Factors influencing absorption. J.Lab.clin.Med., 51, 24.

Bothwell, T.H., van Doorn-Wittkampf, H.van W., Du Preez, M.L. and Alper, T. (1953) Radioiron studies in idiopathic hemochromatosis, malnutritional cytosiderosis and transfusional hemosiderosis. J.Lab.clin.Med., 41, 836.

Bradlow, B.A., Dunn, J.A., and Higginson, J. (1961) The effect of cirrhosis on iron storage. Amer.J.Path., 39, 221.

Brise, H. and Hallberg, L. (1962) Iron absorption studies: II. Acta med.scand., Suppl. 376.

Brown, E.B., Dubach, R. and Moore, C.V. (1958) Studies in iron transportation and metabolism. XI. Critical analysis of mucosal block by large doses of organic iron in human subjects. J.Lab.clin.Med., 52, 335.

Brown, H. (1964) The absorption of iron in rats and the effect of the pancreas and liver upon this. B.Sc. Thesis, Oxford.

Cachin, M., Pergola, F., Debesse, B. and Levillain, R. (1958) Cirrhoses alcooliques et hémochromatose. Bull.Mem.Soc.méd., Hôp.Paris, 74, 530.

Callender, S.T. (1964) Digestive absorption of iron in iron metabolism. Ciba Symposium. Springer-Verlag, Berlin . p.69.

Callender, S.T., Mallet, B.J. and Smith, M.D. (1957) Absorption of haemoglobin iron. Brit.J.Haemat., 3, 186.

Callender, S.T. and Malpas, J.S. (1963) Absorption of iron in cirrhosis of liver. Brit.med.J., 2, 1516.

Cantrill, S. and Walsh, R.J. (1962) Iron absorption from the gastrointestinal tract: A reassessment of the 'mucosal block' theory and the influence of the rate of erythropoiesis. Austr.J.exp. Biol.med.Sci., 40, 17.

Cantrill, S., Walsh, R.J., Brading, I. and George, E.P. (1962) Iron absorption from the intestinal tract: The influence of the iron stores. Aust.J.exp.Biol.med.Sci., 40, 25.

Ceppell, D.F., Hutchison, H.E. and Jowett, M. (1957) Transfusional siderosis: The effects of excessive iron deposits on the tissues. J.Path.Bact., 74, 245.

Caroli, J. and André, (1964) Surcharge ferrique dans les cirrhoses (à l'exclusion de l'hémochromatose idiopathique). In Iron Metabolism. Ciba Symposium. Springer Verlag, Berlin. p.327.

Caroli, J. and Étevé, J. (1954) La foie dans les hémochromatoses. Arch.Biol.méd.Sem.Hôp.Paris, 30, 19.

Chodos, R.B., Ross, J.F., Apt, L., Pollycove, M. and Halkett, J.A.E. (1957) The absorption of radio-iron labelled foods and iron salts in normal and iron-deficient subjects and in idiopathic haemochromatosis. J.clin.Invest., 36, 314.

Conn, H.O. and Lindenmuth, W.W. (1962) Prophylactic portacaval anastomosis in cirrhotic patients with oesophageal varices: A controlled study. New Engl.J.Med., 266, 743.

Cooke, W.T. (1956) The diagnosis and investigation of chronic diarrhoea excluding tropical diarrhoea. Proc.roy.Soc.Med., 49, 627.

Conrad, M.E., Berman, A. and Crosby, W.H. (1962) Iron kinetics in Laennec's cirrhosis. Gastroenterology, 43, 385.

Dacie, J.V. (1963) Practical Haematology, 3rd edition. Churchill, London.

Davis, A.E. and Badenoch, J. (1962) Iron absorption in pancreatic disease. Lancet, 2, 6.

Dubach, R., Callender, S.T.E. and Moore, C.V. (1948) Studies in iron transportation and metabolism. VI. Absorption of radioactive iron in patients with fever and with anaemias of varied aetiology. Blood, 3, 526.

Finch, S.C. and Finch, C.A. (1955) Idiopathic haemochromatosis an iron storage disease. Medicine, 34, 381.

Gaskell, J.F., Mackenzie Wallis, R.L., Sladen, A.F., Vaile, P.T. and Garrod, A.E. (1914) A contribution to the study of bronzed diabetes. Quart.J.Med., 7, 129.

Cappell, D.F., Hutchison, H.E. and Jowett, M. (1957) Transfusional siderosis: The effects of excessive iron deposits on the tissues. J.Path.Bact., 74, 245.

Caroli, J. and André, J (1964) Surcharge ferrique dans les cirrhoses (à l'exclusion de l'hémochromatose idiopathique). In Iron Metabolism. Ciba Symposium. Springer Verlag, Berlin. p.327.

Caroli, J. and Étevé, J. (1954) La foie dans les hémochromatoses. Arch.Biol.méd.Sem.Hôp.Paris, 30, 19.

Chodos, R.B., Ross, J.F., Apt, L., Pollycove, M. and Halkett, J.A.E. (1957) The absorption of radio-iron labelled foods and iron salts in normal and iron-deficient subjects and in idiopathic haemochromatosis. J.clin.Invest., 36, 314.

Conn, H.O. and Lindenmuth, W.W. (1962) Prophylactic portacaval anastomosis in cirrhotic patients with oesophageal varices: A controlled study. New Engl.J.Med., 266, 743.

Cooke, W.T. (1956) The diagnosis and investigation of chronic diarrhoea excluding tropical diarrhoea. Proc.roy.Soc.Med., 49, 627.

Conrad, M.E., Berman, A. and Crosby, W.H. (1962) Iron kinetics in Laennec's cirrhosis. Gastroenterology, 43, 385.

Dacie, J.V. (1963) Practical Haematology, 3rd edition. Churchill, London.

Davis, A.E. and Badenoch, J. (1962) Iron absorption in pancreatic disease. Lancet, 2, 6.

Dubach, R., Callender, S.T.E. and Moore, C.V. (1948) Studies in iron transportation and metabolism. VI. Absorption of radioactive iron in patients with fever and with anaemias of varied aetiology. Blood, 3, 526.

Finch, S.C. and Finch, C.A. (1955) Idiopathic haemochromatosis an iron storage disease. Medicine, 34, 381.

Garrod, A.E., Gaskell, J.F., Mackenzie Wallis, R.L., Sladen, A.F. and Vaile, P.T. (1914) A contribution to the study of bronzed diabetes. Quart.J.Med., 7, 129.

Gervertz, N.R., Rubin, E., Wasserman, L.R. and Jacobson, J.H. (1964) Hepatic storage iron and portal blood flow. Abstracts Xth Congress of the International Society of Haematology.

Gillman, J., Gillman, T., Mendelstam, J. and Gilbert, C. (1947) Cytosiderosis and the genesis of arteriosclerosis and other fibrous tissue reactions. Nature (Lond.), 159, 875.

Granick, S. (1946) Increase of the protein apoferritin in the gastrointestinal mucosa as a direct response to iron feeding. The function of ferritin in the regulation of iron absorption. J.biol.Chem., 164, 737.

Greenberg, M.S., Strohmeyer, G., Hine, G.J., Keene, W.R., Curtis, G. and Chalmers, T.C. (1964) Studies in iron absorption: III. Body radioactivity measurements of patients with liver disease. Gastroenterology, 46, 651.

Hahn, P.F., Bale, W.F., Ross, J.F., Balfour, W.M. and Whipple, G.H. (1943) Radioactive iron absorption by gastrointestinal tract. Influence of anaemia anoxia and antecedent feeding. Distribution in growing dogs. J.exp.Med., 78, 169.

Hanot, V. and Chauffard, A. (1882) Cirrhose hypertrophique pigmentaire dans le diabète sucré. Rev.Médecine, 2, 385.

Haverback, B.J., Dyce, B.J., Gutentag, P.J. and Montgomery, D.W. (1963) Measurement of trypsin and chymotrypsin in stool. A diagnostic test for pancreatic exocrine insufficiency. Gastroenterology, 44, 588.

Hegsted, D.M., Finch, C.A. and Kinney, T.D. (1949) The influence of the diet on iron absorption: II. The interrelation of iron and phosphorus. J.exp.Med., 90, 147.

Herbut, P.A. and Tamaki, H.T. (1946) Cirrhosis of the liver and diabetes as related to hemochromatosis. Amer.J.clin.Path., 16, 640.

Howard, C.P. and Stevens, F.A. (1917) The iron metabolism of haemochromatosis. Arch.int.Med., 20, 896.

Howatt, H.T. (1964) Personal communication.

Josephs, H.W. (1939) Iron metabolism in infancy: I. Factors influencing iron retention in ordinary diets. Johns Hopk.Hosp. Bull., 65, 145.

Kalow, W. and Genest, K. (1957) A method for the detection of atypical forms of human serum cholinesterase: Determination of dibucaine numbers. Canad.J.biochem.Physiol., 35, 339.

Kinney, T.D., Finch, C.A., Kaufman, N., Hegstead, M. and Partington, P.F. (1950) The relationship of the pancreas to the absorption of iron. Amer.J.Path., 26, 746.

Kinney, T.D., Kaufman, N. and Klavins, J. (1955) Effect of ethionine-induced pancreatic damage on iron absorption. J.exp.Med., 102, 151.

Letulle, M. (1897) Note sur trois observations de cirrhose hypertrophique pigmentaire non diabetique. Bull.Soc.méd.Hôp. Paris, 14, 205.

Leverton, R.M. and Roberts, L.J. (1937) The iron metabolism of normal young woman during consecutive menstrual cycles. J.Nutr., 13, 65.

Libre, E.P. and MacFarland, W. (1964) Mechanism of increased iron absorption associated with pancreatic damage. Abstracts Xth Congress of the International Society of Haematology.

Linscheer, W.G., Greenberg, M.S., Moore, E.W. and Chalmers, T.C. (1964) Studies in iron absorption: IV. Absorption in the proximal small intestine in patients with cirrhosis. Gastroenterology, 46, 662.

MacDonald, R.A. and Mallory, C.K. (1960) Study of 211 autopsied cases of haemochromatosis and haemosiderosis. Arch.int.Med., 105, 686.

Marks, I.N. and Bank, S. (1963) The aetiology, clinical features and diagnosis of pancreatitis in the South-Western Cape. S.Afr.med.J., 37, 1039.

McCance, R.A. and Widdowson, E.M. (1937) Absorption and excretion of iron. Lancet, 2, 680.

McCance, R.A. and Widdowson, E.M. (1937) The fate of the elements removed from the bloodstream during the treatment of polycythaemia by acetyl-phenyl hydrazine. Quart.J.Med., 6, 277.

McClure, C.W. (1918) Metabolism in a case of haemochromatosis. Arch.int.Med., 22, 610.

Moore, C.V. (1955) The importance of nutritional factors in the pathogenesis of iron-deficiency anaemia. J.clin.Nutr., 3, 3.

Moore, C.V. and Dubach, R. (1951) Observations on absorption of iron from foods tagged with radioiron. Tr.Ass.Amer.Phscns., 64, 245.

Nadler, S.B., Hidalgo, J.U. and Bloch, T. (1962) Prediction of blood volume in normal human adults. Surgery, 51, 224.

Noyes, W.D. and Jordan, P.H. (1964) Iron absorption following portacaval anastomosis in dogs. Abstracts Xth Congress of the International Society of Haematology.

Oliver, R. (1953) Use of a scintillation gamma counter for the estimation of iodine^{131} in liquid samples. Brit.J.Radiol., 27, 663.

Perry, S.W. and Warner, G.T. (1963) A method of sample preparation for the estimation of Fe^{55} in whole blood by the liquid scintillation counting technique. Int.J.appl.Rad.Isotopes, 14, 397.

Peters, T., Giovaniello, T.J., Apt, L. and Ross, J.R. (1956) A new method for the determination of serum iron-binding capacity. J.Lab.clin.Med., 48, 274.

Peterson, R.E. and Ettinger, R.H. (1953) Radioactive iron absorption in siderosis (haemochromatosis) of the liver. Amer.J.Med., 15, 518.

Pirzio-Biroli, G., Bothwell, T.H. and Finch, C.A. (1958) Iron absorption: II. The absorption of radioiron administered with a standard meal in man. J.Lab.clin.Med., 51, 37.

Pirzio-Biroli, G. and Finch, C.A. (1960) Iron absorption: III. The influence of iron stores on iron absorption in the normal subject. J.Lab.clin.Med., 55, 216.

Pitcher, C.S. (1962) Haemolysis in liver disease. D.M. Thesis, Oxford.

Recklinghausen, F. von (1889) Uber hämochromatose. Tageblatt der (62) Versamml.dtsch.Naturf.Heidelberg, p.324.

Recklinghausen, F. von (1889) Quoted by Letulle, M. (1897) in Note sur trois observations de cirrhose hypertrophique pigmentaire non diabetique. Bull.Soc.méd.Hôp.Paris, 14, 205.

Richardiere, M. (1895) Cirrhose pigmentaire avec melanodermie, diabète bronzé? Un.med.No.49, 577, 4e ser., Tome 1.

Robscheit-Robbins, F.S. (1929) The regeneration of hemoglobin and erythrocytes. Physiol.Rev., 9, 666.

Saunders, S.J., Bank, S., Airth, E. and Williams, J. (1963) Iron absorption in pancreatic diseases. S.Afr.med.J., 37, 1106.

Schaeffer, J.W., Amick, C.J., Oikawa, Y. and Schiff, L. (1962) The development of haemochromatosis following portacaval anastomosis. Gastroenterology, 42, 181.

Sephton Smith, R. (1964) Iron absorption in cystic fibrosis. Brit. med.J., 1, 608.

Sheldon, J.H. (1935) Haemochromatosis. London: Oxford University Press.

Sherlock, S. (1964) Introduction to the general discussion on iron overload in iron metabolism. Ciba Symposium. Springer-Verlag, Berlin. p.392.

Sobel, H.J. and Waye, J.D. (1963) Pancreatic changes in various types of cirrhosis in alcoholics. Gastroenterology, 45, 341.

Soothill, J.F. (1962) Estimation of eight serum proteins by a gel diffusion precipitin technique. J.Lab.clin.Med., 59, 859.

Sørenson, E.W. (1964) Studies on iron absorption. Acta med.scand., 175, 763.

Stinson, J.C., Baggenstoss, A.H. and Morlock, C.G. (1952) Pancreatic lesions associated with cirrhosis of the liver. Amer.J.clin.Path., 22, 117.

Stojceski, T.K. Malpas, J.S. and Witts, L.J. (1964) To be published.

Strachan, A.S. (1929) M.D. Thesis, Glasgow. Quoted by Wainwright, J. in Siderosis in the African. S.Afr.J.Lab.clin.Med., 3, 1.

Taylor, J., Stiven, D. and Reid, E.W. (1931) Haemochromatosis in a depancreatised cat. J.Path.Bact., 34, 793.

Taylor, J., Stiven, D. and Reid, E.W. (1935) Experimental and idiopathic siderosis in cats. J.Path.Bact., 41, 397.

Tisdale, W.A. (1961) Parenchymal siderosis in patients with cirrhosis after porta-systemic-shunt surgery. N.Engl.J.Med., 265, 928.

Troissier, M. (1871) Diabète sucré. Bull.de la Soc.anat., 16, 231.

Trousseau, A. (1865) Glycosurie: Diabète sucré. Clin.méd.Hôtel Dieu de Paris, 2, 663.

Tuttle, G.S., Figueroa, W.G. and Grossman, M. (1959) Development of hemochromatosis in a patient with Laennec's cirrhosis. Amer.J.Med., 26, 655.

van Goidsenhoven, G.E., Henke, W.J., Valla, J.B. and Knight, W.A. (1963) Pancreatic function in cirrhosis of the liver. Amer.J. dig.Dis., 8, 160.

van Hoek, R. (1961) The Turnover of Radio-Elements in Clinical Medicine in Radioactivity in Man. Charles C. Thomas, Springfield, Illinois. p.299.

van de Kamer, J.H., Huinink, H. ten B. and Weyers, H.A. (1949) J.biol.Chem., 177, 347.

Varley, J. (1963) Practical Clinical Biochemistry. Heinemann, London and New York.

Villaret, M., Justin-Besançon, L. and Even, R. (1932) Étude anatomoclinique des cirrhoses pigmentaire du foie. Press. méd., 40, 672.

Wainwright, J. (1957) Siderosis in the African. S.Afr.J.Lab.clin. Med., 3, 1.

Warner, G.T. and Oliver, R. (1962) A plastic phosphor well counter for sample volumes up to 400 ml. Brit.J.Radiol., 35, 349.

Wheby, M.S., Conrad, M.E., Hedberg, S.E. and Crosby, W.H. (1961) The role of bile and pancreatic juice in the control of iron absorption. Clin.Res., 9, 168.

Wheby, M.S., Conrad, M.E., Hedberg, S.E. and Crosby, W.H. (1962) The role of bile in control of iron absorption. Gastroenterology, 42, 319.

Williams, R. (1964) Personal communication.

Williams, Pitcher, Williams and Sherlock (1964) Quoted by Sherlock, S. in Introduction to the general discussion on iron overload in iron metabolism. Ciba Symposium. Springer-Verlag, Berlin. p.392.

Williams, R., Scheuer, P.J. and Sherlock, S. (1962) The inheritance of idiopathic haemochromatosis. Quart.J.Med., 31, 249.

Wohler, F., Heilmeyer, L., Emrich, R. and Kang, S.H. (1957) Zur function des ferritins bei des eisenresorption. Arch.exp.Path., 230, 107.

Woldman, E.E., Fishman, D. and Segal, A.J. (1959) Relation of fibrosis of the pancreas to fatty liver and/or cirrhosis. I.A.M.A., 169, 1281.

Zimmerman, H.J., Chomet, B., Kulesh, M.H. and McWhorter, C.A. (1961) Hepatic haemosiderosin deposits, incidence in 558 biopsies from patients with and without intrinsic hepatic disease. Arch.int.Med., 107, 494.

ABSTRACT

ABSTRACT

The purpose of this study has been to investigate the absorption of iron in various kinds of liver disease and to determine the influence of some of the factors which might affect absorption. Especial attention has been paid to the possible rôle of the pancreas in influencing the absorption of iron in these conditions.

The absorption of iron was studied in a total of 39 patients with liver disease. Twenty patients with iron-deficiency anaemia were also given iron absorption tests. Data from 61 normal control subjects and 46 patients with iron-deficiency anaemia were used in defining a range of absorption in normal and iron-deficient subjects.

The absorption of iron was studied using the radio-isotope balance technique introduced by Dubach, Callender and Moore (1948). The principle of the method is that a known amount of activity is administered by mouth and the amount of activity appearing in the faeces is determined. The amount of iron retained can then be calculated and equated to the amount absorbed.

Using this method the absorption of labelled inorganic iron and food iron as represented by labelled haemoglobin was determined. The doses of iron were prepared so that 5 mg. of elemental iron was

present in each dose of labelled ferrous sulphate or haemoglobin. The amount of radioactivity in each dose was also comparable and varied from 3 to 5 microcuries.

The percentage of the dose given by mouth which was used for haemoglobin formation was determined from a blood sample taken 11 to 15 days after administration of the dose.

Haematological investigations and biochemical tests were done by standard procedures.

The results of iron absorption studies in normal control subjects given labelled ferrous sulphate showed in 45 observations on 37 subjects a mean absorption of 30 per cent. with a range of 7 to 70 per cent. The mean percentage appearing in the blood was 23 per cent. and the range 1 to 68 per cent.

Iron absorption studies using labelled haemoglobin in normal control subjects showed in 24 subjects a mean absorption of 11 per cent. with a range of 0 to 32 per cent. The mean percentage of the dose which appeared in the blood was 6 per cent. and the range 0 to 21 per cent.

In iron-deficient subjects absorption was increased. In 19 iron-deficient subjects given labelled ferrous sulphate the mean absorption was 54 per cent. with a range of 14 to 94 per cent. The mean percentage of the dose appearing in the blood was 53 per cent.

with a range of 8 to 90 per cent.

The absorption of labelled haemoglobin in 27 patients showed a mean value of 20 per cent. with a range of 1 to 56 per cent. The mean percentage appearing in the blood was 18 per cent. with a range from 0 to 40 per cent.

Eight patients with haemochromatosis were investigated. Seven were untreated. They were all tested with labelled haemoglobin. One patient had completed a full course of venesection and was not iron-laden. In the seven iron-laden patients the mean absorption of labelled haemoglobin was 12 per cent. with a range of 5 to 20 per cent. The amount of the dose utilised for red-cell formation showed a mean of 4 per cent. with a range of 1.5 to 7 per cent. In the patient who had been venesected absorption was 47 per cent. and the patient utilised 13 per cent. of the dose for red-cell formation. The absorption of iron in these patients was comparable to that seen in normal control subjects, but iron overload usually reduces absorption. Therefore this in fact represents a high absorption when the state of the increased tissue iron is taken into consideration.

Seventeen patients with cirrhosis of the liver were studied using either labelled ferrous sulphate or labelled haemoglobin iron. In seven patients with cirrhosis of the liver given labelled ferrous

sulphate the mean absorption was 63 per cent. with a range of 17 to 100 per cent., an increase in absorption which is statistically significant when compared with a group of normal control subjects. In 10 patients with cirrhosis given labelled haemoglobin the mean absorption was 37 per cent. with a range of 3 to 64 per cent. This increase in absorption was also statistically significant.

Examination of this group of cirrhotic patients shows that nine had anaemia or recent bleeding which might be expected to increase absorption. However in the eight others no such complicating factor was present and in addition the saturation of the iron-binding capacity was increased. This group is noteworthy in that it contained patients with cryptogenic cirrhosis and also patients in whom cirrhosis was associated with alcoholism. Previous workers have suggested that increased absorption is specifically associated with alcoholic cirrhosis, but this was not confirmed.

The utilisation of the absorbed iron was remarkably low in nearly all patients with cirrhosis of the liver.

The levels of absorption seen in the cirrhotic patients frequently exceeded the upper limits seen in patients who were iron-deficient. The only other group who showed such high levels of absorption in this study were two chronic alcoholic subjects with

little or no evidence of liver damage who absorbed 46 and 50 per cent. respectively of a dose of labelled haemoglobin.

The low levels of absorption found in general in patients with acute liver disease and transfusion siderosis are in marked contrast to the findings in cirrhosis. In eight patients with acute liver disease the absorption of labelled ferrous sulphate varied markedly during the course of the illness. It is not possible to state a range of absorption but with the exception of lupoid hepatitis other acute forms of liver disease tended to show a low absorption at the beginning of the disease which rose with clinical improvement or administration of steroid hormones.

The mean absorption in four patients with transfusion siderosis given labelled haemoglobin was 5 per cent. with a range of 1 to 11 per cent. Utilisation of the dose of iron in these patients was extremely low.

The findings by previous workers (Davis and Badenoch, 1962) that patients with primary pancreatic disease show an increased absorption of iron and the high incidence of pancreatic fibrosis reported in post-mortem studies on patients with cirrhosis of the liver and haemochromatosis prompted an investigation into the effect of pancreatin on the absorption of iron.

This was studied using sequential iron absorption tests. Either the first or second dose of radioactive iron was accompanied by pancreatin; the order was determined randomly.

In eight patients with haemochromatosis the mean absorption of labelled haemoglobin was reduced by the pancreatin from 17 to 9.7 per cent. In one treated patient with haemochromatosis absorption was reduced from 47 to 16 per cent. Although the reduction in absorption in the group as a whole was not statistically significant a definite trend is evident and further investigation with patients whose haemochromatosis has been treated is warranted.

In six patients with cirrhosis of the liver the absorption of labelled ferrous sulphate was reduced from a mean level of 60 per cent. to 26 per cent. by pancreatin. The absorption of labelled haemoglobin was reduced from 56 to 32 per cent. in four patients.

Thus pancreatin was effective in reducing the absorption of iron in both haemochromatosis and in cirrhosis of the liver, conditions known to be associated with pancreatic fibrosis.

When pancreatin was given to patients with acute liver disease, a condition in which pancreatic fibrosis would not be expected to occur, there was no effect. There was a remarkably

variable effect when pancreatin was given to iron-deficient subjects and the results in this particular group are without explanation at the moment.

The findings in patients with liver disease suggest that the pancreas is normally responsible for producing some agent that has an inhibitory effect on iron absorption.

In preliminary studies it has been shown that this inhibitory factor in pancreatic extract is heat labile and partly resistant to an acid medium. One of the constituents of pancreatin, the enzyme trypsin, has been shown to have an effect, though less marked than pancreatin, in reducing the absorption of iron, which suggests that enzymes are probably involved at some stage in the inhibition of iron absorption.

The increase in the absorption of iron in patients with cirrhosis of the liver affords an explanation for the siderosis sometimes seen in cirrhosis. Although no patients in the present study showed florid manifestations of secondary siderosis, eight showed increased saturation of their iron-binding protein and some excess stainable iron was seen either in liver biopsy or bone-marrow biopsy in some patients. If these patients survive it is likely that they will show the features of the patients with cirrhosis and secondary haemosiderosis

described by Caroli et al. (1964).

The implications of the finding of increased iron absorption in some patients with cirrhosis of the liver are discussed with particular reference to the treatment of these patients with iron.

The present study lends no support to the 'mucosal block' theory of the control of iron absorption but emphasises the rôle of factors acting within the lumen of the intestine which enhance or inhibit the absorption of iron.