COBALT AND IRON ABSORPTION IN MAN

(A Comparative Study of The Factors Regulating Absorption In Subjects With and Without Disorders of Iron Metabolism).

A THESIS SUBMITTED TO THE UNIVERSITY OF IBADAN

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SUMMARY

The similarity between cobalt and iron suggests that the two metals might share at least part of a common intestinal transport pathway. This hypothesis has, however, never been tested in the human subject not even in disorders of iron metabolism.

The intestinal absorption of cobalt and iron was determined in 18 control subjects without disease of the alimentary system, 21 patients with portal cirrhosis of the liver, 6 patients with alcohol induced fatty infiltration and degeneration of the liver, 4 patients with idiopathic haemochromatosis and 2 patients with refractory anaemia and exogenous iron overload from multiple blood transfusions and iron medication. The subjects investigated were further subclassified on the basis of their tissue iron stores into iron normal, iron deficient and iron loaded subgroups.

The intestinal absorption of both metals was measured by a faecal recovery method. A technique employing non absorbable markers $Cr^{51}Cl_3$ and carmine red was devised to ensure completeness of faecal collections. With this technique it was also possible to separate into three components, the process of absorption of the orally administered test dose; namely the initial uptake by the mucosal cell, the sequestration by the cell and the final

transfer to the body or net absorption.

Four test doses of cobalt each containing 20 μ moles of $COCl_2$ tagged with CO^{57} and 4 test doses of iron each containing 20 μ moles of $FeCl_2$ tagged with Fe^{59} were consecutively administered orally to each patient over a four day period. The non absorbable markers were given orally as follows, $Cr^{51}Cl_2$ with the 8th dose, and carmine red two capsules (2 grams) two hours after the 8th dose.

Body retention of cobalt, measured as the percentage of the administered dose not recovered in the faeces and urine in ten days, was determined in 13 patients with normal iron stores (5 controls and 8 patients with liver disease), 5 iron deficient subjects (3 controls and 2 patients with liver disease) and in 3 patients with idiopathic haemochromatosis.

In 12 control subjects with normal iron stores the intestinal uptake of cobalt was greater than of iron being 45.6 per cent and 36.2 per cent respectively. The delayed excretion of cobalt was less (cobalt 2 per cent, iron 8.6 per cent), but its net absorption was greater than that of iron (Co. 43.6 per cent, Fe. 27.5 per cent). The results in the patients with portal cirrhosis of the liver and in those with alcohol induced fatty infiltration and degeneration of the liver but with normal iron stores were similar to those in control subjects.

Iron deficiency enhanced the intestinal uptake and net absorption of both cobalt and iron to a similar level in control subjects without liver disease and in patients with portal cirrhosis. The delayed excretion of iron was reduced in iron deficiency but that of cobalt was unaffected. The significance of the delayed excretion in regulating the intestinal absorption of the two metals is discussed.

A direct correlation was found between the intestinal absorption of cobalt and iron over the range of values where iron absorption is normal or increased. This observation demonstrates that cobalt and iron share at least part of a common absorptive pathway. However, in the two patients with exogenous iron overload, the intestinal absorption of iron was reduced whereas that of cobalt was unaffected. The explanation for this discrepancy is discussed.

In a total of 27 subjects a direct relationship was present between intestinal absorption and urinary excretion of cobalt. As a result of this, no difference was observed in the average body retention of this metal in the iron normal, iron deficient or iron loaded groups. Also, a direct relationship was found between the intestinal absorption of iron and urinary cobalt excretion in 18 subjects.

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From the observations, it is proposed that the measurement of urinary cobalt following its oral administration might provide a simple indirect measurement of the absorptive capacity of the intestinal mucosa for iron.

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INTRODUCTION

HISTORICAL BACKGROUND

Cobalt has been used to colour glass and pottery for centuries. The blue of Ming China and Venetian glass owe their brilliance to cobalt salts. Cobalt has also found wide application in modern technology; alloys of the metal are used in the manufacture of jet engines, rocket nozzles, gas turbines and permanent magnets for high-fidelity loud speakers (1).

However, it is only in the last four decades that the biological importance of cobalt, especially in animal husbandry, has been recognised. In 1934, in Southern Australia, the experimental investigation of Coast Disease, a malady affecting sheep and cattle grazed on certain restricted pastures, revealed that these animals were suffering from dietary cobalt deficiency. The malady was characterised by anaemia, loss of appetite and severe weight loss. Interestingly, it did not affect non-ruminant animals like the horse (2). The response of the affected animals to orally administered cobalt was dramatic; the anaemia was corrected, appetite improved and there was weight gain. However, the administration of cobalt by injection was without effect even though blood and tissue levels were up to ten times the levels in normal sheep (3) (4).

The next major landmark in the elucidation of the biological importance of cobalt in animal and human physiology was the discovery in 1948 (5) (6) that the anti-pernicious anaemia factor in liver extracts (7) was Vitamin B_{12}, which was later shown to contain a cobalt atom in its molecule (8). Subsequent investigations demonstrated that the nutritional cobalt deficiency in ruminants was due to Vitamin B_{12} deficiency (9). The Vitamin is synthesized in the rumen from dietary cobalt by intestinal bacteria (10). Thus came the explanation for the early observation (3) (4) that parenteral cobalt administration was ineffective in correcting the anaemia of Coast Disease.

All attempts to produce manifestations of cobalt deficiency by dietary deprivation of inorganic cobalt in small laboratory animals such as the rat and the rabbit have been unsuccessful (11) (12) (13). These animals, as well as non-ruminants, require Vitamin B_{12} directly in their diets and, provided this is available, a dietary deficiency of inorganic cobalt does not produce symptoms. The main sources of naturally occurring Vitamin B_{12} are micro-organisms (14) and the rat and rabbit largely meet their needs by corprophagy.

Following the discovery that cobalt was a potent erythropoietic agent in laboratory animals and in man, (see Grant and Root (15) for a complete review) attempt were made

to treat anaemias associated with infections, burns, kidney disease and refractory anaemias of adults with large doses of cobaltous chloride (16). Berk et al. (17), found that 300 mg. daily doses of orally administered cobaltous chloride produced a reticulocyte response and an increase in the red cell count in normal subjects and in occasional cases of refractory anaemia. Holly (18) also found that supplementing a daily dose of 0.8 to 1.2 gm. of an iron salt with 60 to 90 mg. of cobaltous chloride prevented a reduction of mean haemoglobin and packed cell volume in some pregnant women; but the administration of 70 to 100 mg. of cobalt alone to another group of pregnant women failed to prevent a decrease in haemoglobin and haematocrit. Thus it appeared that the erythropoetic potency of orally administered cobalt was linked with iron administration.

The administration of large doses of cobalt salts to humans has been associated with toxic side effects such as goitre and depression of thyroid function (19), peripheral neuropathy, and renal tubular damage similar to Wilson's disease due to copper poisoning (20). The addition of small quantities of cobalt salts to draft beer to improve the stability of the foam led to the development of a form of cardiomyopathy in Quebec, Canada (21), the United States (22)

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(23) and in Belgium (24). Thus, the high incidence of toxicity with therapeutic doses of cobalt led to a discontinuation in the use of cobalt salts in the treatment of anaemia.

HUMAN REQUIREMENTS FOR COBALT AND VITAMIN B_{12}

A human requirement for cobalt as such is unproven as is the need for cobalt in non-ruminant animals (25).

Harp and Scoular (26) reported that the total daily cobalt intake, from composite foods and milk, in self-selected diets of 23 school girls ranged from 5.63 to 7.79 µg. A similar range of cobalt intake was reported by Cartwright (27) and by Hubbard et al. (28). Engel et al. (29) reported a daily cobalt intake of 17.4 to 42.8 µg. and recommended a daily allowance of 15 µg. to maintain the body in positive cobalt balance.

Schroeder et al. (1) employed both emission and atomic absorption spectrophotometry to determine the cobalt content in the diets in a hospital and from an institution. They reported a range of 140 to 580 µg. of cobalt per day per diet. The higher values in this study as compared to the others may be due to the different technique of cobalt determination. Their measurements were by colorimetric methods which are known to give lower values.

The cobalt content of common food stuffs have been given by Schroeder et al. (1). Sea foods are very rich in

cobalt whereas vegetables, fresh and canned fruits, dairy products, meats and alcoholic beverages contain very little cobalt. Intermediate amounts are present in cereals, nuts and spices. But it is of interest to note that the recommended dietary sources of Vitamin B_{12}, such as liver and meats are not food items rich in cobalt.

Herbert (30) has proposed 0.1 µg. of Vitamin B_{12} as the minimal daily requirement necessary to prevent deficiency of the vitamin in the adult male. Haematological improvement has been reported following the administration of as little as 1 µg. of Vitamin B_{12} to patients with pernicious anaemia (31) (32).

Since cobalt constitutes only 4.5% by weight of the vitamin B_{12} molecule (33), and since it has been shown that cobalt is firmly bound in the molecule and does not exchange with inorganic cobalt (34), it is evident that the daily intake of the element is far in excess of the requirements for vitamin B_{12}. Engel et al. (29) have estimated that of the total daily intake of cobalt, the amount that can be accounted for in vitamin B_{12} is less than 1%.

THE ABSORPTION, EXCRETION AND TISSUE DISTRIBUTION OF COBALT IN ANIMALS:

Studies with radioactive cobalt have demonstrated in the rat (35) (36) (37) (38), in rabbits, swine and young calves (39), in cattle (36), and in dogs (40), that orally administered cobalt is readily absorbed and excreted mainly in the urine. When administered parenterally, the main path of cobalt excretion is also the urine although a small amount is excreted in the faeces. That bile is the main vehicle of cobalt excretion into the gastro-intestinal tract has been demonstrated in rats by Greenberg et al. (37), and in dogs by Lee and Wolternick (40). In these studies, the site of maximum cobalt absorption was not determined although Lee and Wolternick had previously reported 50% absorption after directly injecting cobalt into the lumen of the second loop of the duodenum in the dog (41).

Following oral or parenteral administration, cobalt is widely distributed in the tissues. The highest concentrations are found in the liver and kidneys and lesser amounts are found in the pancreas, the spleen and other organs (36) (37) (41) (42) (43). The explanation for the high concentrations of cobalt in the liver and the kidneys might be that they provide the main pathways for its excretion by way of the bile and urine respectively.

THE ABSORPTION, EXCRETION AND TISSUE DISTRIBUTION OF COBALT IN HUMAN SUBJECTS:

Early investigations of cobalt absorption in man were directed towards establishing the amount required to maintain the body in positive balance by the measurement of the cobalt content of diets and daily excreta. Kent and McCance (44) reported a total excretion of cobalt in the faeces and urine of 1.25 mg. in one week by a patient on a hospital diet. This was increased to 4.14 mg. during the following week, after 13 mg. of cobalt had been given by intravenous injection. Seventy-four per cent of the total cobalt excretion was in the urine. Cartwright (27) found that normal adults on a self-selected diet containing about 5 to 8 µg. of cobalt per day absorbed 73 to 77%, and 67% of the absorbed cobalt was excreted in the urine. Other cobalt balance studies in man are those of Schroeder et al. (1), Hubbard et al. (28) and Engel et al. (29). The results of these studies all indicate a high faecal and urinary excretion of cobalt but they do not make definite comments in the nature of the intestinal absorption of cobalt in man.

Using cobalt tagged with a radioactive isotope, Parley et al. (45) reported that 10% of an orally administered dose of 1 mg. of cobalt was absorbed and excretion of the isotope was chiefly by the kidneys.

The site of maximum cobalt absorption in man is unknown, and it has not yet been demonstrated whether cobalt is excreted in the bile, as in experimental animals, or secreted directly into the gastro-intestinal tract.

Analysis of human tissues for cobalt indicate that the element is present in almost every organ. Yamagata et al. (46), employing a spectrophotometric method, estimated from 46 accident cases that the total cobalt content in the human body was 1.1 mg. The highest concentrations were found in the liver, bone with marrow and in skeletal muscle. Parr and Taylor (47), employing neutron activation analysis technique, also found the highest cobalt concentration in the liver, 50% of which was estimated to be present in Vitamin B_{12}. The livers of newborn infants have significantly lower cobalt content than those of adults.

Hubbard et al. (28), using a spectrophotometric method, were unable to detect any cobalt in either whole blood or in serum but Thiers et al. (48) with a similar technique reported cobalt concentrations ranging from 0.083 to 0.29 µg. per kg. whole blood. Parr and Taylor (45) reported serum concentrations ranging from 0.08 to 0.58 ug. per litre, and estimated that only 5 to 10% of serum cobalt is in Vitamin B_{12}. The blood cobalt concentrations reported by

Koch et al. (49) were higher than those previously reported but they showed that washed red blood cells contained more cobalt than either whole blood or plasma. What all these studies showed was that blood cobalt was significantly much lower than the levels in tissues; mainly liver and kidney.

Cobalt is transported in the albumin fraction of blood plasma by non-specific binding (50). A similar non specific binding of the cobaltous ion to plasma transferrin has been described (51).

THE RELATIONSHIP BETWEEN COBALT AND IRON

Cobalt and iron belong to the first series of the transition elements in the periodic table. The transition elements have been defined as those, which as elements have partially filled "d" or "f" shells in their orbital electron configuration (52). The physical and chemical properties of the transition elements are adequately described in standard texts (52) (53); but within the group of transition elements, a closer similarity in physical and chemical properties exists particularly between cobalt, iron, and manganese than between them and the other members of the group, namely scandium, titanium, vanadium, chromium, nickel, copper and zinc. A comparison of the physical properties of cobalt and iron

Table 1

COMPARISON OF THE PHYSICAL PROPERTIES OF COBALT AND IRON*

	COBALT	IRON
Atomic Weight	58.94	55.85
Atomic Number	27	26
Number of electrons (3d)	7	6
in two outer shells (4s)	2	2
Covalent Radii	1.16	1.16
Ionic Radii M^{2+} (A)	0.82	0.83
M^{3+} (A)	0.65	0.67
Atomic Volume	6.7	7.1
Specific Gravity (20°C)	8.9	7.85 - 7.88
Common Valencies	2 and 3	2.3 and 6
Solubility of Chloride Salt (gm. litre at 40°C)	410	407
Melting Point 760 mm. Hg.	1495°C	1535°C
Boiling Point 760 mm. Hg.	2900°C	3000°C

*Data taken from Heslop and Robinson (53) and from Hodgman (54).

is given in Table 1 (page 21).

One characteristic of great biological importance which is common to both cobalt and iron is the tendency of both metals to co-ordinate with 6 ligands to form complexes. Ferrous iron forms a large number of complexes, most of them octahedral. One of the most important of all its complexes is the porphyrin complex, haem, which exists associated with a globular protein in haemoglobin (52). Other iron co-ordination compounds of biological importance are the enzymes cytrochrome c, the catalases and the peroxidases (55) which catalyse oxidation reduction reactions.

An important cobaltic complex which occurs in nature is Vitamin B_{12}. The central portion of the vitamin B_{12} molecule consists of four reduced and substituted pyrrole rings surrounding a single cobalt atom. This central structure, known as a "corrin" ring system, is similar to the haemoporphyrins with the difference that two of the pyrrole rings (I and IV) are joined directly rather than through a single methane carbon (56). Other co-ordinate cobalt compounds have been described (57) but their physiological importance is as yet uncertain.

THE SPECIFICITY OF THE INTESTINAL TRANSPORT SYSTEM FOR IRON

Pollack *et al.* (58) have demonstrated that rats rendered iron deficient either by bleeding or by a deficient diet

absorbed increased amounts of both cobalt and iron. This observation suggests that the intestinal transport mechanism for iron might not be specific and could be shared by similar elements including cobalt. There have been no previous studies of cobalt absorption in iron deficiency states in man, and the significance of the findings of Pollack and co-workers has not been fully explored.

ALTERATIONS IN COBALT ABSORPTION IN DISORDERS OF IRON METABOLISM

1. Idiopathic Haemochromatosis

Although the results of iron absorption studies in idiopathic haemochromatosis appear contradictory, it is generally accepted that the excessive deposition of iron in the tissues is a direct consequence of inappropriate absorption from the gut. If one accepts the postulate of Pollack et al. (58) that cobalt and iron share a common absorptive pathway, one might anticipate increased cobalt absorption in idiopathic haemochromatosis. However, there have been no studies of cobalt absorption in idiopathic haemochromatosis. In the studies of Butt et al. (9), which used a spectrochemical method, cobalt was not detected in livers of 10 patients with idiopathic haemochromatosis, although the iron contents were very high.

2. Portal Cirrhosis

Although increased absorption of iron has been reported in 30 to 100% of patients with portal cirrhosis (60 - 66) increased deposition of iron in the liver and other tissues has been found infrequently (67) (68). A review of the English literature reveals that only 11 authentic cases of haemochromatosis secondary to portal cirrhosis have been reported (69 - 77). A summary of these case reports is given in Table II (page 25).

The discrepancy between the reported increase in iron absorption and the rarity of iron overload complicating portal cirrhosis might be explained by the failure to exclude iron deficiency by definitive tests such as bone marrow aspiration for stainable iron in many of the studies.

Cobalt absorption has not been previously investigated in portal cirrhosis but measurements have been made of cobalt concentrations in the liver. Hunt et al. (78), by neutron activation analysis technique, found significantly lower cobalt concentrations in the livers of cirrhotic patients than in control accident victims. Norwood et al. (79), using the same method, found no significant difference in liver cobalt concentration between biliary and other types of liver cirrhosis. Their studies did not however comment on the comparison with normals.

E. II. REVIEW OF CASES IN THE LITERATURE OF IRON OVERLOAD PRECEDED BY PORTAL OBSTRUCTION

AUTHOR	AGE AND SEX	CHRONIC ALCOHOL INGESTION	PORTO-SYSTEMIC SHUNT	MAGNITUDE OF IRON EXCESS	HEMOGLOBIN (gm./100 ml.)	SERUM IRON (μg./100ml.)	TOTAL IRON BINDING CAPACITY (μg./100ml.)	REMARKS
...e Records ...s. Gen. Hospital ...h (65)	48M	Yes	No	Unknown	15.9	-	-	Portal cirrhosis without excess stainable iron in initial biopsy but 4.5 years later at autopsy extensive iron deposition in the liver.
...ke et al ...9 (72)	55M	Yes	Yes	Unknown	14.2	330	430	Portal cirrhosis with no stainable iron at time of portacaval shunt. Died 5 years later with excess iron in liver, heart, pancreas and other organs.
...Thorner et al ...60 (71)	50F	Not given	Yes	Unknown	-	-	-	Eleven years after onset of jaundice a portacaval shunt was carried out for bleeding varices associated with post necrotic cirrhosis. No stainable iron in liver biopsy specimens but at autopsy 18 months' later excessive iron deposits in liver.
...adale ...61 (72)	51F	No	Yes	Unknown	-	-	-	No stainable iron in liver at time of splenorenal shunt. Died 6.5 years later. Dense clumps of hemosiderin in liver and pancreas. The administration of 1.5 grams of iron given in the form of blood transfusion for anemia contributed to the iron overload.
...Saefer et al ...62 (73)	37F	Yes	Yes	Unknown	14.5	-	-	Stainable iron absent in the liver at the time of portacaval shunt but 3 years later abundant iron deposits in pancreas and liver. The administration of 5000 ml. of blood in the 3 weeks prior to death makes it difficult to assess whether an underlying abnormality was present in iron absorption.
...ebstein and ...Sorman 1965) (74)	1954	No	No*	Unknown	-	168	160	No stainable iron in liver biopsy specimen when portacaval shunt was made. At autopsy 3 years later excess iron in liver and pancreas.
...Pace and ...Baint 1966 (75)	62M	Yes	Yes	2.3 gm. in liver	11.7	124	379	Small quantities of hemosiderin in initial liver biopsy specimen. Died 2 years later with marked iron deposition in liver, pancreas and other organs.
...Mann 1966 ...74)	46M	Probable	No*	Unknown	-	138	191	No stainable iron in initial biopsy or 3 years later when portacaval shunt was required. Died 1.5 years later with marked increase in iron in liver and other organs. A total of 7500 ml. of blood and oral iron therapy given following operation makes significance of iron overload difficult to assess.
...Kuhey et al ...1968 (77)	30M	Yes	Yes	Unknown	-	420	420	Presented with encephalopathy. Alcoholic cirrhosis with minimal stainable iron in biopsy. Large amounts of iron in liver, pancreas and other organs 18 months later.
	34M	No	Yes	Unknown	-	-	-	When portacaval shunt was carried out for bleeding varices, there was no stainable iron in liver but 3 years later extensive iron deposits at autopsy in liver and pancreas.
								Large amounts of iron in liver and pancreas at autopsy but some present in biopsy specimens obtained 1.5 years before at time of portacaval shunt.

* Esophageal varices present

STATEMENT OF THE PROBLEMS INVESTIGATED:

AIMS AND OBJECTIVES

It has been postulated that cobalt and iron, on account of their structural and chemical similarity, might share a common intestinal absorptive pathway. Support for this hypothesis has been provided by Pollack et al. (58) who showed that iron deficiency in rats enhanced the absorption of both cobalt and iron. The significance of this observation to the human subject has not been fully explored. There have been no studies in man of cobalt absorption in conditions in which iron absorption is either increased or decreased. It is also not known whether increased cobalt absorption is complicated by increased body retention of cobalt.

The aims and objectives of the studies to be reported in this thesis are as follows:-

I. To compare the intestinal absorption of cobalt and iron in subjects with normal iron stores.

II. To investigate whether alterations in cobalt absorption occur in conditions in which iron absorption is increased or decreased.

(a) To determine whether liver cirrhosis per se alters the intestinal absorption of cobalt as has been reported for iron.

(b) To find out whether cobalt absorption is increased in idiopathic haemochromatosis.

(c) To determine whether cobalt is retained in the body in conditions in which its absorption is increased.

MATERIALS AND METHODS

1. TECHNICAL METHODS

1. Haemoglobin was estimated by a cyanomethaemoglobin method (80). The normal range of values are 12 - 16 gm./100 ml. for females and 14 - 18 gm./100 ml. for males.

2. Packed cell volume (Haematocrit) was measured by a microhaematocrit method (81). The normal range of values are 36-47% for females and 40-54% for males.

3. Red cell count was measured by an electronic Coulter counter (82). The normal range of values are 4.2 - 5.4 million red cells per cu.mm. for females and 4.6 - 6.2 million per cu.mm. for males.

4. Reticulocyte count The method employed was as described by Miale (83). Normal range of values is 0.5% - 3.0%.

5. Serum protein and electrophoresis The total serum protein was determined by the Biuret reaction (84) and a standard electrophoretic technique was employed to estimate the albumin and gamma globulin fractions (85). The range of normal values are - total serum protein 6-8 gm./100 ml.; serum albumin 3.5 - 5.5 gm./100 ml. and gamma globulins

0.8 - 1.2 gm/100 ml.

6. Serum bilirubin This was determined by a modification of the method of Evelyn and Malloy (86). The range of normal values is from 0.1 to 0.8 mg./100 ml.

7. Serum alkaline phosphatase This was measured by the method of Bodansky (87). The normal range of values are 1.5 to 4.0 Bodansky units/100 ml.

8. S.G.O.T. This was determined by the method of Reitman and Frankel (88). The normal range of values are 4 - 40 units/100 ml.

9. Bromsulphthalein retention This was determined by the method of Seligson et al. (90). The normal retention of the dye in the plasma at 45 minutes is less than 6% of the injected dose .

10. Serum iron and unsaturated iron binding capacity Measurements were made by the method described by Schade (91). The normal mean value for serum iron is 100 µg./100 ml. with a range of 55 - 185 µg./100ml.: for unsaturated iron binding capacity 250 µg./100ml. with a range of 185 - 335 µg./100 ml.

II. CLINICAL EVALUATION OF IRON STORES

1. Introduction

The usefulness and the limitations of available methods for the clinical evaluation of iron stores

have been adequately reviewed (91) (92) (93). The absence of stainable iron on histological examination of the bone marrow has been claimed the best method at present for the diagnosis of iron deficiency (94) (95) (96) (97). Subjects with normal iron stores have stainable iron in the bone marrow. The haemoglobin concentration, packed cell volume, blood smear, serum iron and unsaturated iron binding capacity may be within normal limits even though the body iron stores are depleted (98).

Attempts have been made to compare the iron stores in different individuals by grading the stainable iron present in the bone marrow (99) (100) (101). There is a considerable overlap between normal subjects and those with increased iron stores as assessed from histological grading of bone marrow iron (102); furthermore, in anaemias other than iron deficiency there is a shift of iron from the red cells to tissue iron stores which is reflected in increased marrow haemosiderin (103).

Haskins et al. (104) claim that the most accurate method of determining increased body iron stores is by the measurement of iron in blood

removed by multiple venesections however, this is not always practicable and cannot be justified in the presence of anaemia. In this situation, estimation of the stainable iron in a bone marrow aspirate is more satisfactory and presents no great practical problem.

In this study, iron deficiency was established by the absence of stainable iron in the bone marrow. A diagnosis of iron overload was made by the finding of excessive stainable iron in a liver biopsy specimen or storage iron in excess of two grams by phlebotomy. According to Haskins et al. (104) 1 ml. of packed erythrocytes contains 1.1 mg. of elemental iron. Removal of 500 ml. of blood with a packed cell volume of 45% results in depletion of total body iron by 1.1 x 225 mg. of iron. The normal adult male has 1.2 to 1.5 grams mobilisable iron.

2. Technical details

(a) Bone marrow was aspirated from the ilium and smears from the aspirate were stained for iron with the Prussian blue reaction (93). The blue staining haemosiderin granules in the reticulum cells were examined by light microscopy and graded according to the following criteria: 0, absent; 1+, 1-25 per cent of high power fields (500 times magnification) contained stainable iron; 2+, 26-75% of high power fields; 3+, 76-100% of fields had granules in them; and 4+, definite blue staining

visible upon naked eye inspection of slides due to large clumps of haemosiderin in reticulum cells and in extracellular foci. This classification condenses that of previous workers who graded stainable iron 1 to 6 and also showed a correlation between chemical estimation of iron and histological staining (101).

(b) Liver biopsies were taken with a Vim-Silverman needle. Histological sections stained for haemosiderin with the Prussian blue reaction were examined under a light microscope and graded on an increasing 0 - 4+ scale using criteria similar to those of Balcerzak et al. (99). A 4+ stainable iron in the liver biopsy was considered an abnormal increase.

III. SELECTION AND CLASSIFICATION OF PATIENTS

The subjects were selected from patients referred to the Division of Gastroenterology at the Kingston General Hospital. All investigations were carried out in the Special Investigation Unit of the hospital. Details of the criteria for the selection and classification of the patients are as follows:

1. Control subjects with normal iron stores

Twelve patients who had no previous history of excessive alcohol intake and were not suffering from disease of the liver, pancreas, the gastro-intestinal tract

or the haemopoietic system served as normal control subjects. There was no history of blood loss or medication with iron or blood transfusions during the preceding two years. There were five females, all post menopausal, and seven males. The subjects ranged in age from 29 to 83 years. The blood counts, serum iron and unsaturated iron binding capacity and liver function tests were within normal limits (Appendix 1, page 114). All the patients had stainable iron in their bone marrow aspirates within the range 1+ to 4+ reported in normal subjects (100).

2. Control subjects with depleted iron stores

Iron depletion was established on the basis of absent stainable iron in bone marrow aspirates (94) (95) (96) (97). In other respects, the criteria for selecting the patients in this group were similar to those for the normal control group. There were six patients, three females and three males, aged from 35 to 72 years, none of whom was bleeding at the time of study. The results of the blood counts, serum iron and iron binding capacity and liver function tests are given in Appendix II (page 115). Overt anaemia was present in two patients and in the others the haemoglobin concentration was normal. One patient (W.A.) had a slight increase in BSP retention presumably on account of the congestive cardiac failure present in the

time of study. Another patient (G.H.) had an unexplained decrease in serum albumin concentration.

Liver biopsies were not taken from subjects either in this group or in the preceding control group.

3. Patients with alcohol induced fatty infiltration and degeneration of the liver

The six patients in this group were all males aged 45 to 67 years. A history of excessive alcohol intake was obtained from all of them but liver histology revealed only fatty infiltration and degeneration without cirrhotic changes. The alcohol intake was mainly distilled spirits and beer and there was no evidence of wine consumption in excessive amounts. Alcohol had been withdrawn from the patients at least five days before absorption studies were carried out. The stainable iron in the bone marrow, in each patient was 3+, and in the liver it ranged 0 to 2+ for the whole group. The results of blood counts, serum iron and unsaturated iron binding capacity, and liver function tests are given in Appendix III (page 116)

4. Patients with portal cirrhosis of the liver

The diagnosis of portal cirrhosis was established in each case by histological examination of liver biopsy specimens for the characteristic changes in portal

cirrhosis (105). There was clinical evidence of portal cirrhosis, such as ascites, hepatosplenonegaly, palmar erythema and jaundice in most of the patients. The total number of 21 patients were subclassified into three groups according to their estimated iron stores. Patients who presented with portal cirrhosis but who were actively bleeding were excluded from the study.

(a) Portal cirrhosis with normal iron stores

The nine patients in this group consisted of eight males and one females. They ranged in age from 48 to 67 years. Chronic alcoholism was responsible for the cirrhosis in the eight male patients, but in the female subject the aetiology of the disease was unknown. The pattern of alcohol sonsumption did not differ from that of the patients with fatty infiltration and degeneration of the liver described in the previous group.

The stainable iron in the bone marrow aspirate, grades 1+ to 3+, was within the range obtained in the control subjects with normal iron stores. In the liver biopsy specimens, the amount of stainable iron ranged from 0 to 2+. The patients who had no stainable iron in the liver had adequate amounts in the bone marrow, and in none of the patients was increased iron, 4+, found in the liver. Two patients (M.M. and N.S.) had had portocaval shunts 24 months and 6 months respectively before the study was undertaken.

None of the patients had been treated with iron medication in the preceding two years. Occult blood was absent from the stools at the time of study in all the patients. The results of the blood counts, serum iron and unsaturated iron binding capacity and liver function tests are shown in Appendix IV (page 117).

(b) Portal cirrhosis with depleted iron stores

Iron deficiency was established in each case by the absence of stainable iron in the bone marrow aspirates. In addition, none of the patients had stainable iron in the liver biopsy. There were eight patients in this group, six males and two females, whose ages ranged from 40 to 67 years. In seven of the patients chronic alcoholism was responsible for the cirrhosis; in one of them, the aetiology was unknown.

Two patients (P.E. and J.M.) had had porto-caval anastomoses, 18 months and 6 months respectively, for bleeding varices before study. The results of the blood counts, serum iron, and unsaturated iron binding capacity and liver function tests are given in Appendix V (page 118)

(c) Portal cirrhosis with increased iron stores

The four patients in this group were selected on the basis that both portal cirrhosis and iron over load were present during the course of their disease. Portal cirrhosis was established by the usual criteria (105) and

iron overload by the finding of increased stainable iron, 4+, in the liver biopsy specimen. In three patients, V.D., J.S., and R.B., iron overload was confirmed by storage iron in excess of 2 grams by phlebotomy. The values obtained were 2.2, 18.5 and 15.0 grams of iron respectively. The fourth patient, R.W. died four weeks after study and at necropsy extensive deposits of iron were present in body tissues.

Iron overload was preceded by portal cirrhosis in two patients (R.W. and V.D.) and in the other two (R.B. and J.S.) the sequence of events was not known. The case histories of the four patients with portal cirrhosis and iron overload are briefly summarised.

R.W., born in 1910, began drinking mainly beer and spirits at the age of 20. Portal cirrhosis of the liver was established by open biopsy in 1947 but the histology did not show increased amounts of stainable iron in the liver. An end to side porto-caval anastomosis was performed in 1964 to control bleeding oesophageal varices; liver histology showed a worsening of the cirrhotic process but the stainable iron in the liver was not increased. The patient died three years later in 1967 in hepatic coma following an acute bout of alcoholism. At necropsy, increased deposition of iron was present in the liver, spleen, kidneys,

adrenals, pancreas and the testes.

V.D., a chronic alcoholic, had liver cirrhosis established by percutaneous liver biopsy in 1961. A portocaval anastomosis was performed in 1964 to control bleeding from oesophageal varices. Liver biopsies taken in 1961 and 1964 showed only normal amounts of stainable iron. In 1966 the serum iron had increased 197 µg/100ml., with full transferrin saturation and increased amounts of stainable iron were present in the liver biopsy. A course of venesections were started to treat her iron overload, and 2.2 gm. of excess iron were removed six months before present investigations were carried out.

J.S., a chronic alcoholic, had portal cirrhosis and iron overload diagnosed at emergency portocaval shunt surgery for bleeding oesophageal varices in 1963. Gross examination of the abdominal organs during the operation showed brownish discolouration of the pancreas and the liver. Histology later confirmed portal cirrhosis with increased deposition of iron in the liver. A total of 18.5 gm. of iron had been removed by venesections from his body iron stores but the sequence of events between the portal cirrhosis and increased iron stores remained uncertain.

R.B. has been abstemious all his life. Portal cirrhosis with increased iron stores was diagnosed at laparotomy for

cholecystoctony in 1968. Increased stainable iron, 4+, was present in the liver biopsy and although 15 gm. of iron had been removed from the body before absorption studies were carried out, it remained uncertain whether the portal cirrhosis was the preceding event.

5. Patients with idiopathic haemochromatosis

The four patients in this group consisted of three males and one female. They ranged in age from 58 to 66 years. The assumption that iron overload was the primary event was based on the finding of extensive iron deposits out of proportion to the degree of liver damage in all of them. One of them, M.R. had a positive family history of iron overload. Twenty-five and 32 grams of iron were removed from V.C. and E.G. respectively, and histological changes characteristic of haemochromatosis were present in J.W. at necropsy despite the fact that 8 gm. of iron had been removed from his stores during life.

6. Patients with iron overload due to exogenous iron administration

Iron overload in the two patients in this group was due to the administration of oral iron and blood transfusions. The stainable iron in the liver in both patients was increased, 4+. B.M., a 61 year old male with portal cirrhosis due to alcoholism also had a refractory

anaemia which had been treated with oral iron and blood transfusions for eight months before study. The second patient, M.D., a 67 year old female had received oral iron and a total of 94 pints of blood over the preceding years for aplastic anaemia. The liver biopsy showed on histology only mild fibrosis around the portal tracts but increased amounts of stainable iron were present in the Kuppfer cells and the liver parenchyma.

The blood counts, and liver function tests of the patients with increased iron stores are shown in Appendix VI (page 119).

IV. MEASUREMENT OF IRON AND COBALT ABSORPTION

1. Introduction

Crosby has reviewed the various techniques for the measurement of iron absorption in experimental animals and in man (106). Three methods utilizing radioisotopes of iron, at present in common use, are the whole body counting technique (107) (108) (109), the double isotope red cell uptake method (110) (111), and the faecal recovery method (112). The results of iron absorption studies with the three techniques have not been too divergent although Lunn et al. (113) reported a discrepancy between the results obtained by the faecal recovery technique and the other two. Discrepancies between the faecal recovery method and the double isotope technique have been reported by Pitcher et al. (111) and between the faecal recovery method and the whole body counting technique by Callender et al. (109). The main source of error in the faecal recovery method arises from incomplete faecal collections especially when absorption studies are carried out in general hospital wards. It should, however, be pointed out that the double isotope method assumes that the radio-iron administered orally is handled in the same way in the blood stream as the isotope administered intravenously. That this assumption is not

always valid, and could lead to erroneously low iron absorption results, has been demonstrated in studies showing that when the plasma transferrin is fully saturated, or nearly so, most of the newly absorbed iron from the gut is deposited in the liver at its first passage (114) (115) (116).

The faecal recovery method has been employed in this study to measure the intestinal absorption of both iron and cobalt. This method is valid only if the element under investigation is not re-excreted into the gastrointestinal tract after absorption. This has been shown to be valid for iron (117) (118).

To establish whether cobalt is re-excreted from the body into the gastrointestinal tract, 10 μmoles of cobaltous chloride ($CoCl_2 . 6H_2o$) tagged with 1 μc. of Co^{60} in 5 ml. was injected intramuscularly into the right thigh in six control subjects. Surface counting with a 5 inch NaI crystal (Nuclear Chicago Co.) demonstrated that 80% of the injected cobalt was absorbed from the injection site within six hours and 90% within 24 hours. In five days an average of 4.4% of the injected dose appeared in the faeces and in 10 days the average faecal excretion was 5.8%. The average urinary percentage cobalt excretion in five days and ten days was 52 and 58% respectively. The small percentage faecal excretion in

five days provided proof that re-excretion of cobalt into the gastrointestinal tract was small and the faecal recovery method was a valid technique for measuring the intestinal absorption of cobalt in man.

2. Preparation of test solutions

The test solution of iron contained 20 µmoles (about 1 mg.) of iron, in the form of $FeCl_2$ 4 H_2O dissolved in 125 ml. of 0.01N. HCl prepared from concentrated HCl and ion-free distilled water. The pH of the solution was 2. The test solution was labelled with 1 µc. $Fe^{59}Cl_3$ (S.A. 20-25 µc./ug. t 1/2 45 days) obtained from the Nuclear Chemical Engineering Company, Cambridge, Massachusetts, U.S.A. An excess of ascorbic acid, 200 µmoles (about 14 mg.) was added to the test solution to keep the iron in solution (119).

The test solution of cobalt also contained 20 µmoles (about 1 mg.) of cobalt in the form of $CoCl_2 6H_2O$ dissolved in 125 ml. of 0.01N HCl. The test solution was labelled with 0.5 µc. of $Co^{57}Cl_2$ (S.A. 6.2 µc./ug. t 1/2 267 days) obtained from the same source as the radio iron. 200 µmoles of ascorbic acid was also added to the mixture to keep the cobalt in solution. The pH of the test solution was 2. Thomson (120) has demonstrated that ascorbic acid in excess amounts

prevents the precipitation of both cobalt and iron with increasing pH.

After thorough mixing, a standard was prepared from each test dose. Five ml. of the 125 ml. solution was withdrawn into a litre size plastic container and made up to 100 ml. with ion free distilled water. The remaining 120 ml. of the test solution was administered to the patient.

The diluted standard was counted for radioactivity in a well type gamma scintillation counter and the radioactivity administered to the patient was calculated as follows:-

counts per minute in standard (5 ml. of original solution) = x.

Total counts per minute in one 120 ml. dose = $\frac{120x}{5}$

The standards were counted at the same time as the faeces in order to correct for losses of radioactivity due to natural decay of the isotopes.

Measurements of iron absorption in individual patients following oral administration of single test doses, are subject to day to day physiological variations owing to changes in motility and secretions of the gastro intestinal tract. The results obtained

prevents the precipitation of both cobalt and iron with increasing pH.

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Measurements of iron absorption in individual patients following oral administration of single test doses, are subject to day to day physiological variations owing to changes in motility and secretions of the gastro intestinal tract. The results obtained

may therefore, not be reproducible when iron absorption is repeated at a later date. Among several subjects there are also biological variations that may affect comparison of results. To correct for these variations, Brise and Hallberg (121) introduced the multiple dose technique in which 5 doses each of Fe^{59} and Fe55 were administered to a group of patients over a 3 day period. By this technique the absorption of each isotope was from tests done on three separate days. Their results showed that the day to day variation in iron absorption could be reduced by more than half.

However, Kuhn et al. (122), later showed that the variability observed in iron absorption from multiple doses was similar to that with single test doses.

The multiple test dose technique had been started upon before Kuhn et al. reported their work. There are no previous studies on the variability in absorption of oral test doses of cobalt.

3. Administration of the test dose to the patient

Four test doses of the element under investigation, each as 120 ml. of solution, were therefore, administered over a period of two days. After an overnight fast, the first dose was administered at 7 a.m. and fasting continued until 9 a.m. when breakfast was served.

No food or drink was then allowed, the second dose was administered at noon; and after a further two hour fast, normal feeding routine was resumed. This procedure was repeated on the second day for the remaining two doses. Two grams of carmine red were given 2 hours after the last test dose. The significance of carmine red is discussed in a subsequent section. Each test dose was well rinsed with distilled water and counting for radioactivity showed very little unadministered residue.

4. Collection of faeces

Each stool specimen was collected in a litre size plastic container of the same size that was used to prepare the standards. The patients were carefully instructed to avoid contaminating their stools with urine. Stool collection was continued until a 24 hour collection contained less than 0.2% of the total radioactivity ingested. This usually occurred between the 10th and 15th day of study. The individual stool specimens were counted separately at each counting session together with the standards.

5. Measurement of radioactivity in the faeces

The gamma scintillation equipment for counting the radioactivity (Nuclear Chicago Corporation) in the faeces

consisted of two thallium activated sodium iodide crystals, each measuring 5 inches in diameter, placed end to end with 6 inch space between them for inserting the stool containers. By adjusting the pulse height on the scaler it was possible to count two different isotopes at the same time. Ease of separate counting of the isotopes was enhanced by the different radiation energies of Fe^{59}, mEv (γ 1.0); Co^{57}, mEv (γ 1.36); Cr^{51}, mEv (γ 0.32), respectively. Counting was continued long enough as to ensure counting error of less than 3%. Corrections for differences in geometry due to different volumes of the stools were made by diluting the standards to volumes that corresponded to those of the stools being counted.

6. Calculation of results

The intestinal absorption of iron involves three stages (Fig. 1, page 46). Initially more iron is taken up by the mucosal cell than is required by the body; a proportion is transferred to the body and the iron that is not transferred is temporarily sequestered in the cell and subsequently lost with normal exfoliation of the intestinal epithelium (123). It is not known whether a similar 3 stage process is involved in the intestinal absorption of cobalt.

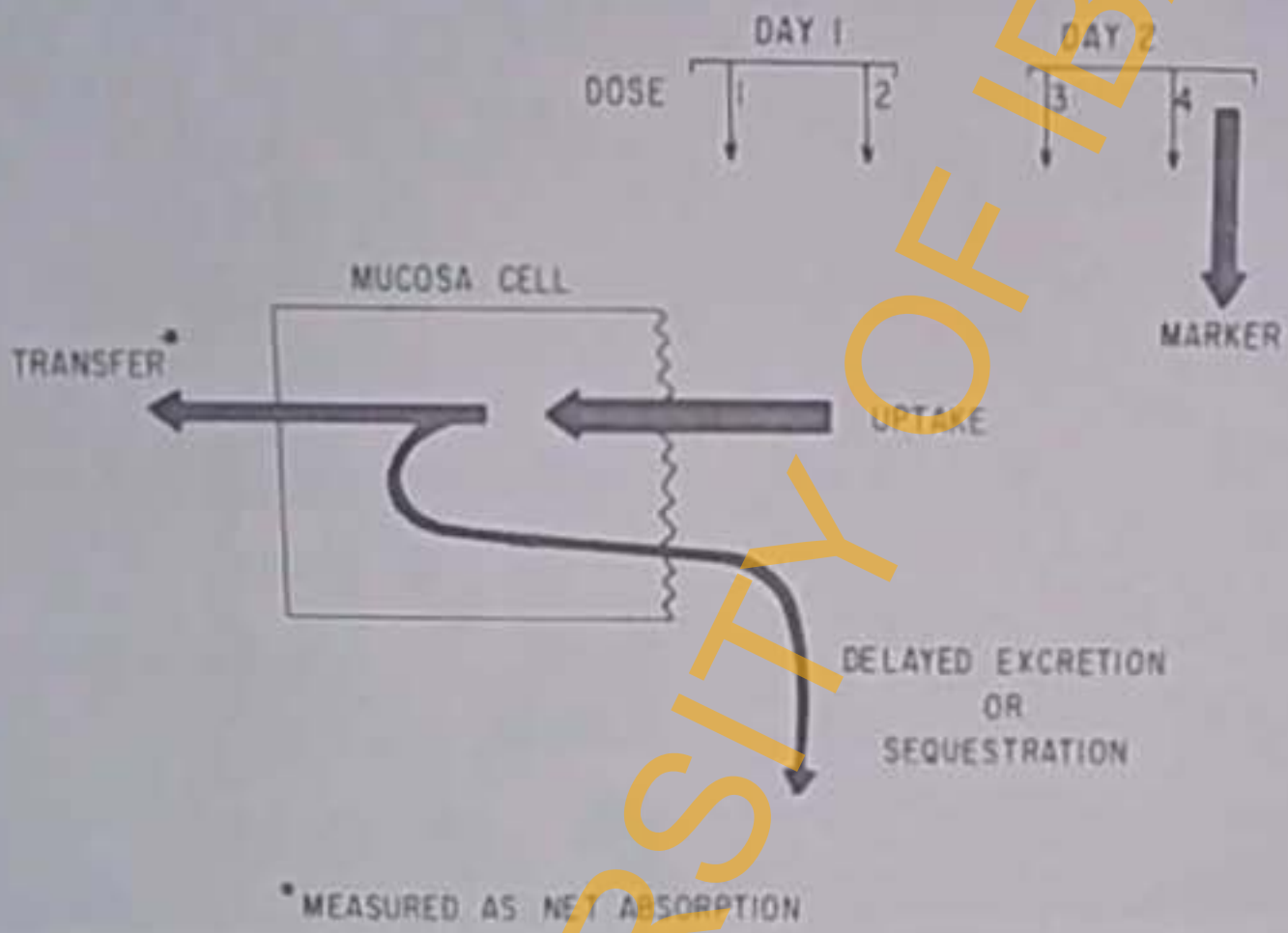

FIGURE 1. INTESTINAL ABSORPTION OF IRON
(Adapted from Conrad and Crosby (123)).

To determine the delayed excretion of both iron and cobalt, an unabsorbable marker, carmine red, was given to the patient after the last test dose. The disappearance of the marker from the faeces, assessed by visual inspection, was used to mark the point at which loss of radioactivity due to desquamation of the epithelium commenced. The efficacy of the method is based on two assumptions: firstly, there is no significant separation of the radioactivity and the marker as they passed down the gastro-intestinal tract, and secondly, the disappearance of carmine red identifies the point at which delayed excretion of the element due to desquamation of the epithelium commenced. The validity of these assumptions was tested with the unabsorbable substance chromic chloride. A solution containing 1.4 umoles of $CrCl_3$ and 1 μc. of $Cr^{51}Cl_3$ (S.A. 13-15 μc./μg; t 1/2 27 days) dissolved in 125 ml. of 0.01N HCl was given by mouth in 4 test doses followed 2 hours later by a carmine red marker. In 10 subjects, the majority of whom had at least one bowel motion a day, the marker appeared in an average of 3 days (1 to 6 days range) and disappeared in an average of 7 days (4 to 9 day range). An average of 98% (91 to 102%) of the radioactivity was passed in the faeces with the marker demonstrating that there was no significant separation of the radioactivity

and the marker as they passed down the gastrointestinal tract. An average of 2.4% (0.2 to 5.7%) of the radioactivity was excreted in the faeces after the marker disppeared, indicating that visual inspection of the stool was adequate for identifying the point at which the marker disappeared.

The total radioactivity in the administered test doses was taken as 100%. The percentage of the test dose transferred to the body, or the net absorption, was calculated as 100 - total radioactivity excreted in the stool specimens. The delayed excretion was the percentage of the test dose excreted after the disappearance of the marker from the faeces. The intestinal uptake was computed as 100 - the percentage of the test dose excreted prior to the disappearance of the marker from the faeces. It is apparent from Fig. 1 that the intestinal uptake is the sum of the delayed excretion and the percentage transfer (net absorption). "Absorption" is used to cover all three stages involved in the process although reports in the literature use the term to describe what is referred to as "net absorption" in this thesis.

The data presented in this thesis were obtained from patients in whom recovery of the Cr^{51} Cl_2 was greater than 98% indicating completeness of faecal collections.

V. MEASUREMENT OF BODY RETENTION OF COBALT

The most direct method of measuring body retention of cobalt is to use a whole body counter to determine the percentage of an oral test dose retained in the body at a given time. Whole body counting equipment was not available at the time these investigations were conducted.

As has been pointed out in the review of the literature, the main excretory routes for cobalt are the urine and the faeces. To determine body retention of cobalt therefore, 24 hour urines were collected separately for 10 days at the same time as the faeces were being collected for cobalt absorption studies.

An aliquot of the test solution was diluted to 500 ml. to prepare a standard and 500 ml. samples from a 24 hour urine collection were counted for radioactivity in the gamma scintillation counter.

The administered cobalt was taken as 100%, and the body retention at ten days was calculated as 100 minus percentage excretion in both the urine and the faeces.

VI. STATISTICAL METHODS

Student's t test (124) was used to evaluate the difference between results. The equality of the variances was tested by the F test (124) and whenever a significant difference was found between the variances the Cox Cochrane correction was applied (125). In the study of the relationship between iron and cobalt absorption the F distribution test was carried out to determine whether the regression was sufficiently linear to enable a valid estimate to be made (124). When the computed F -distribution was statistically significant the coefficient of correlation was calculated.

The crude data were transferred to punch cards and were processed by an IBM Model 360 Computer.

RESULTS

COBALT AND IRON ABSORPTION

1. PATIENTS WITH NORMAL IRON STORES

(a) Control subjects

The average intestinal cobalt uptake was 45.6 percent with a range of 33.7 to 60.3 per cent. The average delayed excretion of cobalt was 2 per cent (range 0.0 to 4.2 per cent) and the mean net absorption was 43.6 percent with a range of 31.4 to 56.2 percent. (Table III page 54).

The average intestinal iron uptake was 36.2 percent with a range of 31.4 to 41.3 percent. The mean delayed excretion of iron was 8.6 percent (range 4.5 to 15.6 percent) and the average net absorption was 27.5 percent with a range of 16.9 to 36.8 percent (Table IV page 55).

The individual results for the control subjects with normal iron stores are given in Appendix 1 (page 114) one patient, M.D., did not participate in the cobalt absorption studies.

The average intestinal uptake of cobalt, 45.6 percent, was significantly greater than that of iron, 36.2 percent, ($p < 0.001$), but the mean delayed excretion, 2 percent, was significantly lower than that of iron 8.6 percent ($p < 0.001$). The average net absorption of cobalt,

III. COBALT ABSORPTION IN SUBJECTS WITH NORMAL AND DEPLETED IRON STORES

GROUP	NO. OF SUBJECTS	AGE (years) mean and range	HEMO-GLOBIN (gm. /100ml.)	MARROW IRON (grade)	LIVER IRON (grade)	PERCENTAGE OF ORAL TEST DOSE (mean and range ± SD)		
						INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION
MAL IRON STORES								
ontrols	11	58.5 (29 - 83)	14.1 ± 0.9	1+ - 4+	-	45.6 ± 8.7 (33.7 - 60.3)	2.0 ± 1.6 (0.0 - 4.2)	43.6 ± 8.8 (31.4 - 56.2)
atty Infiltration f the Liver	5	53.3 (42 - 67)	14.1 ± 0.9	3+	0 - 2+	39.7 ± 2.3 (36.8 - 42.4)	2.3 ± 0.8 (1.5 - 3.6)	37.4 ± 2.8 (34.1 - 39.8)
Portal Cirrhosis	6	58.3 (40 - 67)	13.5 ± 1.3	1+ - 3+	0 - 2+	52.9 ± 9.5 (36.5 - 61.0)	1.2 ± 0.9 (0.4 - 1.6)	51.8 ± 9.7 (35.9 - 60.1)
PLETED IRON STORES								
Controls	6	48.2 (35 - 72)	12.2 ± 3.6	0	-	72.3 ± 10.2[a] (61.1 - 87.2)	2.2 ± 1.2 (1.0 - 3.9)	70.1 ± 10.7[a] (60.4 - 86.2)
Portal Cirrhosis	8	53.4 (40 - 67)	12.3 ± 1.8	0	0	71.3 ± 10.7[a] (53.4 - 81.6)	0.8 ± 0.8 (0.2 - 2.5)	70.1 ± 10.3[a] (52.4 - 80.7)

here (a) is not given as a superscript, the difference between the ean value in this group and the comparable mean value for the ntrol group with normal iron stores is not statistically gnificant, $p>0.05$.

) Difference between mean value for this group and mean value r control group with normal iron stores statistically significant. <0.001.

III. COBALT ABSORPTION IN SUBJECTS WITH NORMAL AND DEPLETED IRON STORES

GROUP	NO. OF SUBJECTS	AGE (years) mean and range	HEMO-GLOBIN (gm. /100ml.)	MARROW IRON (grade)	LIVER IRON (grade)	PERCENTAGE OF ORAL TEST DOSE (mean and range ± SD)		
						INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION
AL IRON STORES								
ntrols	11	58.5 (29 - 83)	14.1 ± 0.9	1+ - 4+	-	45.6 ± 8.7 (33.7 - 60.3)	2.0 ± 1.6 (0.0 - 4.2)	43.6 ± 8.8 (31.4 - 56.2)
atty Infiltration the Liver	5	53.3 (42 - 67)	14.1 ± 0.9	3+	0 - 2+	39.7 ± 2.3 (36.8 - 42.4)	2.3 ± 0.8 (1.5 - 3.6)	37.4 ± 2.8 (34.1 - 39.8)
ortal Cirrhosis	6	58.3 (40 - 67)	13.5 ± 1.3	1+ - 3+	0 - 2+	52.9 ± 9.5 (36.5 - 61.0)	1.2 ± 0.9 (0.4 - 1.6)	51.8 ± 9.7 (35.9 - 60.1)
LETED IRON STORES								
Controls	6	48.2 (35 - 72)	12.2 ± 3.6	0	-	72.3 ± 10.2[a] (61.1 - 87.2)	2.2 ± 1.2 (1.0 - 3.9)	70.1 ± 10.7[a] (60.4 - 86.2)
Portal Cirrhosis	8	53.4 (40 - 67)	12.3 ± 1.8	0	0	71.3 ± 10.7[a] (53.4 - 81.6)	0.8 ± 0.8 (0.2 - 2.5)	70.1 ± 10.3[a] (52.4 - 80.7)

ere (a) is not given as a superscript, the difference between the an value in this group and the comparable mean value for the ntrol group with normal iron stores is not statistically gnificant, $p>0.05$.

) Difference between mean value for this group and mean value r control group with normal iron stores statistically significant, <0.001.

IV. IRON ABSORPTION IN SUBJECTS WITH NORMAL AND DEPLETED IRON STORES

GROUP	NO. OF SUBJECTS	AGE (years) mean and range	HEMOGLOBIN (gm. /100ml.)	MARROW IRON (grade)	LIVER IRON (grade)	PERCENTAGE OF ORAL TEST DOSE (mean and range ± SD) INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION
IAL IRON STORES								
ntrols	12	60.0 (29-83)	14.0 ± 0.9	1+ - 4+	-	36.2 ± 7.1 (31.4 - 41.3)	8.6 ± 7.1 (4.5 - 15.6)	27.5 ± 6.5 (16.9 - 36.8)
atty Infiltration the Liver	6	53.3 (45-67)	14.3 ± 0.9	3+	0 - 2+	33.5 ± 7.4 (26.4 - 42.0)	9.7 ± 7.2 (3.8 - 19.1)	23.8 ± 2.3 (20.5 - 26.4)
Portal Cirrhosis	9	54.3 (48-67)	12.8 ± 1.3	1+ - 3	0 - 2+	36.6 ± 7.0 (29.6 - 52.9)	7.8 ± 3.8 (2.5 - 13.4)	28.4 ± 7.1 (16.9 - 37.9)
PLETED IRON STORES								
Controls	6	48.3 (35-67)	12.2 ± 3.6	0	-	65.8 ± 12.7[a] (55.4 - 87.8)	3.9 ± 2.4[c] (1.0 - 7.0)	61.9 ± 13.9[a] (49.0 - 84.2)
Portal Cirrhosis	8	53.4 (40-67)	12.3 ± 1.8	0	0	61.1 ± 17.3[a] (42.1 - 86.6)	3.7 ± 2.7[c] (1.4 - 7.2)	57.5 ± 18.1[b] (40.0 - 84.1)

re (a), (b) and (c) are not given as superscripts, the difference
reen the mean value in this group and the comparable mean value
the control group with normal iron stores is not statistically
ificant, $p>0.05$.

(b) and (c) Difference between mean value for this group and
an value for control group with normal iron stores statistically
nificant. (a) = $p<0.001$, (b) = $p<0.01$, and (c) = $p<0.05$.

43.6 percent, was significantly greater than the average net iron absorption of 27.5 percent ($p < 0.001$).

The results are graphically represented in Fig. 2 (page 57). The delayed excretion of iron amounted to an average of 23.8 percent of its uptake whilst the delayed excretion of cobalt was only 4.4 percent of uptake. This demonstrates that in control subjects with normal iron stores the intestinal mucosa sequestrates a greater proportion of iron taken up from the lumen than it does for cobalt. The significance of this is discussed later.

The results of the absorption studies in this group of patients also demonstrate that when equimolecular amounts of cobalt and iron are administered to control subjects with normal iron stores significantly more cobalt than iron is taken up by the mucosal cell; less is temporarily stored in the cell; and as a result, the net absorption of cobalt is greater.

FIGURE 2. COBALT AND IRON ABSORPTION SHOWING INTESTINAL UPTAKE, DELAYED EXCRETION AND NET ABSORPTION.

KEY: Hatched areas: Net Absorption of Iron

Stipled areas: Net Absorption of Cobalt.

Open spaces above each column show delayed excretion of the metal.

* Preceded by Portal Cirrhosis

** Sequence of Events Uncertain.

(b) Patients with alcohol induced fatty Infiltration and Degeneration of the liver

The average intestinal uptake of cobalt was 39.7 percent (range 36.8 to 42.4 percent), the mean delayed excretion was 2.3 percent (range 1.5 to 3.6 percent) and the average net cobalt absorption was 37.4 percent (range 34.1 to 39.8 percent). (Table III page 54).

The average intestinal uptake of iron was 33.5 percent (range 26.4 to 42.0 percent), the average delayed excretion was 9.7 percent (range 3.8 to 19.1 percent) and the average net iron absorption was 23.8 percent (range 20.5 to 26.4 percent). Table IV page 55).

The individual results on each patient are given in Appendix III (page 110), one patient, WS, did not participate in the cobalt absorption studies. The mean intestinal cobalt uptake, 39.7 percent, was significantly higher than the mean iron uptake of 33.5 percent ($p < 00.5$). The delayed excretion of cobalt, 2.3 percent, was significantly lower than of iron, 9.7 percent ($p < 0.001$).

The net absorption of cobalt (mean 37.4 percent) was significantly greater than that of iron (mean 23.8 percent) ($p < 0.001$).

As in the control group with normal iron stores, the delayed excretion of iron in the subjects in this group represented a higher proportion of the intestinal uptake

than the delayed excretion of cobalt, 25.9 percent as compared to 5.6 percent (See Fig. 2, page 57).

All of the findings are similar to those observed in the control subjects.

(c) Portal Cirrhosis

The average cobalt uptake was 52.9 percent (range 36.5 to 61.0 percent), the average delayed excretion was 1.2 percent (range 0.4 to 1.6 percent) and the mean net absorption was 51.8 percent (range 35.9 to 60.1 percent). (Table III, page 54).

The average intestinal iron uptake was 36.6 percent (range 29.6 to 52.9 percent), the average delayed excretion was 7.8 percent (range 2.5 to 13.4 percent) and the mean net iron absorption was 28.4 percent (range 16.9 to 37.9 percent). (Table IV, page 55).

The average intestinal cobalt uptake was significantly greater than the mean iron uptake ($p < 0.001$), the delayed excretion of cobalt was less than of iron ($p < 0.001$), and the net cobalt absorption was greater than that of iron ($p < 0.001$).

The individual results are given in Appendix IV (page 117) and for the whole group they are graphically shown in Fig. 2 (page 57). Three patients (JH, CS, and NS) did not take part in the cobalt absorption studies.

The delayed excretion of cobalt amounted to only 2.2 percent of the intestinal uptake whilst the delayed excretion of iron was 21.0 percent of its uptake.

The results in this group were similar to those in control subjects and patients with alcohol induced fatty infiltration and degeneration of the liver. They indicate that in patients with normal iron stores, associated liver disease does not directly influence the handling of either cobalt or iron by the intestinal mucosa.

2. PATIENTS WITH DEPLETED IRON STORES

(a) Control Subjects

The average intestinal cobalt uptake 72.3 percent (range 61.1 to 87.2 percent) (Table III, page 54) was similar to the average intestinal iron uptake 65.8 percent (range 55.4 to 87.6 percent) (Table IV, page 55).

The average delayed excretion of cobalt 2.2 percent (range 1.0 to 3.9 percent (Table III, page 54) was similar to the average delayed excretion of iron, 3.9 percent (range 1.0 to 7.0 percent) (Table IV, page 55).

The average net absorption of cobalt 70.1 percent (range 60.4 to 86.2 percent) was similar to that of iron 61.9 percent (range 49.0 to 84.2 percent).

In comparison with the results in the control group with normal iron stores, the intestinal uptake and net absorption of both cobalt and iron were significantly increased ($p < 0.001$ in each case). The delayed excretion of iron was significantly reduced ($p < 0.05$) but the delayed excretion of cobalt was similar to that in controls with normal iron stores.

The individual results are given in Appendix II (page 115), and are graphically presented for the whole group in Fig 2 (page 57).

These findings demonstrate that in iron deficiency the uptake of both cobalt and iron are increased. The increase in iron uptake is accompanied by a dimunition in its delayed excretion and the result is an increase in the net absorption of iron. In constrast, the increase in cobalt uptake is not accompanied by a decrease in its delayed excretion, and the net absorption tends to approximate to the uptake just as in the control subjects with normal iron stores.

(b) Portal Cirrhosis

The average intestinal uptake of cobalt 71.3 percent (range 53.4 to 81.6 percent (Table III, page 54) was similar to the average intestinal iron uptake of 61.1 percent(range 42.1 to 86.6 percent)

(Table IV, page 55).

The delayed excretion of cobalt, mean 0.8 percent (range 0.2 to 2.5 percent) was also similar to that of iron, mean 3.7 percent (range 1.4 to 7.6 percent).

The average net cobalt absorption 70.1 percent (range 52.4 to 80.7 percent) was similar to that of iron mean 57.5% (range 40.0 to 84.1 percent).

In comparison with the results in the control group with normal iron stores, the intestinal uptake and net absorption of both cobalt and iron were significantly increased, the delayed excretion of iron was reduced but that of cobalt was similar. The results were similar to those in iron deficient control subjects, but they show increased absorption when compared with cirrhotics with normal iron stores.

Individual results are shown in Appendix V (page 118).

The results thus show that in patients with depleted iron stores there is an increase in the intestinal absorption not only of iron but of cobalt as well. The degree of increase in the absorption of both elements is similar in control subjects and those with portal cirrhosis (see Fig. 2, page 57). Liver disease in the iron depleted subject seems not to affect the mechanism regulating the intestinal absorption of these two elements.

PATIENTS WITH INCREASED IRON STORES

(a) Idiopathic Haemochromatosis

The average intestinal cobalt uptake was 85.7 percent (range 79.9 to 92.4 percent), the average delayed excretion was 0.8 percent (range 0.2 to 2.0 percent) and the average net cobalt absorption was 84.9 percent (range 79.4 to 92.1 percent).

In comparison with the results in the control subjects with normal iron stores, both the intestinal uptake and net absorption of cobalt were significantly increased ($p < 0.001$ in each case) but the delayed excretion was similar.

The average intestinal uptake of iron was 73.7 percent (range 58.2 to 90.3 percent), the average delayed excretion was 8.9 percent (range 2.5 to 20.5 percent) and the average net iron absorption was 64.8 percent (range 57.7 to 84.8 percent).

In comparison with the results in the control group with normal iron stores, the average intestinal uptake and net absorption of iron were both significantly increased ($p < 0.001$ in both cases). The average delayed excretion of iron was similar to that in controls, however, in two patients who had been treated by venesections (V.C. & E.G.) the values, 3.8 percent and

ABLE V. COBALT AND IRON ABSORPTION IN PATIENTS WITH INCREASED IRO

AETIOLOGY OF IRON OVERLOAD	NO. OF PATIENTS OR INITIALS	AGE (years) mean and range	HGB (gm. /100ml.) mean and range	MARROW IRON (grade)	Li Le Ta
Idiopathic Haemochromatosis	4	61.2 (58 - 66)	12.8 (12.1 - 13.6)	3 - 4+	0
Secondary to Portal Cirrhosis	R. W.	56	10.4	1+	
	V. D.	65	12.4	1+	
Sequence of Cirrhosis and Iron Overload Uncertain	J. S.	59	11.8	3+	
	R. B.	63	14.3	4+	
Exogenous	B. M.	63	14.4	4+	
	M. D.	60	10.8	4+	

* 15 grams of iron subsequently removed from iron stores

These findings suggest that there is an abnormality in

BLE V. COBALT AND IRON ABSORPTION IN PATIENTS WITH INCREASED IRON STORES

AETIOLOGY OF IRON OVERLOAD	NO. OF PATIENTS OR INITIALS	AGE (years) mean and range	HGB (gm. /100mL) mean and range	MARROW IRON (grade)	LIVER IRON (grade)	IRON REMOVED BY VENESECTION (grams)	COBALT ABSORPTION PERCENTAGE OF ORAL TEST DOSE (mean and range)			IRON ABSORPTION PERCENTAGE OF ORAL TEST DOSE (mean and range)		
							INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION	INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION
Idiopathic Haemochromatosis	4	61,2 (58 - 66)	12,8 (12,1 - 13,6)	0 - 4+	0 - 4+	0 - 12,5	85,7 (79,5-92,4)	0,8 (0,2-2,8)	84,9 (79,4-92,1)	71,7 (58,2-90,3)	8,9 (2,5-20,5)	64,8 (37,7-84,8)
Secondary to Portal Cirrhosis	R. W.	56	10,4	1+	4+	0	90,2	0,2	90,0	77,8	2,6	75,2
	V. D.	67	12,4	1+	4+	2,2	71,0	0,1	70,9	49,0	1,4	47,6
Sequence of Cirrhosis and Iron Overload Uncertain	J. S.	59	13,8	2+	4+	18,5	85,4	0,2	85,2	80,2	2,9	77,3
	R. D.	63	14,3	4+	4+	0*	65,1	0,5	64,6	74,5	2,6	71,9
Exogenous	B. M.	63	14,4	4+	4+	0	60,4	1,3	59,1	28,4	7,4	21,0
	M. D.	60	10,8	4+	4+	0	22,2	0,2	22,0	9,0	4,4	4,6

* 15 grams of iron subsequently removed from iron stores

2.5 percent respectively, were within the range in iron deficient subjects.

The individual results are given in Appendix VI (page 119) and graphically for the whole group in Fig. 2, (page 57).

(b) Iron Overload Secondary to Portal Cirrhosis

The intestinal uptake of cobalt in the two patients were 90.2 and 71.0 percent respectively, the delayed excretion 0.2 and 0.1 percent respectively, and the net cobalt absorption were 90.0 and 70.9 percent respectively.

The intestinal uptake of iron were 77.8 and 49.0 percent respectively, the delayed excretion 2.6 and 1.4 percent respectively and net absorption 75.2 and 47.6 percent respectively.

The values for the intestinal uptake and net absorption of both metals were increased in comparison with corresponding values in control subjects with normal iron stores and were similar to those with iron deficiency or idiopathic haemochromatosis.

The results show that an inappropriate increase in intestinal absorption of iron has occurred in these patients because their iron stores were not diminished. These findings suggest that there is an abnormality in

the intestinal mechanism regulating cobalt and iron absorption in these patients. The fact that liver disease had been shown in the previous groups of patients to be without direct effect on the mechanisms regulating the intestinal absorption of cobalt and iron indicates that the disorder of iron metabolism may reside in the intestinal mucosa. The cirrhotic process seems to exert no influence on this.

(c) Portal Cirrhosis and Iron Overload
(Sequence of events uncertain)

The intestinal uptake of cobalt in the two patients were 85.4 and 65.1 percent respectively; delayed excretion, 0.2 and 0.5 percent respectively, and net absorption 85.2 and 64.6 percent respectively.

The intestinal uptake of iron in the two patients were 80.2 and 74.5 percent respectively, delayed excretion 2.9 and 2.6 percent respectively and net absorption 77.3 and 71.9 percent respectively.

In comparison with the results in the control subjects with normal iron stores the intestinal uptake and net absorption of both cobalt and iron were significantly increased. The delayed excretion of cobalt was similar to the values obtained in the controls with normal iron stores but the delayed excretion of iron

was reduced to similar levels obtained in iron deficient subjects.

When these two patients were investigated the iron stores were increased. One of them J.S. whose iron stores had been depleted by 18.5 grams of iron still had increased amounts of iron in his liver biopsy (4 + deposition). The other patient, R.B., who had not been treated at the time absorption studies were carried out subsequently had 15 grams. of iron removed from his body stores.

The results in these patients demonstrate in appropriate increase in iron absorption which is closely reflected by increased cobalt absorption. The pattern of absorption points to a disorder in the intestinal mechanism regulating the absorption of these metals. It is suggested that liver cirrhosis is not likely to be responsible for this.

(d) Exogenous Iron Overload

In B.M., the results for the intestinal uptake, delayed excretion and not absorption of cobalt were 60.4, 1.3 and 59.1 percent respectively. The corresponding values for iron absorption results were 26.4, 7.4 and 21.0 percent respectively.

In comparison with the results in the control group with normal iron stores, the values for the cobalt

absorption studies in B.M. were similar. (Mean values in controls:- cobalt uptake, 45.6 percent, delayed excretion 2.0 percent, net absorption 43.6 percent). The iron absorption results in B.M. were within the lower range of values observed in control subjects with normal iron stores.

In M.D., the results for the intestinal uptake, delayed excretion and net absorption of cobalt were 22.2, 0.2 and 22.0 percent respectively. The corresponding values for the iron absorption results were 9.0, 4.4 and 4.6 percent respectively.

In comparison with the results in the control group with normal iron stores the uptake and net absorption of both metals in ~~these~~ this two patients was diminished.

The results in these two patients indicate a lowering in the intestinal absorption of both metals when body iron stores are exogenously increased in contrast to results obtained in patients with increased stores from endogenous causes. Iron absorption is lowered to a greater degree than cobalt absorption. It seems likely that body iron stores when artificially expanded exert a greater control over the mechanisms regulating the intestinal absorption of iron than of cobalt.

In summary, cobalt and iron absorption studies were carried out on a total of ten patients with increased iron stores. In two of the patients, the increase in body iron stores was a result of iron being introduced into the body in the form of blood transfusion and iron medication. The intestinal absorption of iron was reduced in the two patients. Cobalt absorption was normal in one and lowered in the other.

In the remaining eight patients consisting of 4 cases of idiopathic haemochromatosis and 4 cases of iron overload and portal cirrhosis, cobalt absorption was increased in all of them. Iron absorption was increased in seven out of the eight patients. The observation of a consistently raised cobalt absorption will be commented on later.

CORRELATIONS BETWEEN THE INTESTINAL ABSORPTION OF COBALT AND IRON

1. Intestinal Uptake

A highly significant direct correlation was present between the intestinal uptake of cobalt and iron in the control subjects with normal iron stores and iron depletion ($p < 0.001$). The correlation coefficient was 0.83 with the intercept on the cobalt axis at 20 percent (see Fig. 3, page 69).

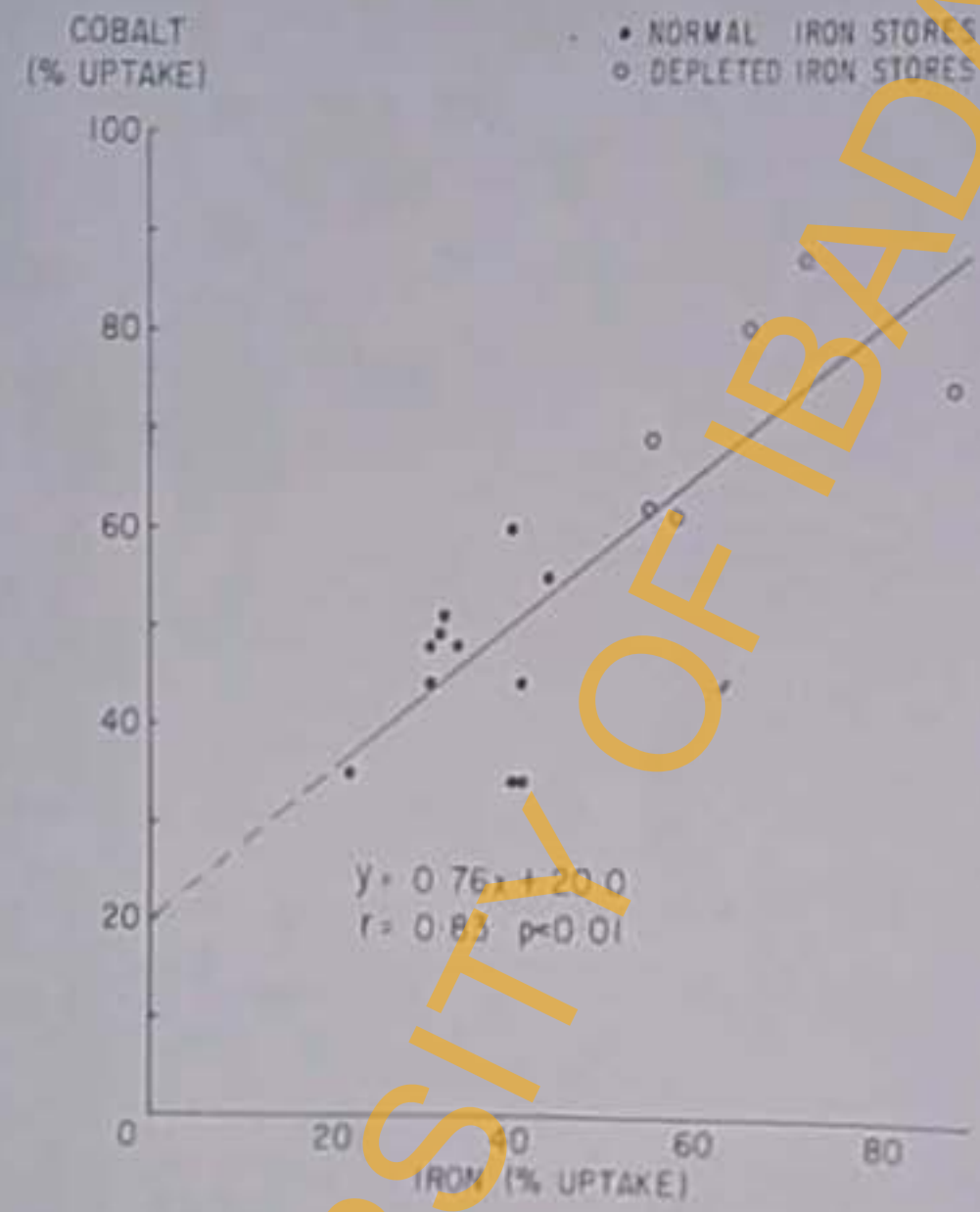

FIGURE 3. CORRELATION OF INTESTINAL UPTAKE OF COBALT AND IRON IN CONTROL SUBJECTS.

A similar direct correlation was observed between the intestinal uptake of cobalt and iron in the patients with fatty infiltration and degeneration of the liver and portal cirrhosis ($p < 0.001$). The correlation coefficient was 0.72 with the intercept on the cobalt axis at 27.8 percent (see Fig. 4, page 71). This represents no significant difference in the regression coefficient and intercept between the patients with liver disease and the control subjects.

The regression line for the combined results in the control subjects, patients with liver disease and idopathic haemochromatosis is given in (Fig. 5, page 72). The relationship between the intestinal uptake of cobalt and iron in all the patients was highly significant ($p < 0.001$). The correlation coefficient was 0.77 and the intercept on the cobalt axis was at 26.0 percent.

These results demonstrate the existence of a direct relationship between the uptake of cobalt and iron and indicate that these elements share part of a common uptake step in the intestinal mucosa. Liver disease does not appear to affect this relationship.

2. Net Absorption

A highly significant direct correlation was present between the net absorption of cobalt and iron in the

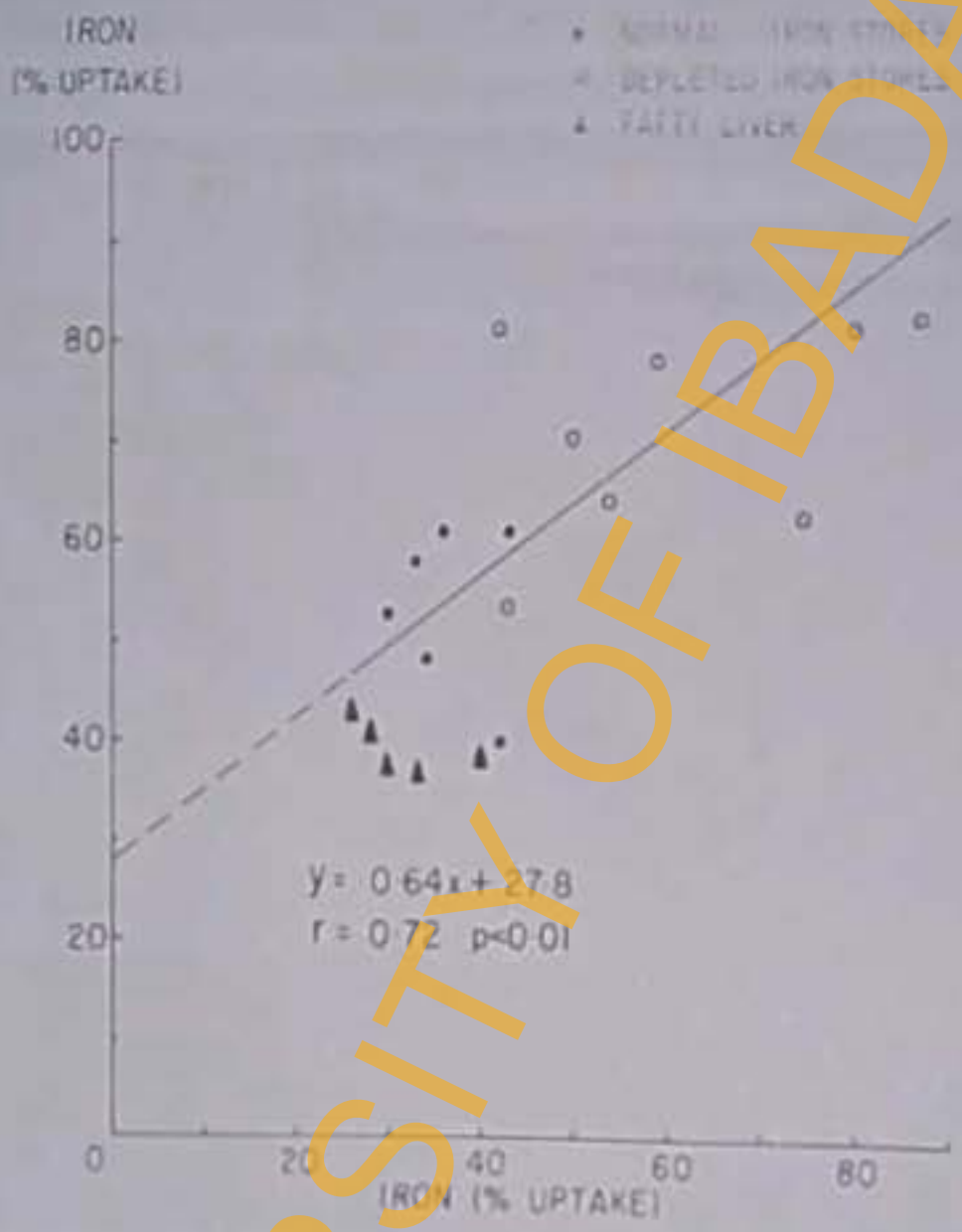

FIGURE 4. CORRELATION INTESTINAL UPTAKE OF COBALT AND IRON IN PATIENTS WITH PORTAL CIRRHOSIS AND ALCOHOL INDUCED FATTY INFILTRATION OF THE LIVER.

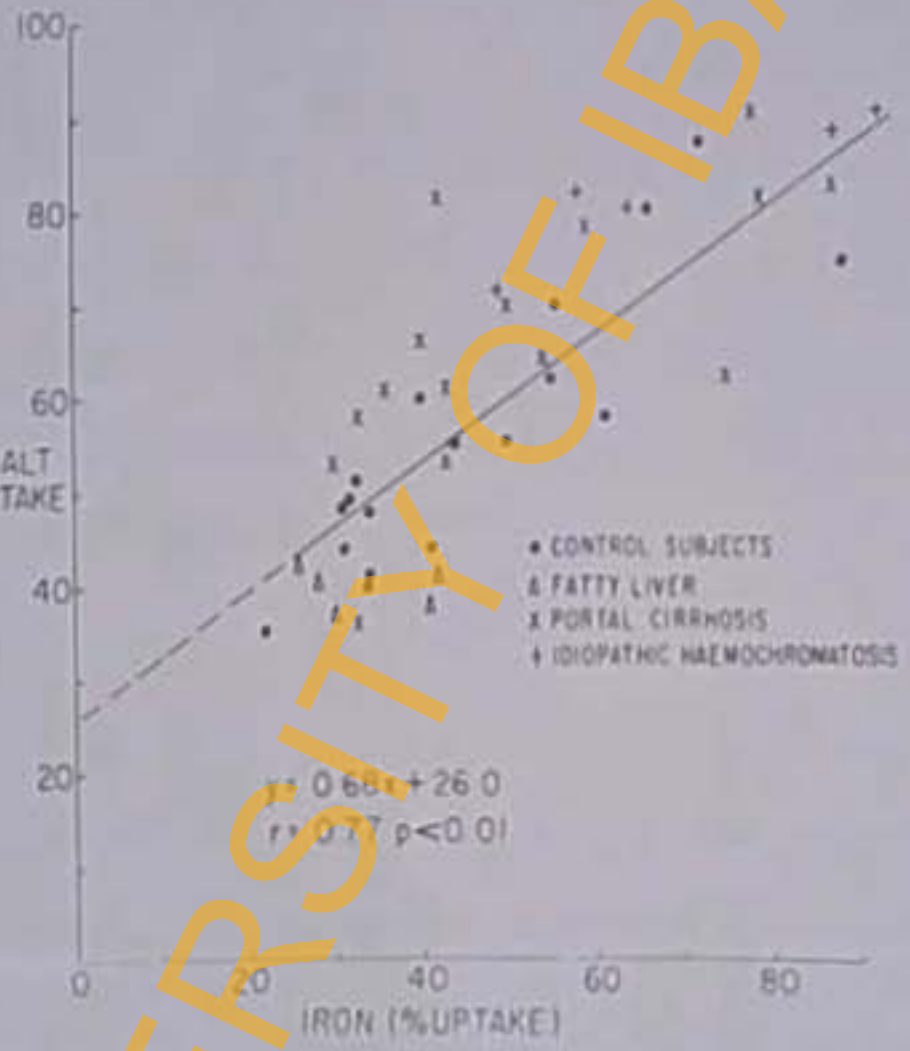

FIGURE 5. CORRELATION OF INTESTINAL UPTAKE OF COBALT AND IRON IN CONTROL SUBJECTS, PATIENTS WITH LIVER DISEASE, AND IN IDIOPATHIC HAEMOCHROMATOSIS.

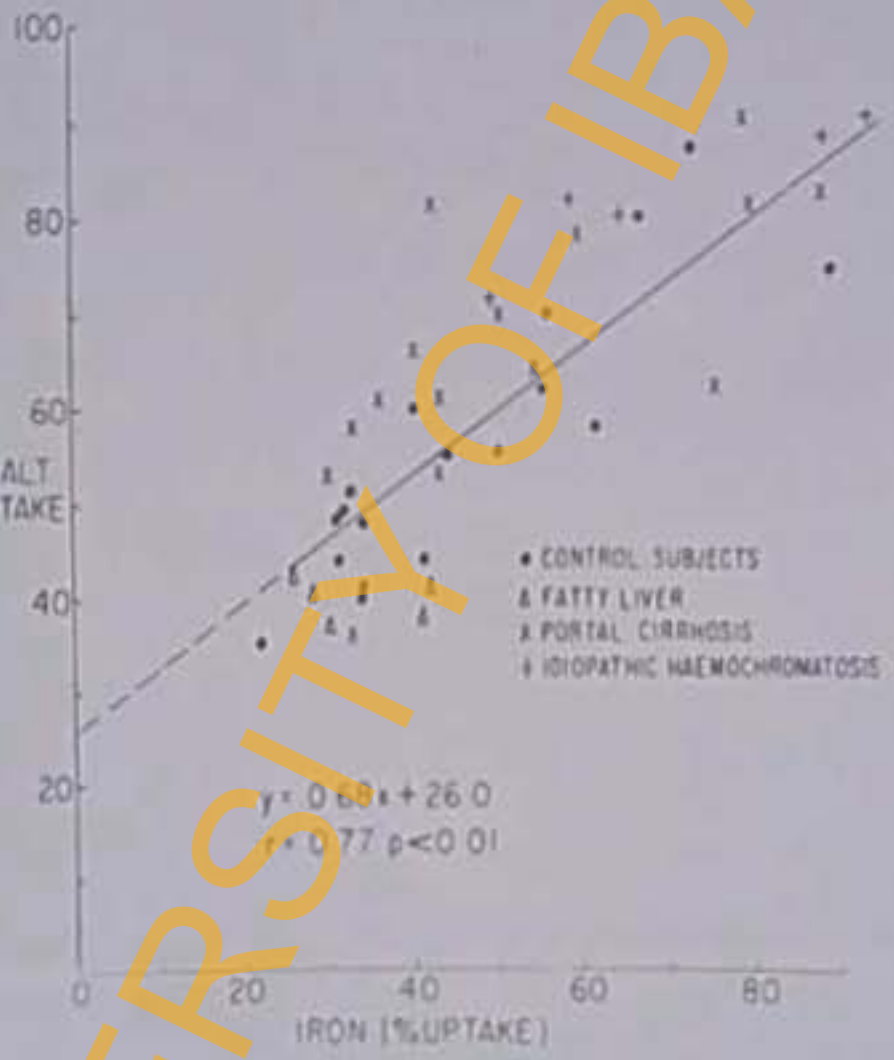

FIGURE 5. CORRELATION OF INTESTINAL UPTAKE OF COBALT AND IRON IN CONTROL SUBJECTS, PATIENTS WITH LIVER DISEASE, AND IN IDIOPATHIC HAEMOCHROMATOSIS.

control subjects with normal iron stores and iron depletion ($p < 0.001$). The correlation coefficient was 0.85 with the intercept on the cobalt axis at 25.4 percent. (See Fig. 6, page 74).

A similar direct correlation was observed between the net absorption of cobalt and iron in the patients with fatty infiltration and degeneration of the liver and portal cirrhosis ($p < 0.001$). The correlation coefficient was 0.76 with the intercept on the cobalt axis at 31.7 percent (see Fig. 7, page 75).

An analysis of the combined results in the control subjects, patients with liver disease and idiopathic haemochromatosis reveals similar results, the correlation coefficient was 0.83 with the intercept on the cobalt axis at 29.4 percent (Fig. 8, page 76).

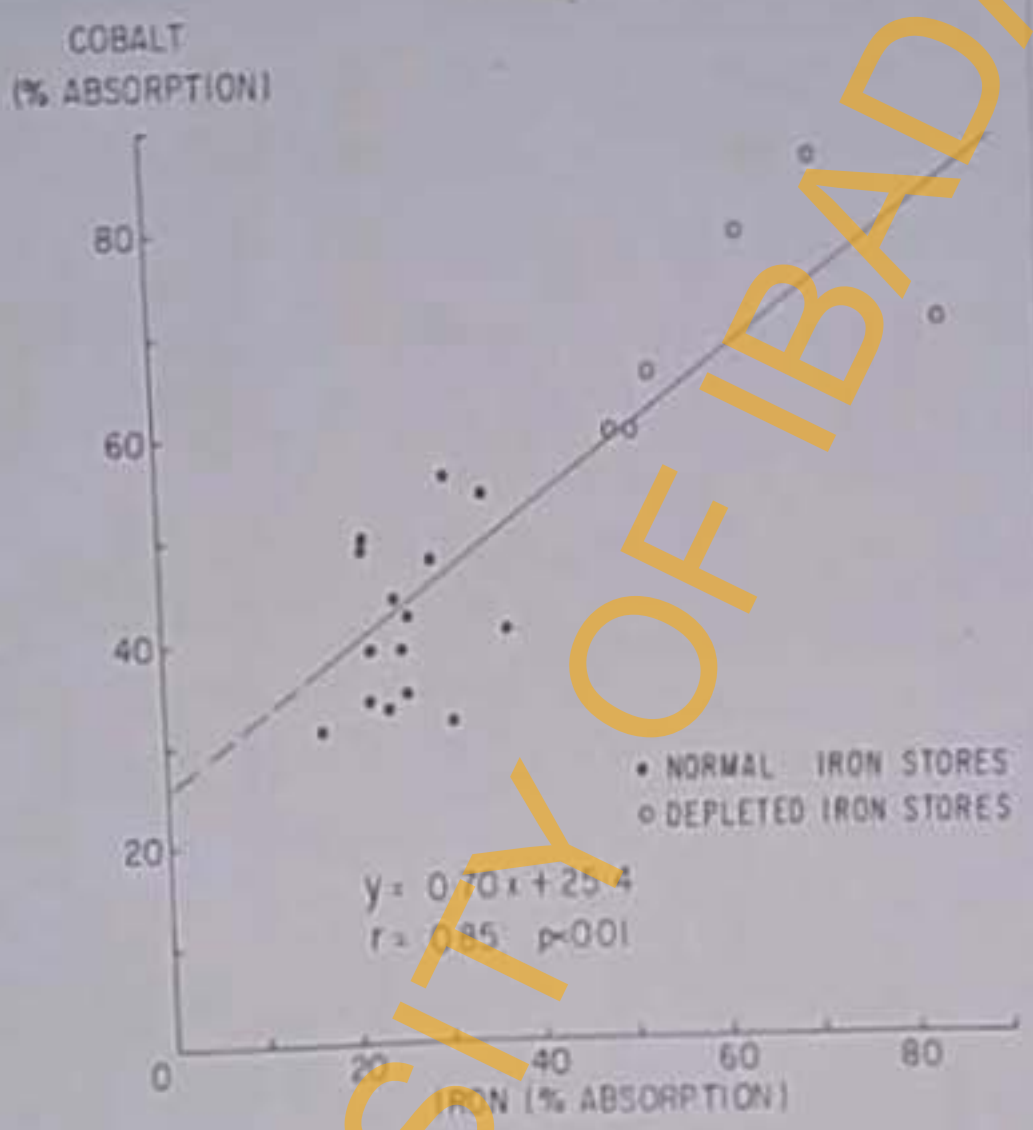

FIGURE 6. CORRELATION OF NET ABSORPTION OF COBALT AND IRON IN CONTROL SUBJECTS.

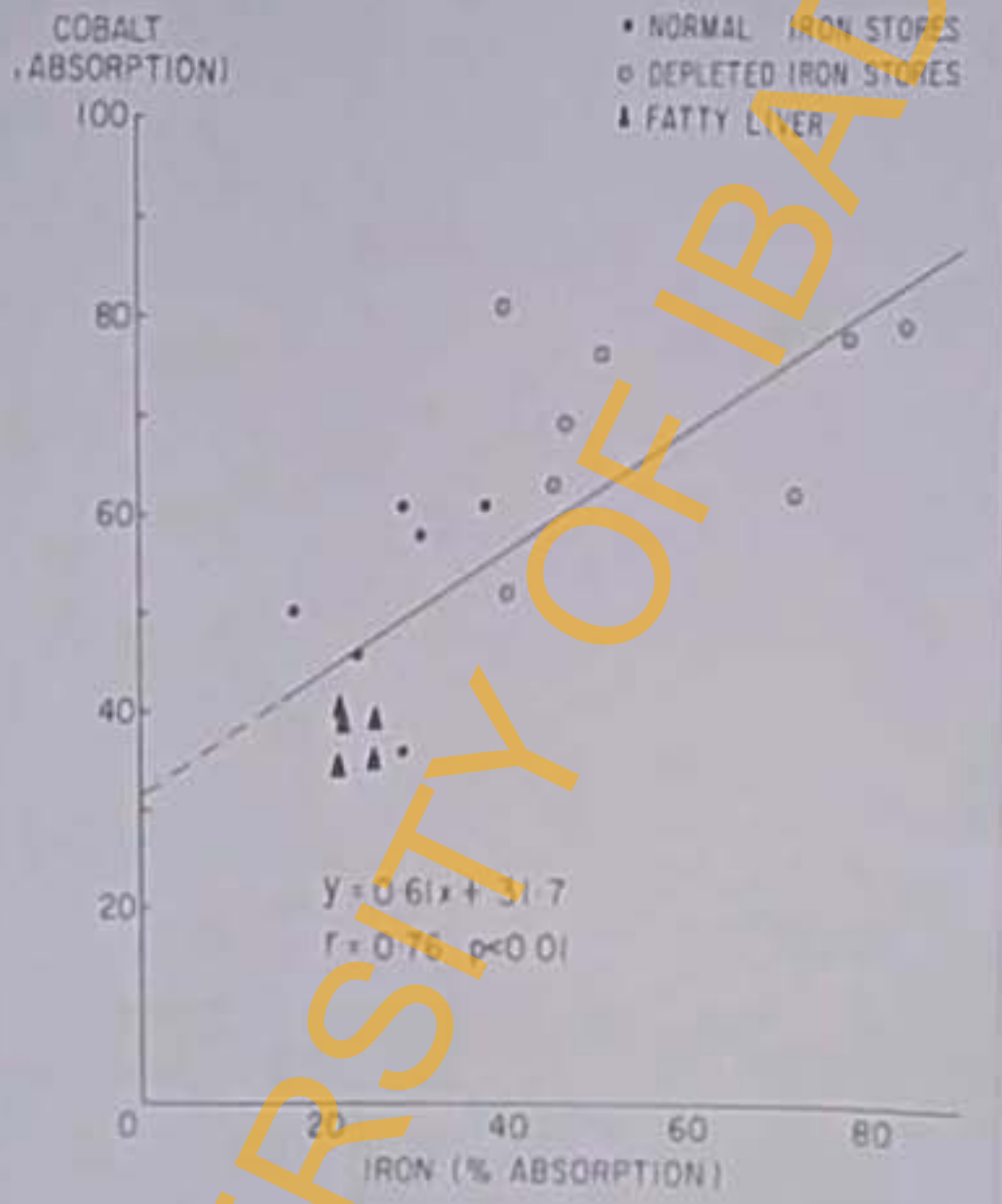

FIGURE 7. CORRELATION OF NET ABSORPTION OF COBALT AND IRON IN PATIENTS WITH PORTAL CIRRHOSIS AND ALCOHOL INDUCED FATTY INFILTRATION OF THE LIVER.

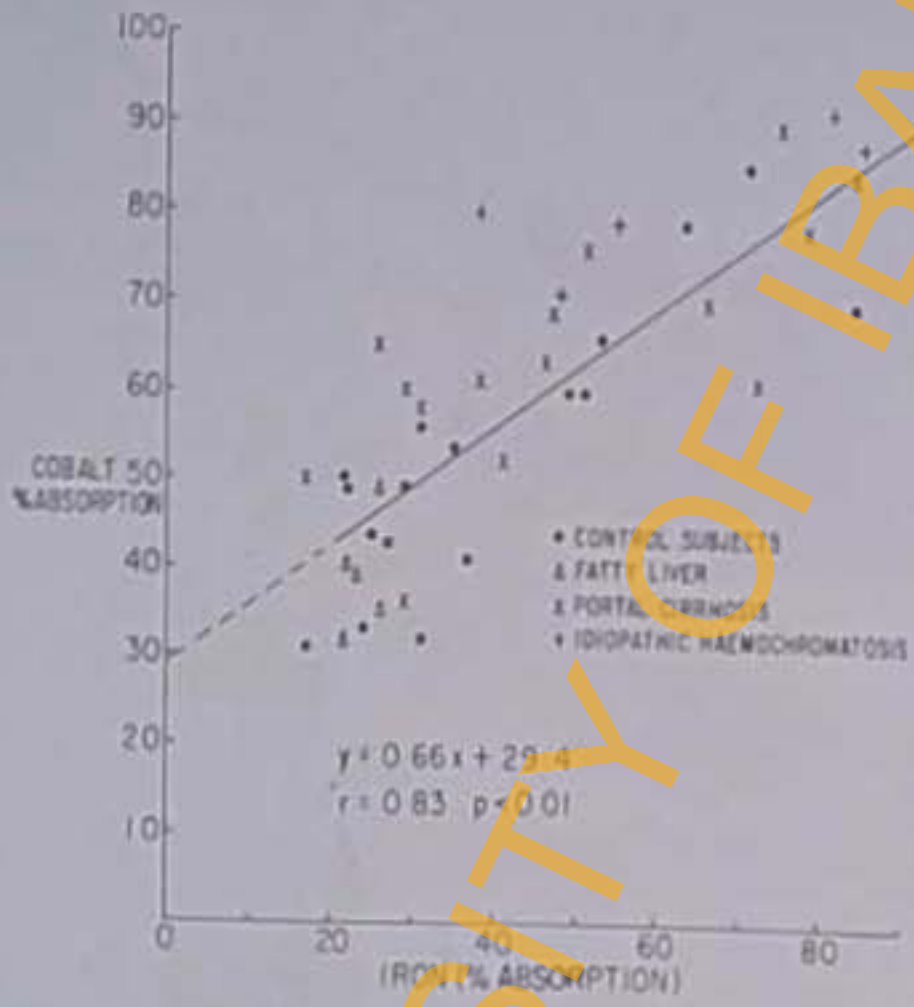

FIGURE 8. CORRELATION OF NET ABSORPTION OF COBALT AND IRON IN CONTROL SUBJECTS, PATIENTS WITH LIVER DISEASE AND IN IDIOPATHIC HAEMOCHROMATOSIS.

III. BODY RETENTION OF COBALT

A direct correlation was present between the net percentage of the test dose of cobalt that was absorbed and the percentage excreted in the urine of subjects with normal iron stores, iron deficiency or endogenous iron over load. (See Fig. 9, page 78). An average of 20 percent of the radio activity was retained in the body 10 days after administration of the labelled test dose, and no significant difference was observed between the results in subjects with normal iron stores and those with either iron depletion or iron overload. (See Fig. 10, page 79). In addition, it was found that there was no significant difference between the body retention of radioactivity in control subjects and those with cirrhosis.

In summary, the results indicate no differences in the relationship between cobalt and iron absorption in control subjects without liver disease and in patients with liver diseases and idiopathic haemochromatosis.

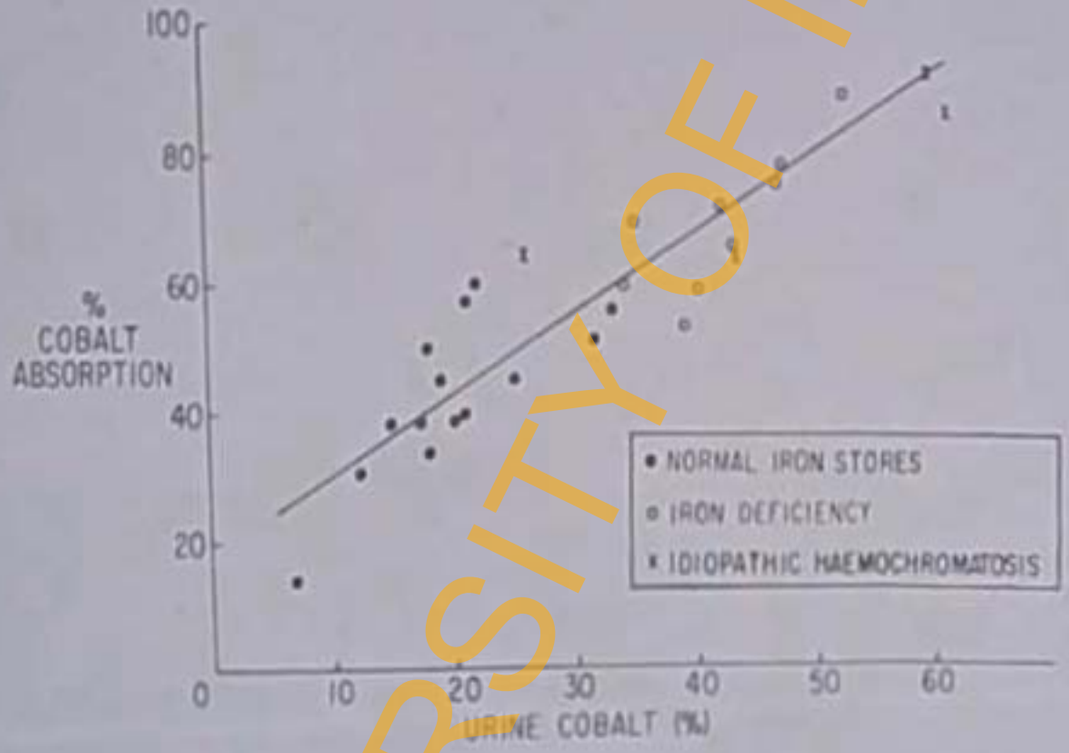

FIGURE 9. RELATIONSHIP BETWEEN NET ABSORPTION AND URINARY EXCRETION OF COBALT.

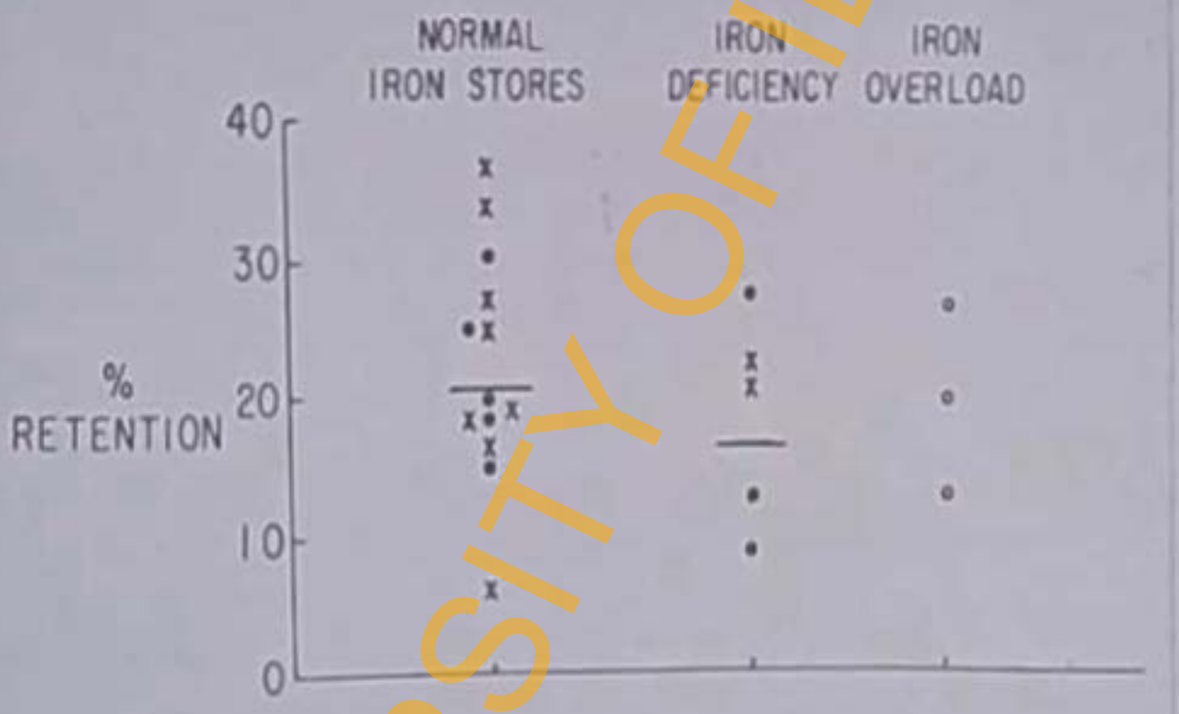

FIGURE 10. BODY RETENTION OF COBALT

KEY: Solid circles o Control Subjects

Crosses x Liver Disease

Open circles o Idiopathic Haemochromatosi

DISCUSSION

When equimolecular amounts of inorganic cobalt and iron were orally administered to subjects with normal iron stores significantly more cobalt was taken up from the intestinal lumen. Very little of the cobalt taken up was sequestrated in the intestinal mucosal cell. As a result of this and the greater uptake of cobalt from the lumen, the net absorption of cobalt was significantly greater than that of iron (Fig. 2, page 57). The reason for this is not clear. One possibility however, is that whereas the mechanism for iron absorption is most efficient in the duodenum, that for cobalt absorption is efficient in the duodenum as well as in the lower small intestine.

Wheby (126), by comparing percentage iron absorption from an orally administered test dose with absorption from chosen sites in the small bowel, demonstrated that iron was absorbed through out the length of the small bowel. The mechanism for iron absorption was most efficient in the duodenum but when iron was directly instilled into the proximal jejunum its absorption was reduced by 50 percent.

No studies of cobalt absorption similar to those of Wheby have been carried out in man; however,

Thomson et al. (127), using isolated gut segments, have shown in the rat, that cobalt and iron are equally well absorbed in the duodenum, but in addition, cobalt absorption is greater than that of iron in the lower small intestine. It is likely that in the human subject the mechanism regulating the intestinal absorption of both metals is similar to that in the rat. The greater intestinal uptake of cobalt than iron in man could, therefore, be a result of efficient absorption over a greater anatomical area of the small intestine.

The significance of mucosal cell sequestration in the regulation of the intestinal absorption of cobalt and iron is not fully understood. Conrad and Crosby (123) from auto-radiographic studies in the rat pronosed a model for the mucosal mechanisms regulating the intestinal absorption of iron. According to this model iron absorption is regulated primarily through the columnar epithelium of the small intestine. In normal, iron replete subjects the mucosal cells may contain a variable amount of iron supplied from the body store. The deposit regulates - within limits - the quantity of iron that can enter the cell from the intestinal lumen. After the iron has entered the cell it may proceed into the body to fulfill a requirement. Alternatively, a portion of the iron may

become fixed in the epithelial cytoplasm, to be lost when the cell is sloughed at the end of its life span. In iron-deficient subjects there appears to be little or no mechanism to inhibit entrance of iron or to retain it in the villous epithelial cells. Thus dietary iron readily proceeds into the body. In iron loaded subjects a significant amount of the body iron is incorporated in the villous epithelial cells, which is eventually lost with sloughing; but during the life span of the cells its presence inhibits the entrance of iron into the cells.

The results of the iron absorption studies in this thesis are largely in agreement with this concept. In control patients with normal iron stores the average delayed excretion of iron was about 9 percent of the orally administered dose and approximately one quarter of the uptake from the lumen. The results in patients with alcohol induced fatty infiltration of the liver and portal cirrhosis with normal iron stores were similar to those of control subjects. This means that out of every 4 parts of iron taken up from the lumen by the mucosal cell, one part is sequestrated and three parts are transferred to the body.

The average intestinal uptake of iron was increased to 65.8 percent in iron deficient controls and to 61.1 percent in iron deficient cirrhotics. The delayed

excretion was however reduced from a mean of about 9 percent in subjects with normal iron stores to approximately 4 percent in iron deficiency. The delayed excretion of iron in iron deficiency therefore amounted to about 7 percent of the initial uptake from the lumen as compared to 25 percent in subjects with normal iron stores. These observations confirm Conrad and Crosby's hypothesis that in iron deficiency there is little or no mechanism in the cell to inhibit entrance of iron into the villous epitholial cells or to retain it (123). However, the fact that diminution in iron sequestration in the mucosa in iron deficiency accounts for only a small proportion of the increase in net iron absorption suggests that sequestration of iron is not a prime factor in the control of iron absorption.

Furthermore, in exogenous iron overload, the reduction in intestinal uptake was the main factor responsible for the reduction in iron absorption because the delayed excretion was within the range observed in normal subjects. This observation is a departure from the model of Conrad and Crosby because the delayed excretion of iron was not increased.

In idionathic haemochromatosis, and in patients with iron overload associated with portal cirrhosis, the intestinal uptake of iron was increased in all the patients. The delayed excretion of iron however, covered a wide range. In two treated patients with idionathic haemochromatosis, it was similar to that in iron deficiency and in the untreated patients it was within the range observed in control subjects with normal iron stores (Appendix VI, page 119). The delayed excretion was also similar to that in iron deficiency in the 4 patients with portal cirrhosis associated with iron overload. The reason for the variation in the sequestration of iron in the mucosa of the subjects with iron overload from endogenous causes, is not clear. The similarity of the results in 3 patients (PM, VD and JS) with porto-caval shunt to those in (PH) with a comparable degree of liver disease but not evidence of portal hypertension suggests that the shunting of blood around the liver does not have a direct bearing on the amount of iron sequestrated in the mucosa. The reduction in delayed excretion in the treated patients lends support to the hypothesis, that the degree of sequestration of iron in the mucosa is related to the magnitude of the iron stores. Perhaps the variation in the intestinal uptake and sequestration

In idiopathic haemochromatosis, and in patients with iron overload associated with portal cirrhosis, the intestinal uptake of iron was increased in all the patients. The delayed excretion of iron however, covered a wide range. In two treated patients with idiopathic haemochromatosis, it was similar to that in iron deficiency and in the untreated patients it was within the range observed in control subjects with normal iron stores (Appendix VI, page 119). The delayed excretion was also similar to that in iron deficiency in the 4 patients with portal cirrhosis associated with iron overload. The reason for the variation in the sequestration of iron in the mucosa of the subjects with iron overload from endogenous causes, is not clear. The similarity of the results in 3 patients (PM, VO and JS) with porto-caval shunt to those in (PB) with a comparable degree of liver disease but not evidence of portal hypertension suggests that the shunting of blood around the liver does not have a direct bearing on the amount of iron sequestrated in the mucosa. The reduction in delayed excretion in the treated patients lends support to the hypothesis, that the degree of sequestration of iron in the mucosa is related to the magnitude of the iron stores. Perhaps the variation in the intestinal uptake and sequestration

of iron in these patients with iron overload from endogenous causes is due to a difference in the aetiology of the iron overload. In one group of patients the intestinal uptake is increased whilst delayed excretion is reduced; in the other group the intestinal uptake is increased whilst delayed excretion is within normal limits. Further studies involving greater number of patients are required to elucidate this apparent difference.

The reason for the failure of the intestinal mocusa to sequestrate a significant proportion of cobalt taken up from the lumen in human subjects is not known. Schade et al. (128) made a similar observation in the rat. They clearly demonstrated an incorporation of a proportion of orally administered iron into the ferritin in the intestinal mucosa but none of cobalt. It is possible that cobalt once taken up by the cell rapidly diffuses through it into the blood stream but there is no experimental evidence for this at present. The studies of Schade and co-workers (128) also showed that the common pathway for the intestinal absorption of cobalt and iron is not ferritin which seems to be playing a role only in temporary sequestration of iron rather than in primary regulation of its absorption. The absence of correlation between net iron absorption and the delayed excretion in this study lends support

to this view.

The increase in net absorption of iron in subjects with depleted iron stores is in keeping with previous reports by Bothwell et al. (129) and Pirzio-Biroli et al. (130). In iron deficiency this study has shown that the intestinal epithelial cells are in some unknown manner made to alter the handling of iron. The mechanism involved leads to increase in the mucosal uptake and transfer of iron to the body whilst less iron is sequestrated in the mucosa. This mechanism also leads to enhanced cobalt absorption which has been shown to be directly proportional to iron absorption (Fig. 8, page 76). This suggests that cobalt and iron share at least part of a common absorptive pathway and that acceleration of their transport is probably governed by the same mechanism.

Iron absorption was regulated by body iron stores in a reciprocal manner in most of the patients with liver disease and in the control subjects that were studied (see Fig. 11, page 87). However, there were four patients with inappropriate increase in absorption inspite of an increase in body iron stores (4 + stainable iron in liver biopsy). In two of the patients V.D. and R.W. the liver disease ante-dated iron overload whereas in J.S. and R.B., who presented in the late stages of their disease, it was

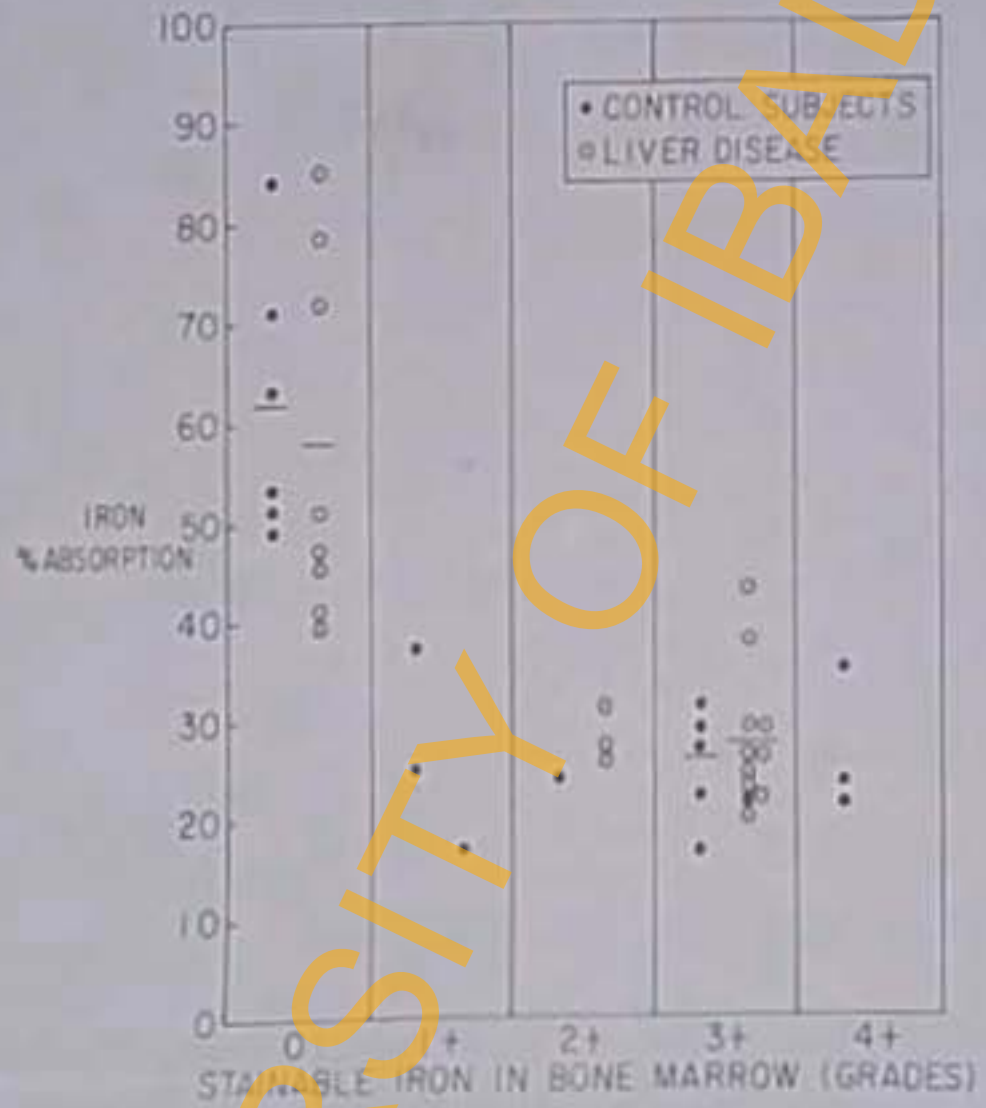

FIGURE 11. RELATIONSHIP BETWEEN IRON ABSORPTION AND STAINABLE IRON IN THE BONE MARROW.

not clear which was the primary event. As was pointed out in the review of the literature, there have been several reports which tended to suggest that liver disease per se directly influenced the intestinal mucosa to increase iron absorption in some cases of portal cirrhosis. Murray and Stein (131) have reviewed the mechanisms through which liver cirrhosis might enhance intestinal iron absorption. Among these are, liver damage, pancreatic insufficiency, haemolysis as reflected by an increase in plasma iron turn over rate, Vitamin B_{12} deficiency and folic acid deficiency. Balcerzak et al. (132), Charlton et al. (133) have shown that in the absence of iron defiency, pancreatic insufficiency had no influence on iron absorption. Also, liver damage such as in infective hepatitis has been shown not to affect iron absorption in the absence of iron deficiency, (134). This report, and the lack of correlation between iron absorption and indices of liver function such as SGOT and BSP retention in the study, presented in this thesis indicate that liver damage by itself does not directly influence the intestinal handling of iron. The effects of liver disease folic acid deficiency, vitamin B_{12} deficiency and the plasma iron turn over rate on iron absorption in control subjects and patients and patients with liver disease have been previously studied by

Olatunbosun et al. (135). It was shown that these factors had no direct influence on iron absorption. It still remains unclear what mechanism was responsible for the inappropriate increase in iron absorption in the four patients with iron overload associated with portal cirrhosis but it is unlikely to be due to liver disease per se. This is also supported by the increase in cobalt absorption in these patients. There is no known mechanism regulating cobalt metabolism in the human body since there is no requirement for it as a metal. The high correlation between the absorption of cobalt and iron in patients with liver disease, and also in control subjects indicates that the increased cobalt absorption is a reflection of an alteration in iron metabolism and not to liver disease. It also lends support to the thesis that the mechanisms regulating the intestinal absorption of these metals are related.

Notwithstanding the small number studied, the consistently observed increase in cobalt absorption in haemochromatosis indicates that cobalt absorption test may likely prove to be a useful test for the study of the underlying abnormality in intestinal absorption in this disease. Cobalt absorption was abnormal in all four patients studied and in J.W. & P.B. tests

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repeated during the following 12 months showed a persistent increase. The finding here of a rather high percentage of increase in iron absorption in haemochromatosis is in contrast with reports by other authors who have shown that iron absorption may be normal in idiopathic haemochromatosis (136). Thus a study of iron absorption alone may not be enough in revealing the nature of the abnormality in intestinal absorption in haemochromatosis. The confirmation of this probability must await further studies in increased number of patients.

This study has shown a parallelism between the intestinal absorption of cobalt and its excretion in the urine (Fig. 9) which explains why increased absorption is not accompanied by increased body retention of cobalt. Hunt et al. (78) were unable to detect increase liver cobalt concentrations in some patients with various types of cirrhosis, as compared to accident victims without liver disease. However, a few of the patients studied by Hunt et al. had abnormally high liver cobalt concentrations. This was thought to be due to previous Vitamin B_{12} administration. Thus the measurement of cobalt in clinical diagnosis must be based on a study of its absorption. It has been shown in this study that

net cobalt absorption is increased in disease states of increased body iron stores not due to exogenous causes and in controls and cirrhotics with depleted iron stores.

In conclusion there are two clinical applications of the results presented in this thesis. Firstly, even though the number of cases of idiopathic haemochromatosis available for study was small, the result suggest that measurement of cobalt absorption might provide a more sensitive index than iron of the intestinal mucosal abnormality in this disorder, since a number of these patients may be expected to have normal iron absorption. Secondly, a parallelism exists between iron absorption and cobalt excretion in the urine (Fig. 12 page 92) which suggests that the measurement of cobalt in the urine following an oral test dose might provide a simple technique for detecting conditions in which iron absorption is increased, such as iron deficiency and idiopathic haemochromatosis. It takes a minimum of ten days to carry out an iron absorption test but a cobalt excretion study can be completed within 24 hours. Most of the absorbed cobalt is excreted within 6 hours following oral administration of the test dose. Preliminary studies by Valberg, Olatunbosun, Ludwig and Corbett (137) revealed that this may be so.

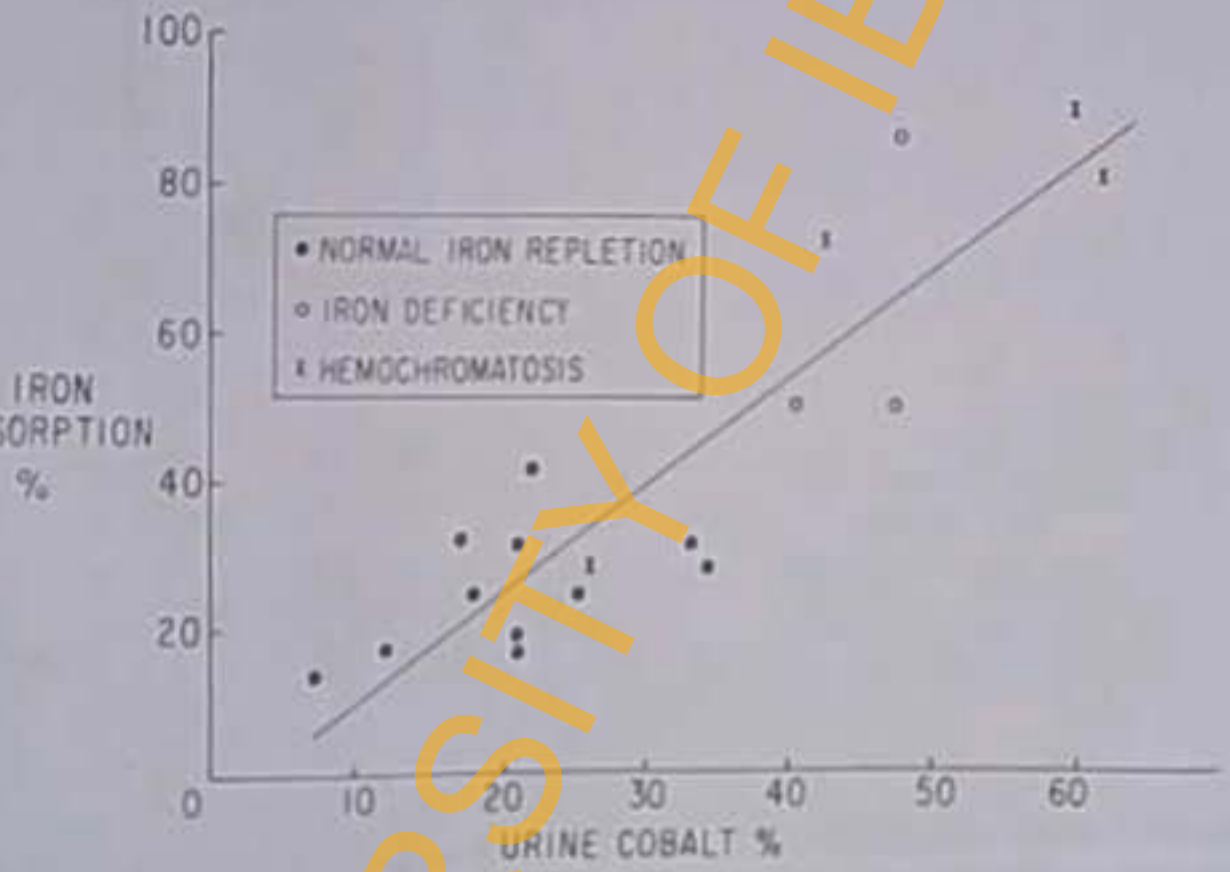

FIGURE 12. RELATIONSHIP BETWEEN IRON ABSORPTION AND COBALT EXCRETION IN THE URINE.

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APPENDIX 3 PRIMARY DATA IN CONTROL SUBJECTS WITH NORMAL IRON STORES

DATE	AGE AND SEX	DIAGNOSIS	BLOOD COUNTS				MARROW IRON (grade)	LIVER IRON (grade)	LIVER FUNCTION TESTS						SERUM IRON	TOTAL IBC	IRON ABSORPTION PERCENTAGE ORAL TEST DOSE			COBALT ABSORPTION PERCENTAGE ORAL TEST DOSE		
			HGB	PCV %	RBC	RETICS %			SERUM ALB.	γ GLOB	BILIRUBIN	ALK. PHOS	SGOT	SGP %			INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION	INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION
12	M/F	Anxiety neurosis	13.6	40.0	4.1	4.0	3+	-	2.6	1.3	0.6	1.9	14	0.6	87	224	33.4	6.9	26.5	41.7	1.2	42.9
23	M/F	" "	12.9	41.3	4.6	2.2	1+	-	3.9	1.2	0.3	4.0	21	4.5	81	273	31.4	6.1	25.3	41.3	4.0	45.3
43	M/M	" "	14.7	45.0	5.1	2.0	1+	-	3.7	1.2	0.3	2.9	18	2.9	103	223	41.3	6.1	34.8	44.2	3.4	48.0
51	M/M	Depression	15.3	48.5	5.5	2.0	3+	-	3.4	1.4	0.6	2.3	40	4.9	141	314	15.2	18.2	22.0	48.7	0.2	48.5
54	F/M	Bronchitis	13.6	41.5	4.6	1.6	2+	-	3.5	1.5	0.8	3.6	25	3.1	55	218	35.9	18.8	24.3	53.7	0.7	53.0
58	M/M	Costochondritis	14.1	44.0	5.0	0.8	3+	-	4.6	1.1	0.8	1.3	18	2.9	72	284	41.0	9.7	31.3	34.0	2.4	31.6
59	M/M	Aortic stenosis	14.5	46.0	5.0	4.2	4+	-	3.4	1.0	0.3	2.4	15	4.0	98	118	41.8	8.5	33.3	55.2	1.0	54.2
79	F/F	Depression	13.1	39.5	4.1	0.8	3+	-	3.9	0.8	0.4	4.0	17	4.0	122	268	41.6	4.8	28.8	48.0	0.0	48.0
98	2/56	"	14.5	46.0	5.3	1.8	3+	-	3.1	0.6	0.3	2.9	20	5.6	78	312	39.7	5.2	30.5	60.9	4.2	56.2
85	7/56	Coronary heart disease	14.8	45.0	4.9	1.6	3+	-	4.0	1.3	1.2	3.0	32	5.0	69	258	22.4	6.4	14.9	18.1	3.7	21.8
249	8/57	Anxiety neurosis	12.2	37.0	5.3	1.8	4+	-	3.6	0.8	0.7	2.8	28	5.0	100	272	12.5	16.2	22.3	51.2	1.3	47.9
251	7/57	" "	14.0	41.5	4.7	1.0	4+	-	3.7	1.0	0.4	1.8	14	4.0	64	248	17.8	11.9	23.9	-	-	-

APPENDIX II. PRIMARY DATA IN CONTROL SUBJECTS WITH DEPLETED IRON STORES

PATIENT DATA	AGE AND SEX	DIAGNOSIS	BLOOD COUNTS				MARROW IRON (grade)	LIVER IRON (grade)	LIVER FUNCTION TESTS						SERUM IRON	TOTAL IBC	IRON ABSORPTION PERCENTAGE ORAL TEST DOSE			COBALT ABSORPTION PERCENTAGE ORAL TEST DOSE		
			HGB g/100ml	PCV %	RBC $\times 10^6$	RETICS %			SERUM ALB. g/100ml	% GLOB g/100ml	BILIRUBIN mg/100ml	ALK. PHOS units	SGOT units	SGP %	µg/100 ml	µg/100 ml	INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION	INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION
[illegible]	12M	Malaria kerala	11.3	41.0	4.3	2.3	0	-	4.0	1.2	0.5	4.0	14	5.3	60	425	55.4	5.4	49.0	62.4	2.0	60.4
KA	41M	Polycythaemia Venesectioma	14.0	50.7	5.4	2.3	0	-	4.3	1.2	0.5	4.0	19	21.6*	49	325	87.8	4.5	84.2	73.9	3.9	70.0
IE	41F	Anxiety neurosis	12.8	40.5	4.1	2.2	0	-	4.7	0.8	0.2	4.0	20	5.2	93	384	55.5	2.6	52.9	68.9	3.4	65.5
CM	52M	Polycythaemia Venesectioma	14.7	47.0	5.0	2.8	0	-	4.7	1.1	0.7	1.9	19	3.8	53	278	72.3	1.9	71.5	87.2	1.0	86.2
JM	46F	Duodenal ulcer	9.1	28.5	3.9	5.2	0	-	4.7	1.1	0.4	2.7	8	2.6	10	310	65.8	2.9	63.1	86.3	1.2	75.1
EC	11F	Menorrhagia	13.4	40.5	4.3	2.6	0	-	4.7	0.9	0.4	1.0	15	2.2	10	211	95.9	5.0	90.9	63.7	2.0	55.1

* Unexplained increase

APPENDIX III. PRIMARY DATA IN PATIENTS WITH ALCOHOL INDUCED FATTY INFILTRATION AND DEGENERATION OF THE LIVER

DIET-ARY HIST.	AGE AND SEX	BLOOD COUNTS				MARROW IRON (grade)	LIVER IRON (grade)	LIVER FUNCTION TESTS						SERUM IRON	TOTAL IBC	IRON ABSORPTION PERCENTAGE ORAL TEST DOSE			COBALT ABSORPTION PERCENTAGE ORAL TEST DOSE		
		HGB g/100 ml	PCV %	RBC x10⁶	RETICS %			SERUM ALB. g/100 ml	γ GLOB g/100 ml	BILIRUBIN mg/100ml	ALK. PHOS units	SGOT units	BSP %	µg/100 ml	µg/100 ml	INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION	INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION
	40M	14.6	44.0	4.2	4.2	3+	2+	3.3	1.6	1.5	2.8	42	35.0	61	314	38.2	1.8	26.4	36.8	2.2	34.6
	35M	14.5	44.0	4.7	1.2	3+	1+	3.1	1.1	1.3	4.4	27	26.4	75	263	40.9	19.1	21.8	37.7	3.6	34.1
	30M	13.4	42.0	4.6	2.4	3+	2+	3.1	1.3	0.6	1.7	25	12.0	63	251	28.2	5.7	22.5	40.3	1.5	39.0
	33M	12.8	40.0	3.9	1.8	3+	2+	4.5	0.9	1.3	2.6	170	24.0	104	254	35.1	14.9	20.5	-	-	-
	42M	13.9	42.0	4.5	1.6	3+	0	2.9	2.1	0.5	3.1	8	15.1	66	240	26.4	4.3	22.1	42.4	2.0	39.8
	39M	15.6	46.5	4.7	2.4	3+	1+	3.5	1.0	0.9	4.3	20	19.4	71	312	42.0	15.7	26.3	40.9	1.9	39.3

APPENDIX IV. PRIMARY DATA IN PATIENTS WITH PORTAL CIRRHOSIS WITH NORMAL IRON STORES

UNIT-CALS	AGE AND SEX	AETIOLOGY OF CIRRHOSIS	BLOOD COUNTS				MARROW IRON (grade)	LIVER IRON (grade)	LIVER FUNCTION TESTS						SERUM IRON	TOTAL IBC	IRON ABSORPTION PERCENTAGE ORAL TEST DOSE			COBALT ABSORPTION PERCENTAGE ORAL TEST DOSE		
			HGB	PCV %	RBC	RETICS %			SERUM ALB.	γ GLOB.	BILIRUBIN	ALK PHOS	SGOT	SGPT %			INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION	INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION
[illegible]	[illegible]	Alcohol	13.4	48.0	5.3	3.2	2+	0	3.4	1.8	0.7	2.1	12	27.0	72	294	[illegible]	3.7	[illegible]	-	-	-
CS	18M	-	14.4	42.5	4.2	1.0	3+	2+	3.0	1.4	0.6	3.7	41	36.5	111	330	42.8	4.9	37.9	41.0	0.9	40.1
JO	11M	-	12.1	37.0	3.7	2.2	3+	0	2.3	2.0	3.8	5.4	39	72.0	121	348	13.2	4.4	28.9	34.5	0.6	33.9
CS	16M	-	11.8	34.5	3.3	5.8	2+	2+	3.1	1.1	2.3	3.7	46	38.8	81	249	32.9	5.9	41.0	-	-	-
NO	47M	-	14.2	46.5	4.9	3.0	3+	0	2.5	1.6	1.6	8.6	7.5	29.6	99	245	34.0	5.6	34.4	47.8	1.6	46.2
CA	16F	Cryptogenic	14.2	42.0	4.9	2.4	3+	0	2.7	1.9	0.8	13.1	88	54.0	93	331	25.9	5.1	28.6	46.7	0.7	46.0
AO	10M	Alcohol	12.2	36.5	3.3	2.9	1+	0	2.2	2.8	0.1	4.2	22	[illegible]	135	221	29.6	14.7	14.9	53.8	2.8	50.2
MM*	16M	Alcohol	15.3	33.0	3.8	6.0	2+	1+	3.4	1.3	2.4	6.1	34	[illegible]	143	382	35.3	2.5	30.8	58.3	0.4	57.9
MI**	45M	-	11.5	35.0	3.7	2.2	2+	0	3.2	1.5	1.9	2.5	[illegible]	33.0	93	434	39.5	15.4	24.1	-	-	-

* Portacaval shunt 24 months before absorption studies

** Portacaval shunt 6 months before absorption studies

APPENDIX V. PRIMARY DATA IN PATIENTS WITH PORTAL CIRRHOSIS WITH DEPLETED IRON STORES

INITIALS	AGE AND SEX	AETIOLOGY OF CIRRHOSIS	BLOOD COUNTS				MARROW IRON (grade)	LIVER IRON (grade)	LIVER FUNCTION TESTS						SERUM IRON	TOTAL IBC	IRON ABSORPTION PERCENTAGE ORAL TEST DOSE			COBALT ABSORPTION PERCENTAGE ORAL TEST DOSE		
			HGB gm./100ml	PCV %	RBC 10^6	RETICS %			SERUM ALB. g/100 ml	γ GLOB g/100 ml	BILIRUBIN mg/100ml	ALK. PHOS units	SGOT units	BSP %	μg/100 ml	μg/100 ml	INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION	INTESTINAL UPTAKE	DELAYED EXCRETION	NET ABSORPTION
OG	45F	Alcohol	11.8	35.8	3.8	7.0	0	0	2.2	2.9	1.9	7.1	48	45.6	66	242	71.5	3.1	75.4	80.7	2.5	78.2
JA	47M	–	11.2	32.5	3.3	6.0	0	0	3.8	1.6	1.4	7.4	52	75.6	54	175	62.7	2.6	60.5	71.4	1.0	52.4
EH	56M	–	13.9	41.0	4.3	4.6	0	0	3.2	2.6	4.3	8.8	81	20.6	52	253	74.5	2.0	71.9	83.7	0.5	83.2
RC	58M	–	12.1	41.0	4.4	2.6	0	0	3.5	1.2	0.8	7.4	28	21.2	75	238	50.1	3.2	46.9	65.5	0.7	65.4
RO	42M	–	7.2	23.6	2.5	5.8	0	0	1.9	2.2	1.3	6.5	42	65.6	17	234	86.5	2.5	84.1	81.6	2.5	77.1
MB	52M	–	13.6	38.5	3.8	0.4	0	0	3.0	2.4	1.6	5.1	43	25.0	65	247	55.0	7.8	51.4	78.0	1.5	74.3
SM*	40M	–	12.2	36.6	3.9	2.9	0	0	3.5	2.1	2.4	4.2	67	65	78	295	62.1	2.1	60.9	80.9	0.2	80.5
PE**	67M	Cryptogenic	13.2	39.0	4.2	4.0	0	0	3.9	2.6	2.7	5.4	38	62.5	17	225	61.5	8.2	45.5	64.0	0.8	63.2

* Portacaval anastomosis 6 months before absorption tests

** Portacaval anastomosis 18 months before absorption tests

APPENDIX VI. PRIMARY DATA IN PATIENTS WITH INCREASED IRON STORES

INITIALS	AGE AND SEX	AETIOLOGY OF IRON OVERLOAD	BLOOD COUNTS: HGB (gm./100ml)	BLOOD COUNTS: PCV %	BLOOD COUNTS: RBC x10⁶	BLOOD COUNTS: RETICS %	MARROW IRON (grade)	LIVER IRON (grade)	LIVER FUNCTION TESTS: SERUM ALB. g/100 ml	LIVER FUNCTION TESTS: γ GLOB. g/100 ml	LIVER FUNCTION TESTS: BILIRUBIN mg/100ml	LIVER FUNCTION TESTS: ALK. PHOS. units	LIVER FUNCTION TESTS: SGOT units	LIVER FUNCTION TESTS: BSP %	SERUM IRON µg/100 ml	TOTAL IBC µg/100 ml	IRON REMOVED BY VENESECTION (grams)	IRON ABSORPTION (PERCENTAGE ORAL TEST DOSE): INTESTINAL UPTAKE	IRON ABSORPTION: DELAYED EXCRETION	IRON ABSORPTION: NET ABSORPTION	COBALT ABSORPTION (PERCENTAGE ORAL TEST DOSE): INTESTINAL UPTAKE	COBALT ABSORPTION: DELAYED EXCRETION	COBALT ABSORPTION: NET ABSORPTION
[illegible]	47F	Idiopathic haemochromatosis	13,3	38,0	4,4	3,6	5	5	3,5	1,2	0,9	11,7	19	2,3	176	176	23,0	55,6	3,4	53,2	75,7	8,5	75,4
[illegible]	70M		13,6	39,3	4,1	2,4	5	5	3,5	5,0	0,5	3,9	33	1,0	183	[illegible]	12,5	87,5	2,5	84,6	88,0	5,2	87,8
[illegible]*	61F	"	12,3	36,0	4,0	3,2	1+	4+	3,5	0,9	0,5	4,1	23	4,4	225	225	0	92,1	0,9	81,1	92,4	0,5	92,1
[illegible]	38M	"	12,1	42,0	4,2	7,0	4+	4+	4,0	1,9	0,6	3,8	27	6,0	200	214	8,0	58,2	25,9	17,7	82,4	2,0	80,4
[illegible]	50M	Secondary to Portal Cirrhosis	13,4	33,0	2,6	2,2	1+	4+	2,8	1,9	7,1	16,8	120	-	150	150	0	77,8	2,6	75,2	90,2	0,2	90,0
[illegible]	47F		12,4	32,5	3,5	2,6	1+	4+	3,7	1,9	0,7	2,3	13	47,2	150	218	1,2	49,0	1,4	47,6	71,0	0,3	70,9
[illegible]	77M	Sequence of events unknown	13,8	41,5	4,7	0,8	2+	4+	3,2	1,1	0,9	4,1	40	17,[illegible]	115	161	18,9	80,2	2,9	77,3	85,4	0,2	85,2
[illegible]**	78M	"	14,3	41,0	4,2	3,0	4+	4+	3,3	2,4	0,9	6,0	13	[illegible]	173	193	0**	74,5	2,6	71,9	64,1	0,5	64,6
[illegible]	41M	Exogenous	13,3	40,0	3,8	1,5	4+	4+	3,1	2,4	0,6	2,0	61	21,4	103	193	-	28,4	7,4	21,0	66,4	1,3	65,1
[illegible]	40F	"	10,8	28,0	3,0	1,0	4+	4+	2,3	1,0	0,7	10,4	150	25,0	111	173	-	6,5	4,9	4,6	22,3	0,3	21,9

* Positive family history of idiopathic haemochromatosis

** 15,5 grams of iron removed since then

ADDENDUM

EFFECT OF ALTERATIONS IN IRON METABOLISM

ON THE INTESTINAL ABSORPTION OF

MANGANESE IN MAN

The work reported in this addendum describes studies of interrelationships between the intestinal absorption of Manganese and Iron. The three metals cobalt Manganese and Iron are chemically related.

SUMMARY

An intestinal perfusion technique was used to measure the rate of absorption of manganese tagged with Mn^{54} from the duodenum and proxinal jejunum. In 8 control subjects with normal iron stores an average of 27% of the infused dose was taken up from one ml. of the infusate per min. The rate of uptake, 67%, was significantly increased in 6 patients with iron deficiency. Increased uptake was also found in 3 patients with endogenous iron overload who were iron deficient at the time of study whereas the rate was normal in one patient who had re-developed iron overload following previous venesections. In 4 patients the addition of unlabelled iron to the infusate led to a prompt decline in manganese uptake. Although the intestinal uptake of manganese was increased in iron deficiency the proportion of an oral test dose retained in the body 10 days after administration was similar to normal controls. It was also normal in patients with iron overload, and neither portal cirrhosis or fatty liver had an effect on the retention of the dose. The results also leads to increased manganese absorption show that in man increased iron absorption and they lend further support to the theory that manganese and iron

share at least part of the same intestinal absorption pathway. The absence of increased body retention of a test dose of radioactive manganese suggests that increased elimination of manganese effectively compensates for the increased absorption.

INTRODUCTION

Alterations in iron metabolism affect manganese metabolism. Iron deficiency in the rat enhances the intestinal absorption of manganese and iron overload depresses it,[1,2]. The total body half-life of radio-manganese is reduced in iron deficiency,[2,3] and a study of the proportionality between the absorption of iron and manganese in man suggests that the absorption of these metals is linked,[4]. Because manganese is excreted mainly by way of the bile into the intestine[5] investigation of its absorption is difficult and direct measurements of the rate of intestinal absorption have not been reported previously in man.

The present study was carried out to investigate the effect of alterations in body iron stores on the intestinal absorption and body retention of manganese.

METHODS AND MATERIALS

MEASUREMENT OF MANGANESE ABSORPTION

An intestinal perfusion technique[6] using a double lumen tube was employed to measure the rate of manganese absorption from a 35 cm. segment of the duodenum and proximal jejunum. After an overnight fast, the patient

swallowed a double lumen polyvinyl tube to which was attached a mercury bag[7]. A balloon was incorporated into the tube as described by Phillips and Summerskill[8] to prevent reflux of fluid proximal to the infusion site. The collecting devise on the distal end of the tube was perforated with multiple small holes and it was vented to the outside by a polyethylene tube to facilitate drainage[9]. Under fluoroscopic control, the tube was positioned in the duodenum and proximal jejunum so that the inflated balloon occluded the first part of the duodenum. A second tube was passed into the stomach through which gastric secretions drained. The position of the tubes was constantly checked during the study by fluoroscopy and in none of the studies was there appreciable movement of the tube. In all except one subject negligible amounts of radioactivity were recovered from the stomach, indicating that the balloon effectively prevented the spread of the infusate in a proximal direction in these patients. The results in the patient in whom appreciable reflux occurred were discarded.

Each 1000 ml. of infusate contained 9.1 μmoles of manganese labelled with Mn^{54}, and 9.1 μmoles of chromium tagged with Cr^{51} as a poorly absorbable marker[10,11]. The exact composition of the infusate is given in Table I. The pH of the infusate was 6.4 to 6.5 and that of the recovered fluid varied between 6.5 and 8.0.

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The test solution, warmed to 37°C, was pumped with a Harvard infusion pump at a constant rate into the duodenum just beyond the balloon at a rate of 7.2 ml. per min. To establish a steady state, perfusion was started and continued for 35 min. before samples were obtained by siphonage and occasional gentle suction. The effluent was continuously collected in 15 min. samples, for one to two hours.

Ascorbic acid, 36.4 µmoles per litre, was added to the infusate to minimize precipitation and delay the formation of insoluble complexes of either manganese or chromium in an alkaline medium. The solubility of manganese and chromium in the infusate and effluent was studied by high speed centrifugation[12]. The pH of the infusate was adjusted to 4, 5, 6 and 8 with either HCl or NaOH. No alteration in solubility of either manganese or chromium occurred over this pH range and both metals remained in solution. Centrifugation of the effluent following perfusion of the duodenum produced no precipitation of either metal.

To establish the validity of chromium as a poorly absorbed marker in patients with variations in iron absorption, 20 µmoles of Na_2CrO_4 tagged with Cr^{51} was given orally with an equivalent amount of sodium ascorbate

to 2 subjects with normal iron stores, one iron deficient patient, and 3 patients with endogenous iron overload. Between 95 and 100 per cent of the radioactivity was recovered from the feces in 10 days and negligible amounts of the administered dose were found in the urine in 5 days.

To exclude the possibility that the labelled manganese absorbed from the intestine re-entered the duodenum during the perfusion period, the infusate without the chromium marker was given intravenously to one subject at the same rate as the Mn^{54} was absorbed from the intestine in a previous study. At the same time, the duodenum was perfused with normal saline. There was no detectable radioactivity in the fluid recovered from the duodenum in 2 hours.

Radioactivity in the samples and appropriate standards of equivalent volume was measured by differential counting in a well-type scintillation counter. Samples were counted for a minimum of 10,000 counts. The intestinal uptake of Mn^{54} was calculated from the difference in radioactivity between the infusate and effluent[7] and expressed as a percentage of the infusate as shown in the following formula:

$$\text{\% } Mn^{54} \text{ uptake per ml. of infusate per min.} = 100 \times \left\{ \frac{\text{CPM } Mn^{54} \text{ per ml. infusate} - \left(\text{CPM } Mn^{54} \text{ per ml. effluent} \times \frac{\text{CPM } Cr^{51} \text{ per ml. infusate}}{\text{CPM } Cr^{51} \text{ per ml. effluent}} \right)}{\text{CPM } Mn^{54} \text{ per ml. infusate}} \right\}$$

The values for the individual 15 min. periods were averaged so the results given represent 60 min. continuous collection times for each study. The results were relatively uniform over this period and analysis of the individual periods led to similar conclusions.

MEASUREMENT OF BODY MANGANESE RETENTION

A fecal recovery method similar to that previously reported for measurement of iron absorption[13] was employed to measure manganese retention. In brief, 4 test doses of manganese were administered to a patient over a 2 day period. Each dose contained 20 μmoles of $MnCl_2 4H_2O$ tagged with 1 μc of $Mn^{54}Cl_2$ and 200 μmoles of ascorbic acid. For comparison iron retention was measured in a similar way. Four test doses of 20 μmoles of $FeCl_2 4H_2O$ with 1 μc of $Fe^{59}Cl_2$ and 200 μmoles ascorbic acid were given over 2 days. The manganese and iron studies were carried out in succession and the radioactivity in the feces was measured by differential counting of the isotopes. Standards prepared from the test doses and specimens of urine and feces were counted with

comparable geometry. The retention of manganese and iron was calculated from the difference between the administered radioactivity and that excreted in the feces. Urinary excretion of both radioisotopes was negligible. In 5 control subjects and one patient with endogenous iron overload the percentage retention of a single dose of 20 μmoles of manganous chloride was measured with a whole body counter[14].

SELECTION OF PATIENTS AND ASSESSMENT OF IRON STORES

The method of assessing iron stores by histological grading of the stainable iron in the bone marrow and in liver biopsies has been described in detail previously[15]. Patients with iron deficiency were selected on the basis of an absence of stainable iron in the bone marrow aspirate, and those with iron overload on the basis of 4+ stainable iron in the liver biopsy. Patients with normal iron stores had grades 1+ to 4+ stainable iron in the marrow.

Statistical methods were similar to those described previously[13].

RESULTS

INTESTINAL UPTAKE OF MANGANESE

Normal controls: In 8 control subjects with normal iron stores an average of 27% of the infused dose was taken up from 1 ml. of infusate per min. by the intestinal mucosa (Table 2).

Iron deficient subjects: The average rate of uptake of manganese from the infusate, 67% per ml. of infusate per min. was significantly higher than in controls ($P < 0.001$) (Fig. 1 and Table 2). The rate manganese uptake was increased in the 2 patients with portal cirrhosis and iron overload, who as a result of previous venesections were iron deficient at the time of study.

EFFECT OF IRON ON THE RATE OF MANGANESE UPTAKE

To investigate the effect of iron on manganese uptake, 91 μmoles of ferrous chloride ($FeCl_2 4H_2O$) and 182 μmoles of sodium ascorbate were added to 1000 ml. of infusate. In each of the 4 patients studied this led to a prompt decline in the rate of manganese uptake (Fig. 2). When the infusate was changed back to the original iron-free solution the manganese uptake rose towards its original rate.

To exclude the possibility that the decline in manganese uptake was an artefact due to iron forming insoluble complexes with either manganese or chromium,

both the infusate and effluent were centrifuged at high speed as described earlier. Precipitation of either chromium or manganese was observed in in either the infusate or the effluent to which cold iron had been added.

The effect of increasing body iron stores on manganese uptake was studied in an iron deficient patient. The rate of uptake; 87% of the infused dose per min., declined to 57% two weeks after treatment with 1.2 gm. of parenteral iron (Inferon, Benger Laboratories, Toronto).

MANGANESE RETENTION

In 8 control subjects an average of 8% with a range of 4 to 13% of the orally administered manganese was retained in the body in 10 days after the test dose, (Table 3). With the whole body counting technique, the average retention in another 5 control was 9% with a similar range. The mean value for iron retention in the control subjects was 28% with a range of 22 to 37%. In both iron deficiency and iron overload the retention of the test dose of manganese was within the range observed in the control group (Table 3), despite the fact t ht iron retention varied widely. Liver disease in the form of eit ker fatty infiltration or portal cirrhosis had no effect on the retention of the test dose.

DISCUSSION

A double lumen perfusion system was employed to measure manganese uptake in the duodenum, which is the major site of iron absorption. Because the infusate is known to spread in a proximal direction with this technique[16] an inflated balloon was used to occlude the first part of the duodenum. The absence of radioactivity from the gastric aspirate indicated that reflux of the infusate was negligible in the patients included in the study.

The results show that in man increased iron absorption is associated with increased manganese absorption. The inhibitory effect of iron on the rate of manganese absorption suggests that manganese and iron compete for a common absorptive pathway in the intestinal mucosa. Cobalt also inhibits iron absorption[13] and iron, manganese and cobalt probably share at least part of the same pathway.

Although manganese absorption was elevated in iron deficiency, the body retention of an oral dose was not increased. This is probably explained by the increased elimination of radiomanganese from the body in iron deficiency,[2,3] which compensates for the increased absorption.

The intestinal uptake of manganese from the duodenum was not studied in exogenous iron overload. Nevertheless the normal retention of the oral dose in a patient with this disorder suggests that either absorption from the gastrointestinal tract as a whole is not impaired, or it is reduced and there is a compensatory diminution in the elimination of radiomanganese from the body similar to that observed in the rat with iron overload[2].

The interpretation of the increased manganese absorption in endogenous iron overload is complicated. In 3 of the patients, body iron stores had been depleted by venesections and it is not possible to discern whether the increased manganese uptake was due to iron deficiency or to the underlying disorder in iron absorption. In one patient, E.G., who had redeveloped iron overload following discontinuation of venesections 3 years previously, the rate of manganese absorption was normal. The explanation of this finding is unknown and it requires confirmation before significance can be attached to it. The failure to find increased manganese^{54} retention in the patients with increased absorption is probably explained by increased excretion of the test dose from the body similar to that observed in iron deficiency[3].

Butt and co-workers[17] and Alstatt et al.[18] found an average of approximately 0.4 mg. per 100 gm. dry weight of manganese in the liver of patients with idiopathic hemochromatosis. The former attributed no significance to this because similar levels were present in patients with various other diseases including cirrhosis. However, the latter considered the levels abnormal because only one half as much manganese was found in the livers of a control group of patients dying of myocardial infarction. In comparison to iron levels, the difference between manganese in the two groups is small, and unlikely to be of any clinical importance.

The results of this study and previous investigations in both man[4, 13, 15] and the rat[1, 2, 19, 22] indicate that iron, cobalt and manganese share at least part of a similar absorptive pathway in the upper small intestine. However, the regulation of body levels and mode of excretion from the body is quite different. The capacity to excrete iron is negligible and iron is maintained within narrow limits in the body by the control of intestinal absorption[21]. In contrast, excretion plays a major part in the control of body manganese levels. The intestinal lumen constitutes the main excretory route

and tissue levels are kept constant primarily by control of excretion of manganese in the bile[5]. However, the rate at which manganese can be excreted from the body is limited and manganese poisoning has been reported in miners exposed for long periods to manganese dust[22]. The kidney is the major route for the elimination of cobalt[15] but small amounts are excreted by way of the gastrointestinal tract[13]. Unlike both iron and manganese, neither ionic or loosely-bound inorganic cobalt appears to be required in the body[23] and it is doubtful whether the level in the body is regulated by the control of either absorption or excretion.

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TABLE I

COMPOSITION OF THE INFUSATE

CONSTITUENTS		CONCENTRATION PER 1000 ml.
$InCl_2 4H_2O$		9.1 μmoles
$In^{54}Cl_2$	(carrier free. Amersham-Searle, Toronto).	25 μC
Na_2CrO_4		9.1 μmoles
$Na_2Cr^{51}C_4$	(S.A. 130 ... per μg. Abbott Laboratories, Chicago, Ill.)	60 μC
NaCl		150 mmoles
Sodium Ascorbate		36.4 μmoles

TABLE 2. RELATIONSHIP BETWEEN BODY IRON STORES AND RATE OF INTESTINAL UPTAKE OF MANGANESE

GROUP	NUMBER OF PATIENTS OR INITIALS	AGE IN YEARS mean and range	HEMOGLOBIN (gm/100 ml) mean and range	MARROW IRON	LIVER IRON	IRON REMOVED BY VENESECTION (grams)	UPTAKE OF Mn^{54} PER MIN. FROM 1 ml OF INFUSATE (% of infused dose) mean ± SD with range
Normal Controls	8	53 (31 - 89)	14.2 (12.1 - 16.0)	3+ - 4+	-	-	27 ± 8 (19 - 38)
Iron Deficiency	6	67 (53 - 89)	11.9 (9.3 - 14.8)	0	-	-	67 ± 17 (47 - 91)
Endogenous Iron Overload							
Idiopathic Haemochromatosis	V.C.	67	12.7	0	0	23	44
	E.G.	62	13.6	4+	4+	32	22
With Portal Cirrhosis Sequence of events Uncertain	R.B.	65	12.0	0	0	15	55
	J.S.	61	14.6	0	0	18	48

TABLE 3. MANGANESE AND IRON RETENTION

GROUP	NO. OF PATIENTS OR INITIALS	AGE (years)	HEMOGLOBIN (gm/100 ml) mean and range	MARROW IRON	LIVER IRON	IRON REMOVED BY VENESECTION (gm)	% ORAL TEST DOSE RETAINED Mean ± SD and Range	
							MANGANESE	IRON
NORMAL IRON STORES								
(a) Controls	8	36 - 83	13.9 (12.2 - 15.3)	1+ - 4+	-	-	8 ± 3.4 (4 - 13)	28 ± 5.4 (22 - 37)
(b) Fatty infiltration of Liver	4	50 - 67	13.5 (13.0 - 13.9)	3+	0 - 3+	-	7 (4 - 9)	25 (22 - 29)
(c) Portal Cirrhosis	3	50 - 67	12.1 (12.0 - 12.4)	1+ - 4+	0	-	10 (3 - 13)	21 (17 - 29)
DEPLETED IRON STORES								
(a) Controls	5	43 - 89	11.9 (5.1 - 14.0)	0	-	-	8 ± 2.9 (4 - 13)	68 ± 11[a] (53 - 82)
(b) Portal Cirrhosis	5	45 - 68	12.2 (11.2 - 13.2)	0	0	-	9 ± 1.8 (7 - 12)	57 ± 17[a] (41 - 78)
ENDOGENOUS IRON OVERLOAD								
(a) Idiopathic Hemochromatosis	V.C.	66 F.	13.3	0	0	23	12	55
	E.G.	60 M.	13.6	4+	4+	32	4.0	85
	J.W.	58 M.	12.1	4+	4+	7.5	11	37
(b) Secondary to Portal Cirrhosis	V.D.	67F.	13.4	1+	3+	2.2	13	48
(c) Associated with Cirrhosis but Sequence of Events Unknown	J.S.	61 M.	12.6	0	0	18.0	8.4[a]	84
EXOGENOUS IRON OVERLOAD	P.D.	85 M.	8.0	4+	4+	-	7.0	6.4

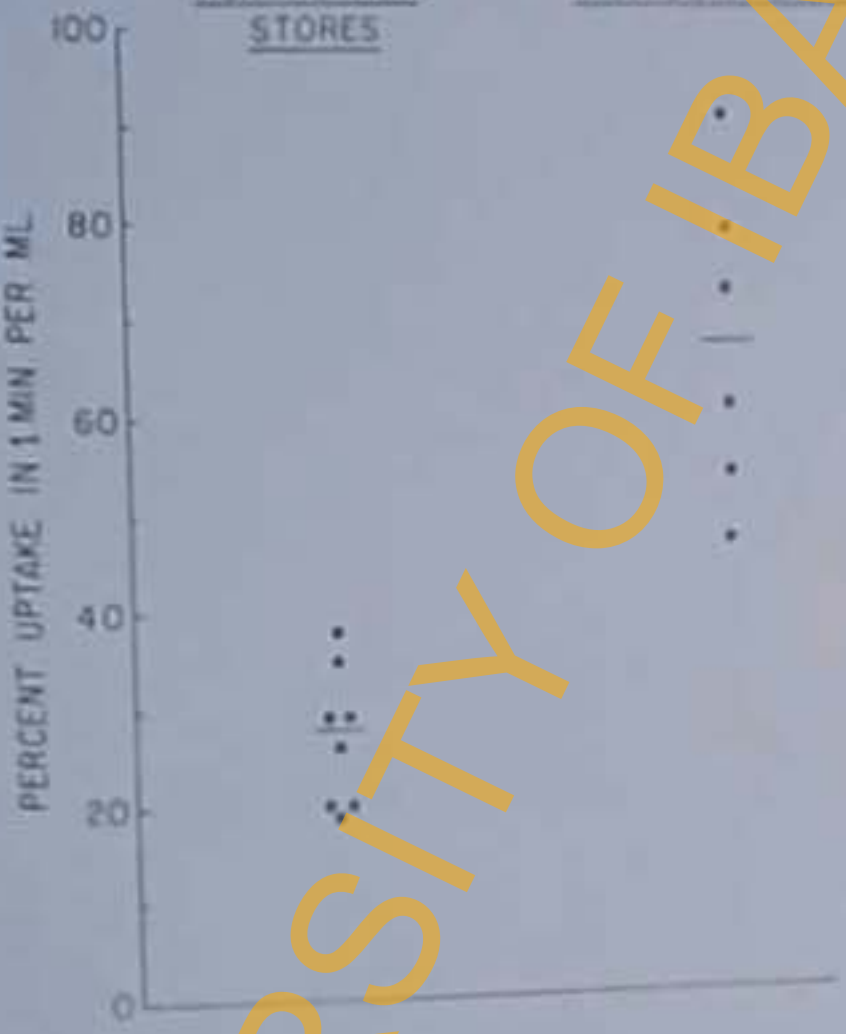

FIGURE 1. INTESTINAL UPTAKE OF MANGANESE II. NORMAL SUBJECTS AND PATIENTS WITH IRON DEFICIENCY.

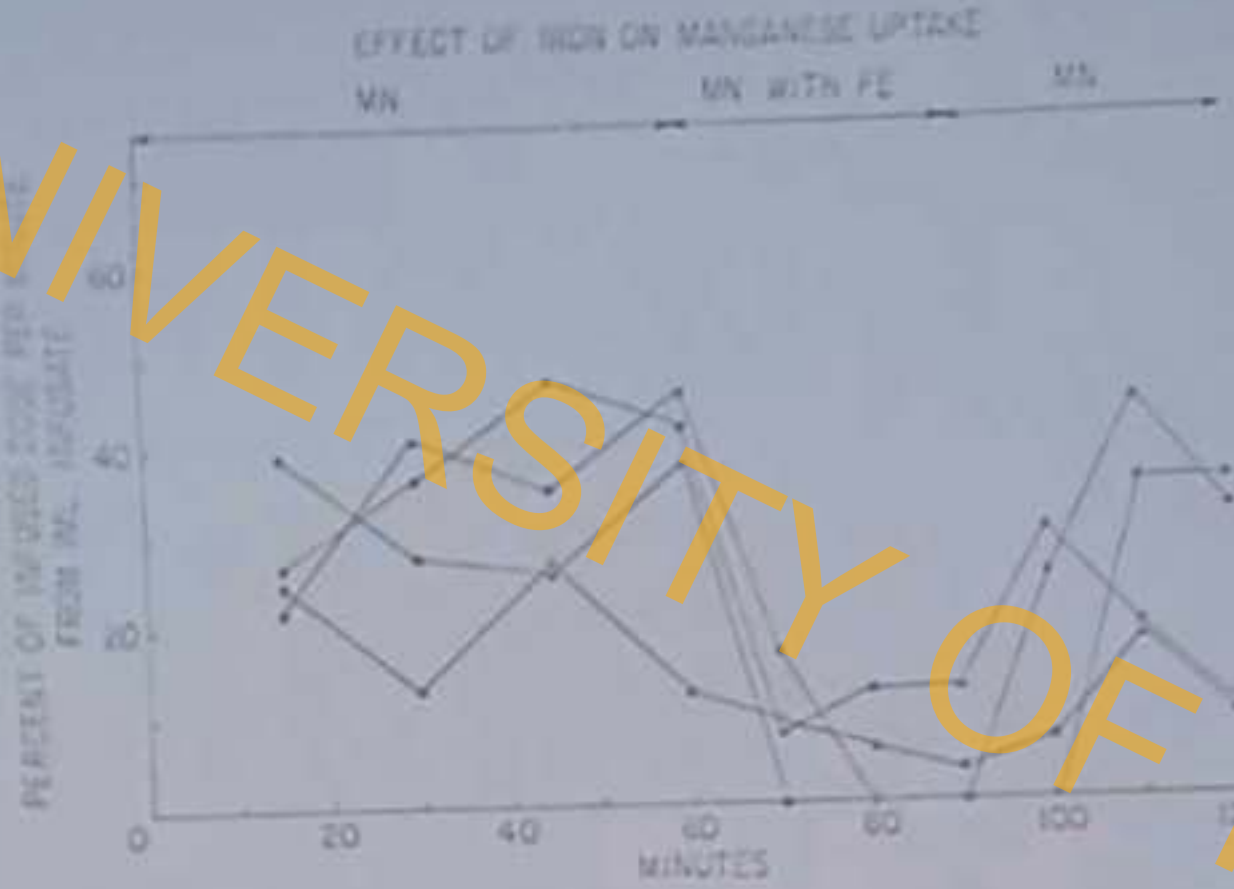

FIGURE 2. EFFECT OF IRON ON THE RATE OF INTESTINAL UPTAKE OF MANGANESE.

PAPERS PUBLISHED AS A RESULT OF WORK PRESENTED IN THIS THESIS

REPRINT 1: VALBERG, L.S., J. LUDWIG and D. OLATUNBOSUN. Alteration in cobalt absorption in patients with disorders of iron metabolism. Gastroenterology, 56, 241 - 251, 1969.

REPRINT 2: OLATUNBOSUN, D., J. LUDWIG, W.E.N. CORBETT and L.S. VALBERG. Alteration in cobalt absorption in portal cirrhosis and idiopathic hemochromatosis. J. Lab. Clin.Med. 75, 754 - 762, 1970.

REPRINT 3: OLATUNBOSUN, D., J. LUDWIG, W.E.N. CORBETT, J.B. SIMON and L.S. VALBERG. The significance of alterations in iron absorption in portal cirrhosis. Submitted for publication in Gastroenterology, 59, 188 - 199, 1970.

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ALTERATION IN COBALT ABSORPTION IN PATIENTS WITH DISORDERS OF IRON METABOLISM

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In 12 normal control subjects given four oral doses of 20 μmoles of radioactive ferrous chloride or an equimolar amount of radioactive cobaltous chloride followed by an unabsorbable marker, the average excretion of radioactivity in the feces prior to disappearance of the marker was 64 and 54%, respectively. An average of 9% of the iron and 2% of the cobalt was lost in the feces after the marker was passed. The average absorption of iron, 27%, was significantly less than the absorption of cobalt, 44%. The results suggest that the intestinal mucosal uptake of cobalt is slightly greater than that of iron, and cobalt, unlike iron, is not sequestered in the mucosa and subsequently lost with desquamation of the intestinal epithelium. In 6 patients with iron deficiency, the average absorption of iron and cobalt, 60 and 71%, respectively, was significantly increased. A direct correlation was observed between the absorption of iron and cobalt in the control and iron-deficient subjects. In 5 patients with exogenous iron overload, the average percentage absorption of iron, 10%, was markedly reduced whereas the absorption of cobalt was within the range observed in control subjects. The average absorption of both iron and cobalt was increased in 2 patients with cirrhosis associated with iron overload and in 4 patients with idiopathic hemochromatosis. These results demonstrate that cobalt absorption is responsive to the physiological mechanisms that enhance iron absorption but not to those that inhibit it.

The electron configurations of iron and cobalt are very close. Each element has two electrons in the outer shell and 14 and 15 electrons, respectively, in the next inner shell; the ionic radii of the elements are similar and both tend to co-ordinate with six ligands to form octahedral complexes. It has been shown recently that the intestinal absorption of cobalt is increased in rats rendered iron deficient by

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either bleeding or diet,[1] but investigation of cobalt absorption has not been undertaken previously in patients with disorders of iron metabolism nor has the biological significance of the observations in the rat been elucidated.

The present study was carried out to determine whether a similar mechanism is involved in the absorption of iron and cobalt in man and to discern whether alterations in cobalt absorption occur in iron deficiency and in iron overload.

Methods

Preparation of iron and cobalt test solutions. The test solution of iron contained 20 μmoles (about 1 mg) of iron in the form of $FeCl_2 4H_2O$ and 1 μc of $Fe^{59}Cl_3$ (SA 20-25 μc per μg) dissolved in 100 ml of 0.01 N HCl, prepared from concentrated HCl and iron-free water. The cobalt solution contained 20 μmoles of cobalt in the form of $CoCl_2 6H_2O$ and 1 μc of $Co^{60}Cl_2$ (SA 100 to 250 μc per μg) or $Co^{57}Cl_2$ (SA 6.2 μc to μg) dissolved in 100 ml of 0.01 N HCl. An excess of ascorbic acid, 200 μmoles, was added to each of the iron and cobalt solutions just prior to administration to maintain the elements in the reduced state.

Administration of test solutions. In the initial studies absorption tests were carried out on subjects who had fasted overnight employing one dose of the test solution. Nothing further was given by mouth for 2 hr. Fecal collections were terminated when less than 1% of the dose was found in two consecutive specimens. The majority of patients had one to two bowel motions daily and the average duration of fecal collections was 7 days, with a range of 5 to 10 days. In subsequent studies, four doses of either iron or cobalt were given to subjects who had fasted overnight at 9 AM and 4 PM on 2 successive days and a light meal was given at 11 AM and 6 PM.

In the studies employing one test dose, the proportion of the element that was lost by delayed excretion due to normal desquamation of the intestinal epithelium was not assessed, but in the studies in which four test doses were given the latter was measured with an unabsorbable marker, carmine red 1.0 g. given 2 hr after the last test dose.[2] The presence of the marker in the feces was assessed by visual inspection and its disappearance was used to identify the point at which the delayed excretion of the element commenced. Fecal collections were continued for a minimum period of 10 days and up to 14 days if >0.2% radioactivity was detectable in a stool specimen. The majority of the patients given the four test doses had one to two bowel motions daily. Radioactivity usually appeared in the stools within 48 hr, reached a maximum in 2 to 4 days, and then declined to levels of approximately 1% daily between days 5 and 8. The radioactive cobalt quickly fell to levels less than 0.2% daily between days 6 and 9 in both the iron-replete and iron-deficient subjects. A similar pattern was observed with radioactive iron in iron deficiency but in iron-replete subjects excretion continued and in many there was a subsequent increase to levels of 1 to 3% daily between days 7 and 9. The radioactive iron in the stool specimens then declined to levels less than 0.2% between days 10 and 14.

The absorptive studies were carried out in the Special Investigation Unit of the Kingston General Hospital to assure completeness of fecal collections. To avoid urinary contamination of feces the patients were advised to urinate prior to defecation and specially designed commode chairs were used that made it difficult for them to inadvertently pass urine into the fecal containers.

Measurement of radioactivity in feces. The radioactivity in individual stool specimens was measured in a large well-type scintillation detector as described previously.[3] In studies in which test doses of iron and cobalt were given simultaneously, the elements were determined by differential counting in a large volume detector equipped with two 5-inch sodium iodide crystals.

Expression of results. In the studies employing one test dose, the results are given as the percentage of the oral dose excreted in the feces. In the comprehensive test in which four doses were administered with carmine red the results are expressed as follows: (a) the percentage of the oral dose excreted prior to the disappearance of the marker from the stools; (b) the percentage of the dose excreted after the marker and; (c) the percentage of total radioactivity recovered in the feces.

The proportion of iron and cobalt lost with desquamation of the intestinal epithelium was estimated from the radioactivity lost in the feces after disappearance of the marker. This estimation is based on three assumptions: first, there was no significant separation of the radioactivity and the marker as they passed down the gastrointestinal tract; second, the disappearance of carmine red identified the

point at which delayed excretion of the element due to desquamation of the epithelium commenced; and third, there was no significant excretion in the feces of radioactivity lost from the body into the gastrointestinal tract during this period. The validity of the first two assumptions was evaluated with the unabsorbable substance, chromic chloride. A solution containing 1.4 nmoles of $CrCl_3$ and 1 μc of $Cr^{51}Cl_3$ (SA 13 to 15 μc per μg) dissolved in 100 ml of 0.01 N HCl was given by mouth in four test doses followed 2 hr later by a carmine red marker. In 10 subjects, the majority of whom had at least one bowel motion daily, the marker appeared in an average of 3 days (1 to 6 day range) and disappeared in an average of 7 days (4 to 9 days). An average of 98% (91 to 102%) of the radioactivity was passed in the feces with the marker demonstrating that there was no significant separation of the radioactivity and the marker as they passed down the gastrointestinal tract. An average of 2.4% (0.2 to 5.7%) of the radioactivity was excreted in the feces after the marker disappeared, indicating that visual inspection of the stool was adequate for identifying the point at which the marker disappeared.

The proportion of iron and cobalt that was taken up by the intestinal mucosa was determined as follows: 100 minus the percentage of radioactivity excreted in the feces prior to disappearance of the marker.

The absorption of the test dose was calculated in the following way: 100 minus the percentage of total radioactivity recovered in the feces.

Selection of normal subjects. The control subjects fulfilled the following criteria: no evidence of disease of the hematological, hepatic, pancreatic, or gastrointestinal system or active disease in other organs; hemoglobin, hematocrit, red blood cell count, and blood smear within normal range; normal amounts of stainable iron in a bone marrow aspirate; serum iron and unsaturated iron-binding capacity within the normal range; and normal gastric acid secretion following maximal histamine stimulation. The serum iron and unsaturated iron-binding capacity were measured by a method previously described.[4]

Selection of iron-deficient subjects. Iron depletion was established on the basis of an absence of stainable iron in a bone marrow aspirate. In 3 of the patients the etiology of the iron deficiency was unknown and in the others it was on the basis of blood loss. None of the patients was bleeding at the time of study.

Selection of patients with exogenous iron overload. Eight patients were studied, 5 with iron overload secondary to the treatment of refractory anemia with iron therapy and blood transfusion and 3 with a previous history of anemia which had been treated with excessive amounts of oral and parenteral iron. A marked increase in stainable iron was present in the bone marrow aspirate in the 5 patients where this examination was carried out and a marked increase in stainable iron in the liver biopsy was present in the 5 patients with refractory anemia.

Selection of patients with endogenous iron overload. Iron overload was established by the finding of a marked increase in stainable iron in both parenchymal and Kupffer cells in histological sections of a liver biopsy and by the subsequent removal of excess iron by venesection. Two of the patients developed iron overload after a portacaval shunt was carried out for repeated bleeding from esophageal varices and in both of these portal cirrhosis preceded the iron overload.

Four patients were classified as idiopathic hemochromatosis on the basis that they had had respectively 18, 32, 14, and 9 g of iron removed by venesection over the preceding 2 to 15 years. Two of them had drunk beer and spirits excessively prior to the diagnosis of iron overload but in the other two there was no previous history of alcoholic excess. One patient was iron depleted at the time that the absorption test was carried out.

Results

Excretion of Cobalt into the Gastrointestinal Tract

The excretion of cobalt from the body into the gastrointestinal tract was studied in 6 control subjects after the intramuscular injection of 10 μmoles of cobalt tagged with 1 μc of Co^{58}. Surface counting demonstrated that 80% of the cobalt was absorbed from the injection site within 6 hr and 90% was taken up within 24 hr. In 5 days an average of 0.44 μmoles appeared in the feces and 5.2 μmoles appeared in the urine (table 1). In 10 days a total of 0.58 μmoles was found in the feces and 5.8 μmoles were present in the urine. The finding of 4.4% of the injected dose in

TABLE 1. *Excretion of Co^{58} in urine and feces of 6 control subjects after parenteral administration of 10 μmoles of cobaltous chloride**

Days	Urine		Feces	
	μmoles	% injected dose	μmoles	% injected dose
1-5	5.2 (3.4-7.8)	52.0	0.44 (0.28-0.64)	4.4
6-10	0.57 (0.44-0.74)	5.7	0.14 (0-0.[illegible])	1.4
Total (1-10)	5.8 (4.1-8.5)	57.7	0.58 (0.41-0.[illegible])	5.8

* Mean and range of values.

the feces in the first 5 days demonstrates that the loss of cobalt from the body by way of the feces is small and estimation of radioactivity in the feces after giving an oral dose of cobalt provides a valid measurement of gastrointestinal absorption.

Effect of Ascorbic Acid on Iron and Cobalt Absorption

Two successive tests employing one test dose were carried out with iron and cobalt in control subjects and patients with iron deficiency and endogenous iron overload. Ascorbic acid, 200 μmoles, was randomly added to one of the iron and one of the cobalt tests. It will be seen in figure 1 that ascorbic acid consistently reduced the amount of iron excreted in the feces. In the control subjects the average excretion was 85% with ascorbic acid and 52% without it. Ascorbic acid had no appreciable effect on the fecal excretion of cobalt; the average excretion in the control subjects was 50% with and 46% without the addition of the vitamin.

Reproducibility of Iron and Cobalt in Which One Oral Dose Was Given

To evaluate the reproducibility of the technique, two successive tests were carried out with iron and cobalt respectively in 6 control subjects and 4 patients with disorders of iron metabolism. The average excretion of iron in the two successive tests in the group as a whole was 40.5 and 42.8%, respectively, and the average results for cobalt were 30.7 and 30.4%, respectively. The coefficient of variation calculated from the difference between duplicates was 8.6% for iron and 4.4% for cobalt. The agreement between the duplicates was good except in 2 patients in whom the results for iron excretion differed by 14 and 18%, respectively.

Fecal Excretion of Iron and Cobalt Following Four Oral Doses Given with an Unabsorbable Marker

Normal controls. In 12 controls subjects the average excretions of iron and cobalt with the carmine marker were 64 and 54%, respectively, and 9.0% of the iron compared to 2.0% of the cobalt was excreted after the dye disappeared from the feces ($P < 0.01$) (table 2). The magnitude of the delayed excretion of cobalt, 2.0%, was similar to the average value of 2.4% obtained with the unabsorbed element, chromic chloride. The average absorption of iron, 27%, was significantly less than the average absorption of cobalt, 44% ($P < 0.01$).

Iron-deficient subjects. In comparison with the controls, the 6 iron-deficient subjects excreted with the marker an average of 37% of the iron ($P < 0.01$) and 27% of the cobalt ($P < 0.01$) and the average excretion following the marker was 3.0% of the iron and 1.8% of the cobalt (table 2). The average absorption of iron and cobalt, 60 and 71%, respectively, was significantly increased ($P < 0.01$). The difference between the mean absorption of iron and cobalt was just statistically significant ($P < 0.05$).

Exogenous iron overloaded subjects. The average percentage of iron excreted

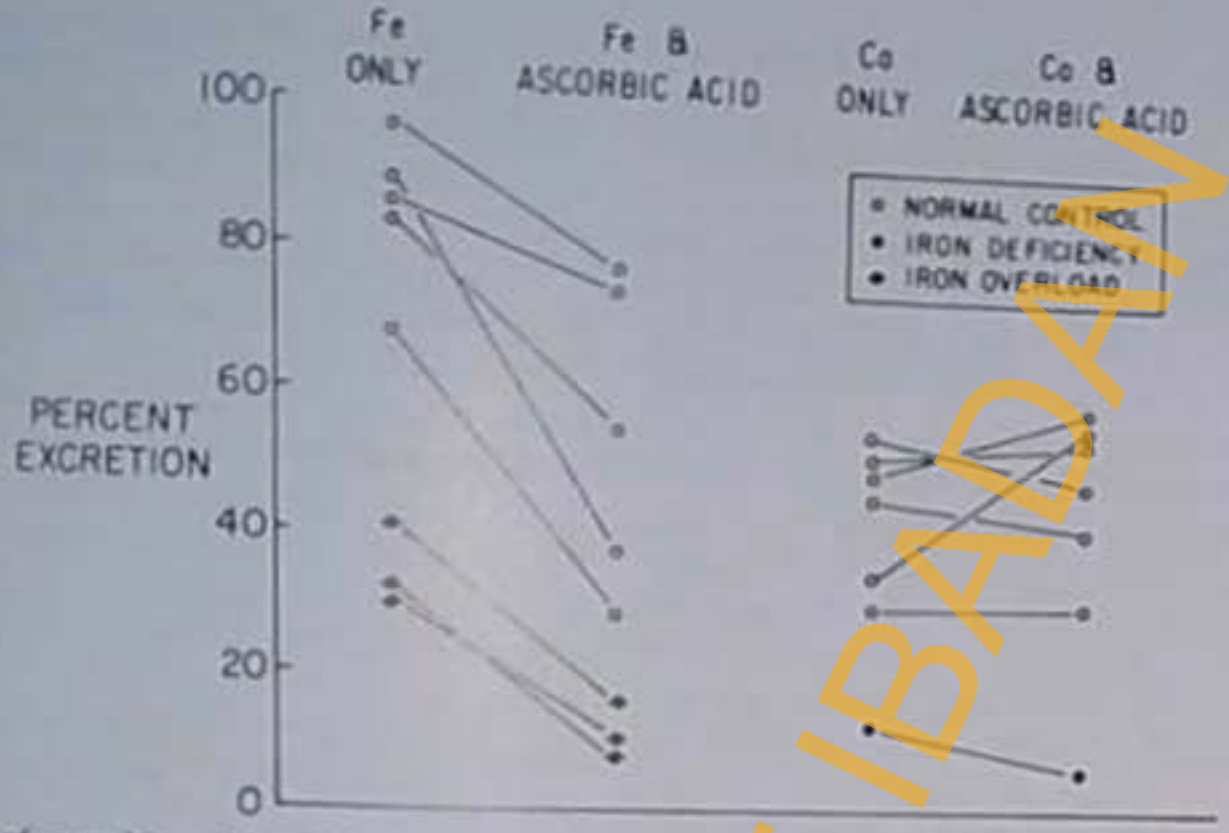

FIG. 1 Effect of ascorbic acid on fecal excretion of iron and cobalt following a single oral test dose.

TABLE 2 *Fecal excretion and absorption of iron and cobalt following four oral doses given with an unabsorbable marker in normal iron repletion, iron deficiency, and exogenous iron overload*[a]

Condition	No. of subjects	Test dose	Percentage of excretion of radioactivity in the feces			Percentage of absorption of radioactivity
			With marker	After marker	Total	
Normal iron repletion	12	Iron	64 ± 7.7 (51-69)	9.0 ± 4.4 (5-20)	73 ± 6.6[c] (58-78)	27 ± 6.6 (22-42)
	12	Cobalt	54 ± 7.5[c] (40-64)	2.0 ± 1.5[c] (0.3-4.0)	56 ± 8.6 (44-69)	44 ± 7.8[c] (31-56)
	10	Chromium	98 ± 3.5 (91-102)	2.4 ± 1.7 (0.2-5.7)	100 ± 3.5 (91-105)	0
Iron deficiency	6	Iron	33 ± 6.7[c] (28-44)	3.0 ± 2.0[c] (1.0-7.0)	40 ± 8.2[c] (29-49)	60 ± 8.2[c] (51-71)
	6	Cobalt	27 ± 11.8[c,d] (13-39)	1.8 ± 0.88 (1.0-3.4)	29 ± 12.5[c,d] (14-41)	71 ± 12.5[c,d] (59-86)
Exogenous iron overload	5	Iron	82 ± 5.7[c] (73-87)	7.4 ± 1.9 (5.0-10.0)	90 ± 5.0[c] (83-94)	10 ± 5.0[c] (6-17)
	5	Cobalt	50 ± 7.4[c] (44-62)	1.3 ± 0.86[c] (0.4-2.7)	51 ± 7.8[c] (44-63)	49 ± 7.9[c] (37-56)

[a] Mean value with range and standard deviation. Where *b*, *e* and *d*, *e* are not given as superscripts, the difference between the mean value for this group and the comparable mean value for the control group, and the difference between the mean value for iron and cobalt in this group are not statistically significant, $P > 0.05$.

[b] Difference between mean value for this group and mean value for control (normal iron repletion) is statistically significant; $b = P < 0.05$, $e = P < 0.01$.

[c] Difference between mean value for iron and cobalt in this group is statistically significant; $d = P < 0.05$, $e = P < 0.01$.

with the marker, [illegible] was markedly increased in the 5 subjects ($P < 0.01$), whereas the average percentage excreted after the marker disappeared, 7.4%, was within the range observed in the controls (table 2). The percentage of excretion of cobalt both with and after the marker was similar to the values in the control subjects. The average absorption of iron, 10%, was significantly reduced ($P < 0.01$),

whereas the absorption of cobalt was normal.

Pattern of excretion of iron and cobalt. To investigate further the difference between the intestinal handling of iron and cobalt, the pattern of daily fecal excretion of radioactivity was studied in a control subject and a patient with iron overload associated with refractory anemia given eight alternate doses of iron and cobalt over a period of 4 days followed by a carmine marker. The results depicted graphically in figure 2 show that in the control subject the cumulative excretion of iron and cobalt in the feces was similar up until the time the marker disappeared on the 6th day, but the excretion of iron, unlike cobalt, continued for another 6 days. In the iron-overloaded subject, 85% of the iron and 40% of the cobalt were excreted with the marker and there was a delayed excretion of 9 and 0% of the iron and cobalt, respectively.

Relationship between mucosal uptake of iron and cobalt. In 10 control subjects and 6 iron-deficient patients in whom both iron and cobalt absorption tests were carried out with four test doses, a linear relationship was observed between the mucosal uptake of iron and cobalt, $r = 0.884$ ($P < 0.01$). The intercept of the least squares line was at 6% on the cobalt axis (fig. 3). The data on the patients with exogenous iron overload did not follow this relationship.

Relationship between absorption of iron and cobalt. In the 10 control subjects and 6 iron-deficient subjects, a linear relationship was also observed between the absorption of iron and cobalt, $r = 0.853$ ($P < 0.01$). The intercept of the least squares line was at 13% on the cobalt axis. The results in patients with exogenous iron overload did not follow this relationship.

Fecal Excretion of Iron and Cobalt Following a Single Oral Dose

Normal controls. In 12 subjects the amount of unabsorbed iron in the feces ranged from 26 to 82% with a mean of 56% and the amount of cobalt ranged from 32 to 72% with a mean of 51% (table 3). The average duration of fecal collection for the iron and cobalt tests was similar, 7.0 and 6.5 days, respectively. The shorter duration of fecal collections in this study failed to pick up the delayed excretion of iron and this masked the significant difference between iron and cobalt absorption ob-

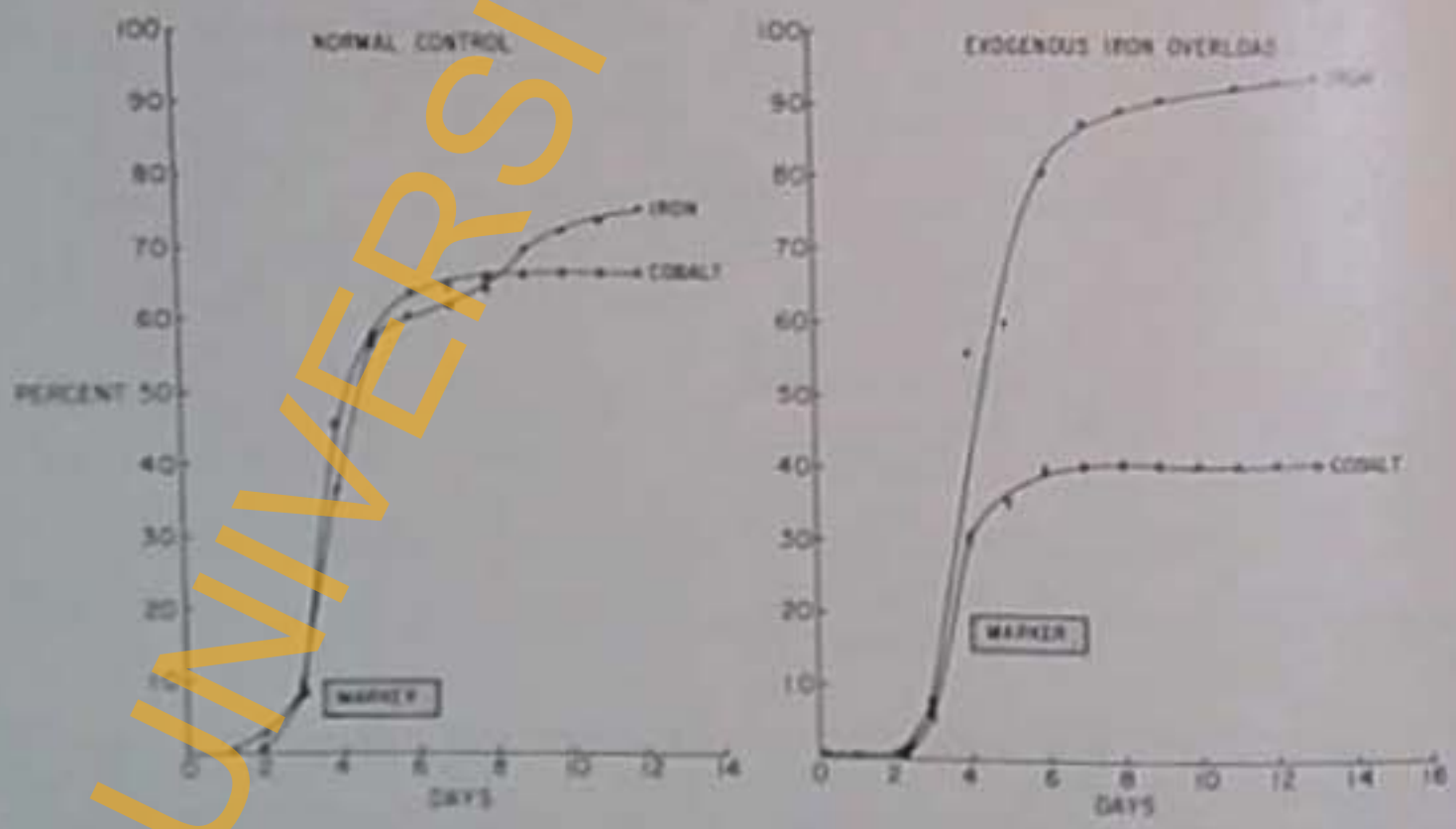

FIG. 2. Pattern of excretion of iron and cobalt in the feces in a normal control subject and a patient with exogenous iron overload following oral test dose given with unabsorbable marker.

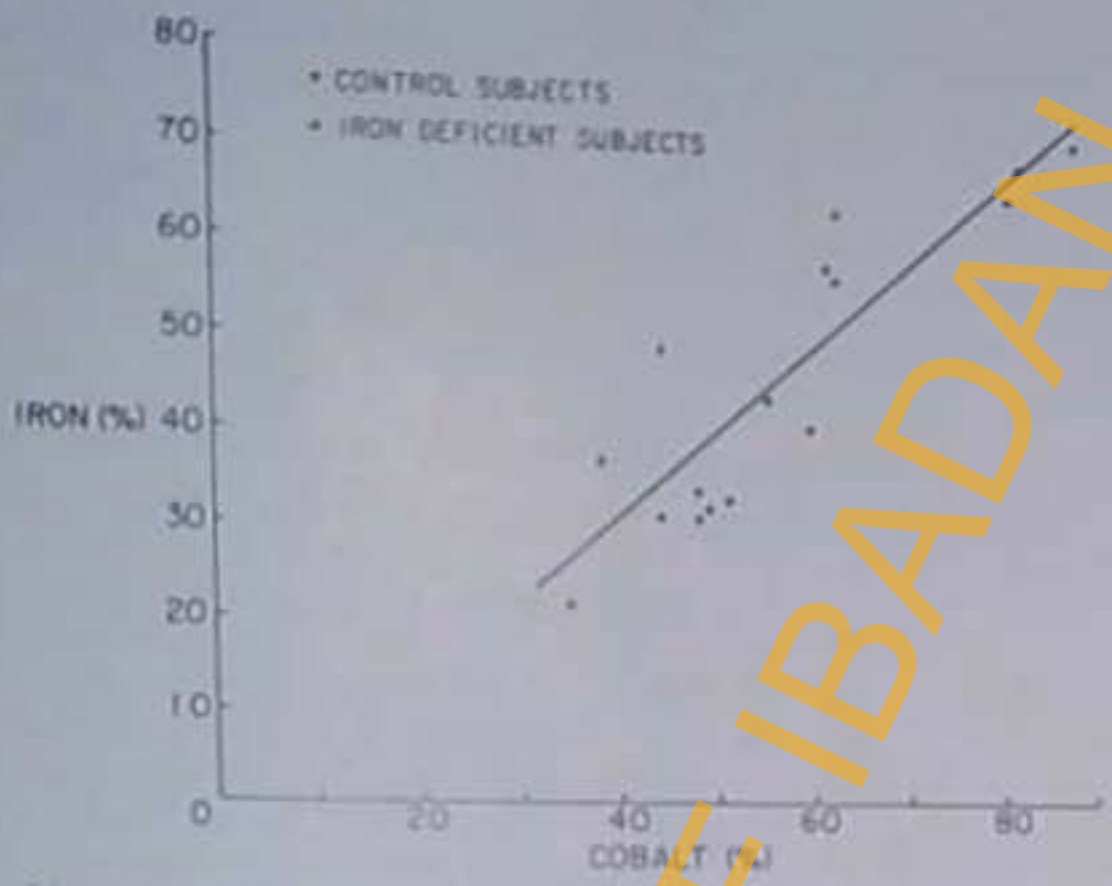

FIG. 3. Relationship between mucosal uptake of iron and cobalt in normal iron repletion and iron deficiency.

TABLE 3. *Fecal excretion of iron and cobalt following a single oral dose in normal iron repletion, iron deficiency, and exogenous iron overload*

Condition	No. of subjects	Test dose	Duration in days of fecal collection	Percentage excretion of radioactivity in feces
Normal iron repletion	12	Iron	7.0 (6-8)	50 ± 18 (26-82)
	12	Cobalt	6.5 (5-8)	51 ± 14 (32-72)
Iron deficiency	8	Iron	7.0 (6-9)	18 ± 11* (6-39)
	8	Cobalt	6.5 (5-8)	22 ± 9.9* (14-45)
Exogenous iron overload	6	Iron	7.5 (6-11)	95 ± 4.0*,† (91-100)
	3	Cobalt	7.0 (5-10)	52 ± 6.8 (46-63)

* Mean value with range and standard deviation. Where *b*, *c* and *d*, *e* are not given as superscripts, the difference between the mean value for this group and the comparable mean value for the control group, and the difference between the mean value for iron and cobalt in this group are not statistically significant, $P > 0.05$.

* - Difference between mean value for this group and mean value for control (normal iron repletion) is statistically significant: *b* — $P < 0.01$, *c* — $P < 0.01$.

* - † Difference between mean value for iron and cobalt in this group is statistically significant: *d* — $P < 0.05$; *e* — $P < 0.01$.

served in the study (table 2) in which feces were collected for 10 to 14 days.

Iron-deficient subjects. The average levels of unabsorbed iron and cobalt, 18 and 22% respectively, were significantly reduced in the 8 subjects ($P < 0.01$) (table 3).

Exogenous iron-overloaded subjects. There was a marked increase in the average proportion of unabsorbed iron in the feces of the 5 patients ($P < 0.01$) but the average amount of cobalt that was unabsorbed was similar to the absorption in normal controls (table 3).

Effect of Carrier Iron on the Fecal Excretion of Cobalt

The effect of 80 μmoles of iron on the fecal excretion of a single test dose of 20 μmoles of cobalt in 6 control subjects and

9 patients with iron deficiency is shown in figure 4. In all but one there was an increase in cobalt excretion. Statistical analysis of the results employing paired observations demonstrated that the average increase, 18%, was significant ($P < 0.01$).

Effect of Carrier Cobalt on Fecal Excretion of Iron

When 80 μmoles of cobalt were given with 20 μmoles of iron, there was a significant increase in iron excretion in 6 iron-deficient and 3 control subjects, $P < 0.01$ (fig. 4). The average increase in excretion, 17%, was similar to that observed when carrier iron was added to the cobalt test solution.

Absorption of Iron and Cobalt in Endogenous Iron Overload

In 2 patients with portal cirrhosis of the liver associated with iron overload, the absorption of both iron and cobalt was increased above the normal range (table 4). In 4 patients with idiopathic hemochromatosis, the average absorption of iron and cobalt was significantly increased ($P < 0.01$ and $P < 0.01$, respectively), but the individual values for iron fell within the upper limits of the normal range in 2 of the subjects.

Discussion

Toxic effects have been reported from the administration of large therapeutic doses of cobalt chloride for the treatment of anemia[6] and there had been speculation that recent deaths due to cardiohepatic failure in beer drinkers may have been due to the addition of cobalt to beer.[7, 8] The tracer doses of cobalt employed in this study were miniscule compared with the amounts ingested by these patients and no untoward effects were encountered.

The enhancing effect of ascorbic acid on iron absorption has been ascribed to the

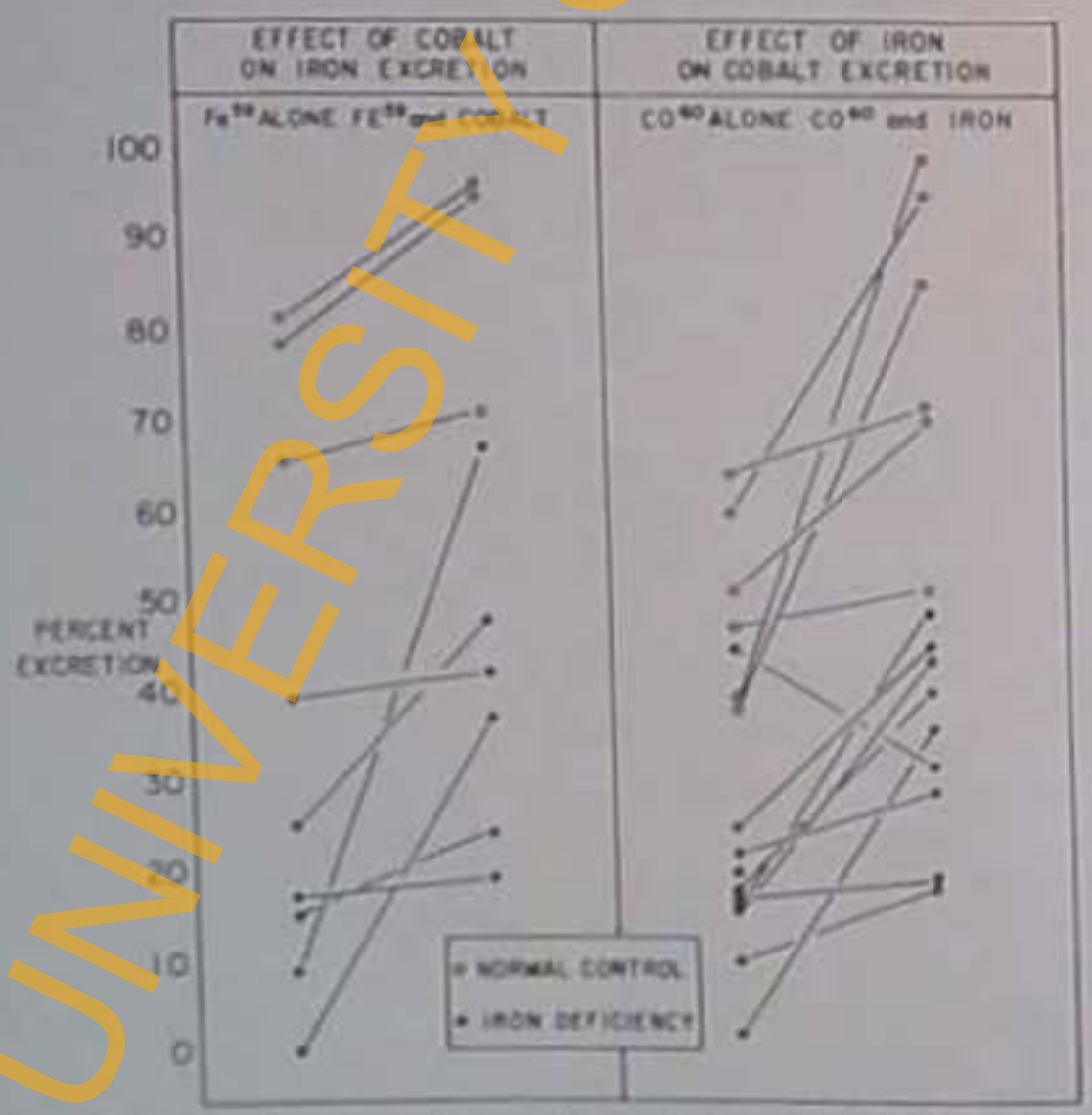

FIG. 4. Effect of carrier iron and carrier cobalt on the fecal excretion of cobalt and iron following a single oral test dose.

TABLE 1. *Absorption of iron and cobalt following four oral doses in patients with endogenous iron overload*

Condition	No. of subjects	Percentage of intestinal absorption	
		Iron	Cobalt
Normal control	8	27 ± 6.3 (22–42)	41 ± 5.3 (32–49)
Cirrhosis and portocaval shunt	2	61[a] (48–75)	81[a] (71–90)
Hepatic hemochromatosis	1	63 ± 21[a] (37–84)	83 ± 3.7[a] (79–84)

[a] Mean value with range and standard deviation. Where *b*, *c* are not given as superscripts, the difference between the mean value for this group and the comparable mean value for the control group, and the differences between the mean value for iron and cobalt in this group are not statistically significant, $P > 0.05$.

[b, c] Difference between mean value for this group and mean value for control is statistically significant: $b = P < 0.05$; $c = P < 0.01$.

effect of a vitamin in delaying the formation of insoluble trivalent iron complexes in the lumen of the upper small intestine.[9] Cobalt, in contrast to iron, is more stable in the divalent state[10] and the failure of ascorbic acid to enhance its absorption may be explicable on the basis that a reducing agent is not needed to maintain the cobalt ion in a soluble form in the gastrointestinal secretions (fig. 1).

The finding of an average of 64% of the test dose of iron and 54% of cobalt excreted in the feces of the control subjects prior to disappearance of the carmine marker (table 2) suggests that approximately 36% of the iron and 46% of the cobalt were taken up by the intestinal mucosa. In addition it suggests that the intestinal uptake of cobalt was greater than that of iron. The validity of this conclusion is based in part on the assumption that the radioactivity transferred from the intestine to the body is not excreted back into the gastrointestinal tract and subsequently lost in significant amounts in the feces. There is ample evidence that this assumption is correct for iron.[11] The finding of an average of 5.8% of a parenterally administered dose of cobalt in the feces within 10 days (table 1) indicates that only small amounts of cobalt are excreted into the gastrointestinal tract. If this is taken into consideration, it suggests that the average mucosal uptake of cobalt was underestimated by approximately 6%, and it strengthens the conclusion that the mucosal uptake of cobalt is greater than iron.

The increase in fecal excretion of cobalt observed when carrier iron was given with cobalt and a similar increase in the excretion of iron when carrier cobalt was administered with iron (fig. 4) suggest that iron and cobalt might compete for a similar uptake pathway in the intestinal mucosal cell. The mutual interference of absorption observed with iron and cobalt might be explained by co-precipitation but this seems unlikely with the low concentrations that were employed and the comparatively large volume of gastrointestinal secretions in which the elements would be diluted after administration. Furthermore, cobalt salts are less likely than iron to precipitate at the *p*H in the small intestine[12] and yet the interference of absorption observed with the two elements was similar. Somewhat similar results were observed by Pollack and coworkers[1] in the rat in which the addition of 40 μmoles of iron to a test dose of 5 μmoles of cobalt produced an increase in the average cobalt excretion from 79 to 84%. In neither this study nor the present one was the test dose large enough to tax to near capacity the absorptive system in the mucosa. Therefore, one cannot conclude with certainty from the results that cobalt and iron compete for the same transport pathway. It has been pointed out elsewhere[1] that to tax the absorptive system to capacity would require unphysiological doses and the results would be of dubious value.

The delayed excretion of an average of

9% of the iron (table 2) following disappearance of the marker lends support to prior studies[8, 10] that have shown that iron taken up from the lumen is in part stored in the intestinal mucosa and subsequently lost from the body with desquamation of the intestinal epithelium. The failure to find a significant delayed excretion of cobalt suggests that this element, unlike iron, is not sequestered and stored in the intestinal mucosa. Recent work[11] indicates that there are no specific binding sites for cobalt on transferrin and it is possible that the failure of the mucosa to retain cobalt may be due to the absence of specific binding sites for cobalt on intracellular protein or cobalt may be in a valence state that does not readily bind to intracellular substances.

In the control subjects the net effect of the smaller mucosal uptake of iron and the higher delayed excretion was that the average absorption of iron, 27%, was significantly less than the average absorption of cobalt, 44% (table 2). The failure to observe a difference between the amount of unabsorbed iron and cobalt in the control subjects given a single test dose (table 3) is due to the shorter duration of the stool collections. The fecal collections in these patients were terminated before delayed excretion of iron in iron-replete patients was taken fully into consideration.

In iron deficiency the intestinal epithelial cells are instructed in some unknown manner to modify their handling of iron, and the mucosal uptake and transfer of iron from the lumen to the plasma is enhanced.[13-17] It is clear from the present study that this also leads to enhanced cobalt absorption (tables 2 and 3). This suggests that iron and cobalt share at least part of the same transport pathway and acceleration of transport of both elements is governed by the same mechanism. Alternative explanations for the results in iron deficiency are less attractive. First, cobalt might follow a different pathway from iron and its transfer might be governed by a mechanism in the mucosal cell which is also activated by iron deficiency or, second, cobalt deficiency might occur in association with iron deficiency and this might be responsible for the enhanced cobalt absorption. The latter seems remote because cobalt deficiency is unlikely to arise from blood loss in view of the fact that blood contains less than 0.03 μg of cobalt per liter.[18] Furthermore, tissue levels of iron and cobalt are unlikely to parallel each other because cobalt, unlike iron, is excreted in the urine.

The absence of a reduction in cobalt absorption in patients with exogenous iron overload (tables 2 and 3) indicates that the instruction given to the intestinal mucosal cells to reduce iron absorption does not inhibit cobalt uptake or transfer to the plasma. The finding of an average of 52% of the iron excreted with the marker in exogenous iron overload (table 2) suggests that the reduction in iron absorption is largely due to a diminution in the mucosal uptake. Despite the reduction in mucosal uptake under conditions of iron overload, the loss of iron after the marker was approximately the same in both normal and iron-overloaded patients. Therefore, it is unlikely that a failure to sequester cobalt in the mucosa, as evidenced by the absence of significant delayed excretion of the isotope, explains the failure of iron overload to inhibit cobalt absorption.

The results suggest that the intestinal mucosal transport of iron and cobalt involves a common transport pathway, but the possibility that each element is also transported by a specific independent mechanism has not been completely excluded. If a single transport system is involved, one might postulate that in a state of normal iron repletion both elements are predominately absorbed in the proximal intestine, but cobalt, which is less apt than iron to form insoluble complexes in a neutral or alkaline medium,[12] is absorbed in the more distal intestine as well. In a state of iron deficiency, the absorption of both elements from the proximal and more distal intestine is increased, whereas in exogenous iron overload absorption of both iron and cobalt in the proxi-

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The absence of a reduction in cobalt absorption in patients with exogenous iron overload (tables 2 and 3) indicates that the instruction given to the intestinal mucosal cells to reduce iron absorption does not inhibit cobalt uptake or transfer to the plasma. The finding of an average of 82% of the iron excreted with the marker in exogenous iron overload (table 2) suggests that the reduction in iron absorption is largely due to a diminution in the mucosal uptake. Despite the reduction in mucosal uptake under conditions of iron overload, the loss of iron after the marker was approximately the same in both normal and iron-overloaded patients. Therefore, it is unlikely that a failure to sequester cobalt in the mucosa, as evidenced by the absence of significant delayed excretion of the isotope, explains the failure of iron overload to inhibit cobalt absorption.

The results suggest that the intestinal mucosal transport of iron and cobalt involves a common transport pathway, but the possibility that each element is also transported by a specific independent mechanism has not been completely excluded. If a single transport system is involved, one might postulate that in a state of normal iron repletion both elements are predominately absorbed in the proximal intestine, but cobalt, which is less apt than iron to form insoluble complexes in a neutral or alkaline medium,[19] is absorbed in the more distal intestine as well. In a state of iron deficiency, the absorption of both elements from the proximal and more distal intestine is increased, whereas in exogenous iron overload absorption of both iron and cobalt in the proxi-

nal intestine is decreased but the absorption of cobalt from the more distal intestine is not affected. This interpretation of the data is supported by the results of experiments in the rat which have shown that the changes that take place in iron absorption in iron deficiency and iron overload occur predominately in the duodenum and that iron absorption from the jejunum and ileum is not inhibited by an increase in body iron stores.[19]

The finding of an increased absorption of cobalt in cirrhosis patients with secondary iron overload and in idiopathic hemochromatosis subjects provides direct evidence that the abnormality in the absorptive process in these disorders is not restricted to iron. The enhanced cobalt absorption in these conditions is unlikely to lead to cobalt overload because the average daily intake of cobalt from food is less than 25 μg per day,[18] and, in addition, cobalt is excreted from the body by the kidneys. However, there appears to be a limit to the rate of excretion (table 1) and cobalt given in therapeutic doses has led to toxic effects.[4, 6]

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ALTERATION OF COBALT ABSORPTION IN PORTAL CIRRHOSIS AND IDIOPATHIC HEMOCHROMATOSIS

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Alteration of cobalt absorption in portal cirrhosis and idiopathic hemochromatosis

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Inorganic cobalt or iron tagged with Co^{57} and Fe^{59}, respectively, were given by mouth to selected patients, and the absorption and body retention of the dose were measured by the recovery of radioactivity in the urine and feces. In 5 patients with fatty infiltration of the liver and 7 with portal cirrhosis associated with normal iron stores, the average absorption of cobalt was similar to the absorption in 11 control subjects with normal iron stores. In contrast, cobalt absorption was increased in 6 patients with portal cirrhosis complicated by iron deficiency and in 6 control subjects with iron deficiency. It was also increased in 4 patients with portal cirrhosis associated with endogenous iron overload and in 4 with idiopathic hemochromatosis. A direct correlation was present between the absorption of iron and cobalt in both the control subjects and patients with liver disease. Unlike iron, cobalt was excreted in the urine, and the body retention of the test dose of cobalt was not increased in patients with enhanced absorption. The results indicate that cobalt absorption is increased in idiopathic hemochromatosis and portal cirrhosis complicated by either iron deficiency or endogenous iron overload. The increase in cobalt absorption in patients with liver disease appears to be secondary to a disturbance in iron metabolism.

Recent studies have shown that iron deficiency enhances the absorption of cobalt. In rats with iron deficiency, Pollack and associates[1] found the absorption of cobalt chloride was increased, and Valberg and co-workers[2] made a similar observation in patients with iron deficiency. The relevance of this finding to clinical disorders in which iron absorption is increased has not been fully explored. This study was carried out to determine whether the intestinal absorption of cobalt was increased in fatty infiltration and degeneration of the liver, portal cirrhosis, and idiopathic hemochromatosis and to establish whether cobalt is retained in the body in conditions in which its absorption is increased.

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Methods and materials

Technical methods.

LIVER FUNCTION TESTS. Standard procedures were employed for the assessment of liver function with the Bromsulphalein test, protein electrophoresis, prothrombin time, serum bilirubin, serum alkaline phosphatase, and serum glutamic oxaloacetic transaminase (SGOT).

ASSESSMENT OF IRON STORES IN BONE MARROW. Bone marrow was aspirated from the ilium, and smears from the aspirate were stained for iron with the Prussian blue reaction. The blue staining hemosiderin granules in the reticulum cells were examined by light microscopy and graded according to the following criteria: 0, absent; 1+, 1 to 25 per cent of the high power fields (500 times magnification) contained iron; 2+, granules in 26 to 75 per cent of the fields; 3+, 76 to 100 per cent of the fields had granules; 4+, blue stain evident upon naked eye inspection of slides due to large clumps of hemosiderin in reticulum cells and in extracellular foci.

ASSESSMENT OF IRON STORES IN THE LIVER. Histological sections of a liver biopsy specimen stained for hemosiderin with the Prussian blue reaction were examined under a light microscope and graded on an increasing 0 to 4+ scale by criteria described by Bakerezak and colleagues.[3] In this grading system, 4+ is considered abnormal.

MEASUREMENT OF INTESTINAL ABSORPTION OF COBALT AND IRON. The technique for the measurement of absorption of iron and cobalt was the same as the fecal recovery method described previously.[3] In brief, 4 test doses of either cobalt or iron were administered to a patient over a 2 day period. Each dose contained 20 μc/ml of either $CoCl_2$ or $FeCl_3$ tagged with either 1 μc of $Fe^{59}Cl_3$ or $Co^{57}Cl_2$, respectively, and 200 μmoles of ascorbic acid. The iron and cobalt absorption studies were carried out in succession over a 4 day period, and the radioactivity in the feces was measured by differential counting. A standard prepared from the dose and specimens of urine and feces was counted with comparable geometry.[3] The absorption of iron and cobalt was determined from the difference between the radioactivity administered and the radioactivity excreted in the stool.

MEASUREMENT OF BODY RETENTION OF COBALT. To determine the percentage of the administered dose of cobalt that was retained in the body, the urine was collected as well as the feces for 10 days in 20 of the subjects. The radioactivity in the urine was measured in a large well-type gamma counter. The retention of cobalt in the body was calculated from the difference between the radioactivity administered and the radioactivity excreted in the urine and feces.

Selection of patients.

CONTROL SUBJECTS WITH NORMAL IRON STORES. The basis for the selection of the 11 control subjects has been described previously.[3] The amount of stainable iron in the bone marrow aspirate was within the range of 1+ to 4+ found in normal subjects.[4]

CONTROL SUBJECTS WITH IRON DEFICIENCY. The 6 patients were selected on the basis of an absence of stainable iron in the bone marrow aspirate. Liver function tests were within normal limits. None of the patients was pregnant at the time of study.

PATIENTS WITH FATTY INFILTRATION AND HEMOSIDEROSIS OF THE LIVER AND NORMAL IRON STORES. The 5 chronic alcoholic patients in this group had fatty livers but no evidence of cirrhosis in the liver biopsy specimen.

PATIENTS WITH CIRRHOSIS AND NORMAL IRON STORES. The 7 patients in this group were chronic alcoholics. Two patients, N. M. and N. S., had had a portacaval shunt. The diagnosis of cirrhosis was established by the finding of abnormalities in liver function and features characteristic of portal cirrhosis in the liver biopsy specimen.[3] The patients were selected on the basis of 1+ to 2+ stainable iron in the bone marrow aspirate and the absence of increased staining iron, 4+, in the liver biopsy specimen. Only one patient, N. S., gave a history of overt bleeding from esophageal varices. Normal blood was absent from the stool at the time of study in all of the cases.

PATIENTS WITH CIRRHOSIS OF THE LIVER AND IRON DEPLETION. Eight patients with portal cirrhosis established by liver biopsy were selected on the basis that stainable iron was absent in the bone marrow aspirate and liver biopsy specimen. In 7 of them, the liver disease was due to chronic alcoholism, and in the other, F. E., the cause was unknown. Two patients, J. M. and F. K., had had a portacaval shunt for bleeding esophageal varices.

PATIENTS WITH PORTAL CIRRHOSIS AND IRON OVERLOAD. The 4 patients in this group were selected on the basis that both portal cirrhosis and iron overload were present during the course of their disease. Portal cirrhosis was established by the usual criteria,[5] and iron overload was established by the finding of an increased, 4+, staining iron in the liver biopsy specimen. In 3 out of 4 cases iron overload was confirmed by the removal of excessive amounts of iron by venesection, and in the other, R. W., extensive iron deposits were found in the liver and other organs at necropsy.

PATIENTS WITH IDIOPATHIC HEMOCHROMATOSIS. The 4 patients in this group had marked iron overload as evidenced by the removal of iron stores in excess of 20 Gm. from 3 of them and in the other, J. W., by extensive deposition of iron in the liver and other tissues at necropsy, even though 8 Gm. had been removed by venesection in the preceding year. The assumption that iron overload antedated the cirrhosis was based on the finding in the initial biopsy specimens of extensive iron deposits out of proportion to the degree of liver damage, the absence of a history of chronic alcoholism in 3 of them, and a positive family history of hemochromatosis in M. R.

Statistical analyses. Student's t test[6] was used to evaluate the difference between results. The equality of the variances was tested by the F test[6] and wherever a significant difference was found between the variances, the Cox-Cochrane correction was applied.[6] In the study of the relationship between iron and cobalt absorption, the F-distribution test was carried out to determine whether the regression was sufficiently linear to enable a valid estimate to be made.[6] When the computed F-distribution was statistically significant, the coefficient of correlation was calculated.[6]

Results

Cobalt absorption in patients with fatty infiltration and degeneration of the liver. An average of 44 per cent of the test dose of cobalt and 27 per cent of the iron was absorbed in the 11 control subjects with normal iron stores (Table I). No significant difference was found in the absorption of either cobalt or iron between the control subjects and patients with fatty infiltration of the liver with normal iron stores. The average absorption of cobalt was significantly greater than the absorption of iron.

Cobalt absorption in patients with portal cirrhosis. The average intestinal absorption of both cobalt and iron in 5 patients with portal cirrhosis and normal iron stores showed no significant deviation from the average results in the control subjects (Table I). In the 2 patients with portacaval shunt, cobalt absorption was just above the upper limit of normal in one, N. M., and definitely increased in the other, N. S., whereas iron absorption fell within the normal range.

The mean intestinal absorption of both cobalt and iron in the patients with iron deficiency was significantly increased in comparison with the results in subjects with normal iron stores. The results in the 2 patients with portacaval shunts were similarly increased (Table I).

Cobalt absorption in portal cirrhosis and iron overload.

PORTAL CIRRHOSIS WITH SECONDARY ENDOGENOUS IRON OVERLOAD. The intestinal absorption of both cobalt and iron was increased in R. W. (Table II). Absorption of these elements was also increased in V. D. when she was studied in 1967 and again in 1969 when the serum iron had risen to 291 and iron stores were replete.

PORTAL CIRRHOSIS AND EXOGENOUS IRON OVERLOAD—SEQUENCE OF DEVELOPMENT UNCERTAIN. The absorption of both cobalt and iron was increased in J. S. when iron stores had reaccumulated 3 years after venesections and later

Table I. Cobalt absorption in control subjects and patients with liver disease

Subjects	No. of cases or patient's initials	Age (yr.)	Hemoglobin (Gm./100 ml.)	Marrow iron	Liver iron	Intestinal absorption mean value ± S.D. (% of oral dose)	
						Cobalt	Iron
Iron excess							
Control subjects	11	39-63	14.0 ± 0.9	1+ – 4+	—	44 ± 6.8* (31-56)	27 ± 6.0 (17-37)
Fatty infiltration of liver	5	45-67	14.1 ± 0.9	3+	0-2+	37 ± 2.9* (31-40)	24 ± 2.3 (22-26)
Portal cirrhosis	5	41-65	13.5 ± 1.2	1+ – 3+	0-3+	51 ± 9.1* (36-62)	30 ± 8.6 (17-38)
Portal cirrhosis with porta-caval shunt	M. M.	40	13.5	2+	1+	58†	31
	M. B.	48	11.9	2+	0	62†	28
Iron deficiency							
Control subjects	6	33-72	13.2 ± 3.6	0	–	70 ± 11† (59-86)	62 ± 14† (51-84)
Portal cirrhosis	6	41-67	11.4 ± 2.6	0	0	69 ± 9.8† (59-79)	63 ± 15† (49-85)
Portal cirrhosis with porta-caval shunt	J. M.	57	13.5	0	0	70†	66†
	F. E.	69	13.2	0	0	62†	48†

*Difference between mean value of cobalt and iron in this group statistically significant ($p < 0.01$).
†Difference between mean value for this group and mean value for control subjects with normal iron stores statistically significant ($p < 0.01$).
‡Above the upper limit of the range in control subjects with normal iron stores.

Table II. Cobalt absorption in patients with iron overload

Condition	*Patients' initials*	*Date*	*Age, sex*	*Iron removed by venesection (Gm.)*	*Hemoglobin (Gm./100 ml.)*
Normal values					F, 12-16† M, 14-18†
Portal cirrhosis with secondary endogenous iron overload	R. W.	1967	56, M	0	10.4
	V. D.	1967	67, F	2.2	12.4
		1969		2.2	12.1
Portal cirrhosis and endogenous iron overload:	J. S.	1967	59, M	17	14.6
		1969		18.5	15.4
Sequence of development uncertain	R. B.	1968	62, M	0	14.3
		1969		4.5	13.5
		1969		11.5	9.9
Idiopathic hemochromatosis	V. C.	1967	66, F	25	13.3
	E. G.	1967	60, M	32	13.6
		1969		32	15.9
	M. R.	1969	61, F	>20	12.3
	J. W.	1967	58, M	8	12.1

*Unsaturated iron-binding capacity.
†F = female; M = male.

when further venesections and intermittent bleeding from a duodenal ulcer led to depletion of iron stores (Table II).

In R. B. the absorption of both metals was increased prior to treatment and during therapeutic venesections.

Cobalt absorption in idiopathic hemochromatosis. There was increased absorption of both iron and cobalt in 3 out of the 4 patients (Table II). In the fourth, J. W., the absorption of cobalt was raised, but iron absorption was within normal limits.

Relationship between cobalt and iron absorption. A highly significant direct correlation was present between the intestinal absorption of iron and cobalt in the control subjects with normal iron stores and iron depletion ($p < 0.001$). The correlation coefficient was 0.845, and the intercept was 25 per cent on the cobalt axis. A similar direct correlation was observed between the absorption of iron and cobalt in the patients with fatty infiltration and degeneration of the liver and portal cirrhosis. The correlation coefficient was 0.755, and intercept was 32 per cent on the cobalt axis. The regression line for the combined results in the control subjects and patients with liver disease is given in Fig. 1.

In the patients with iron overload, the results, with one exception, J. W., fell along the upper part of the regression line found in the control subjects and patients with liver disease. However, when the results were considered separately, their relationship was not sufficiently linear for valid estimate of regression to be made.

Relationship between the intestinal absorption and urinary excretion of co-

Iron iron (µg/100 ml.)	Serum UIBC* (µg/100 ml.)	Bone marrow iron stain	Liver iron stain	Intestinal absorption (% of oral dose)	
				Cobalt	Iron
33-185	185-335	1+-4+	0-3+	31-56	17-35
130	0	1+	4+	90	75
165	50	1+	—	71	48
291	0	1+	3+	58	55
117	26	2+	4+	84	77
108	0	0	—	77	73
173	20	4+	4+	65	72
251	0	3+	—	67	70
67	198	1+	—	86	92
178	0	0	4+	79	55
183	13	0	3+	88	85
207	5	2+	4+	84	82
229	0	1+	4+	92	81
209	14	4+	4+	80	37

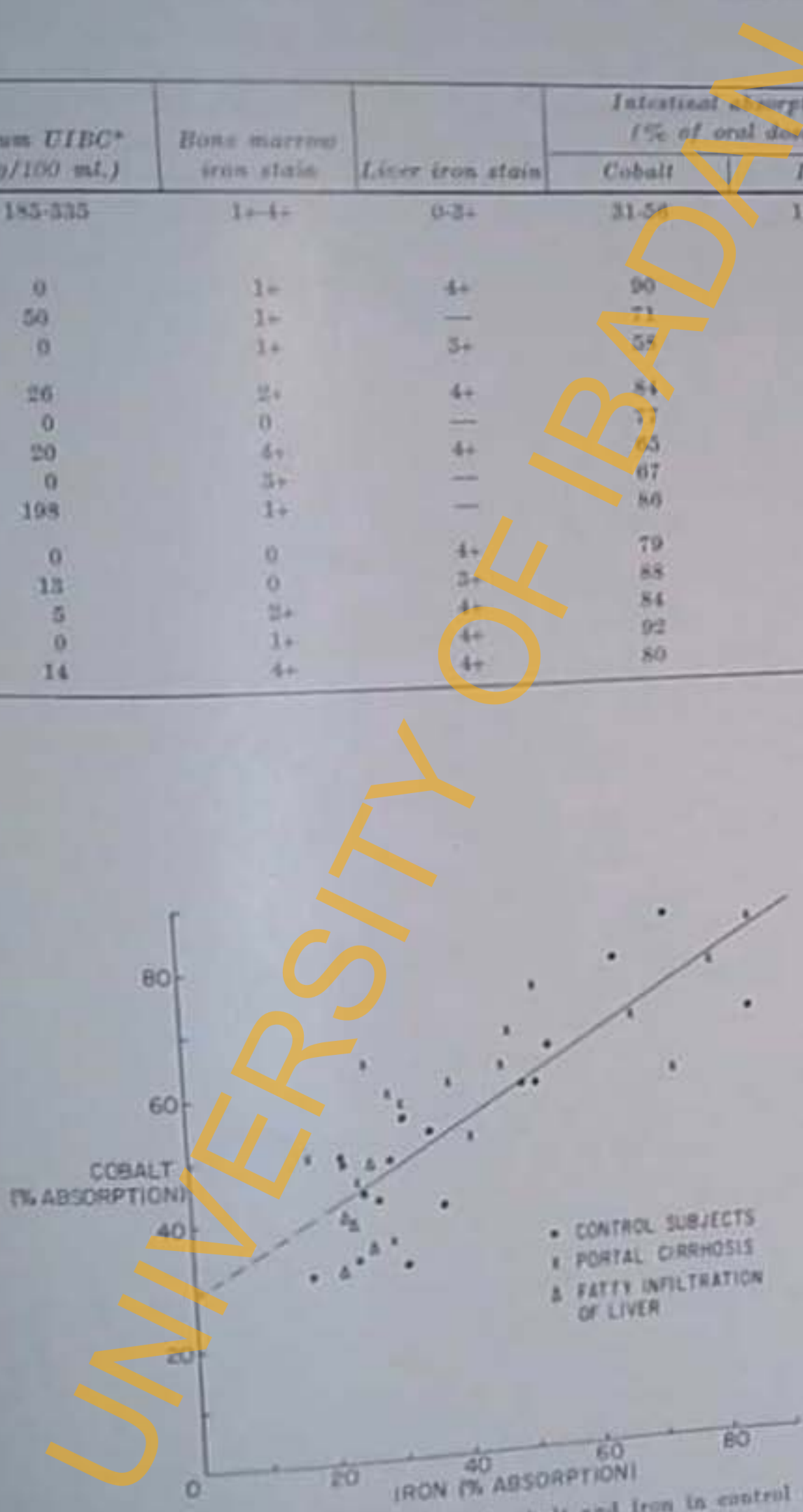

FIG. 1. Relationship between intestinal absorption of cobalt and iron in control subjects and patients with liver disease.

balt. A direct correlation was found between the per cent of the test dose of cobalt that was absorbed and the per cent excreted in the urine of subjects with normal iron stores, iron deficiency, and endogenous iron overload (Fig. 2).

Body retention of cobalt. An average of 20 per cent of the radioactivity was retained in the body 10 days after administration of the labeled test dose, and no significant difference was observed between the results in subjects with normal iron stores and those with either iron depletion or iron overload (Fig. 3). In addition, there was no difference between the body retention of radioactivity in control subjects and those with cirrhosis.

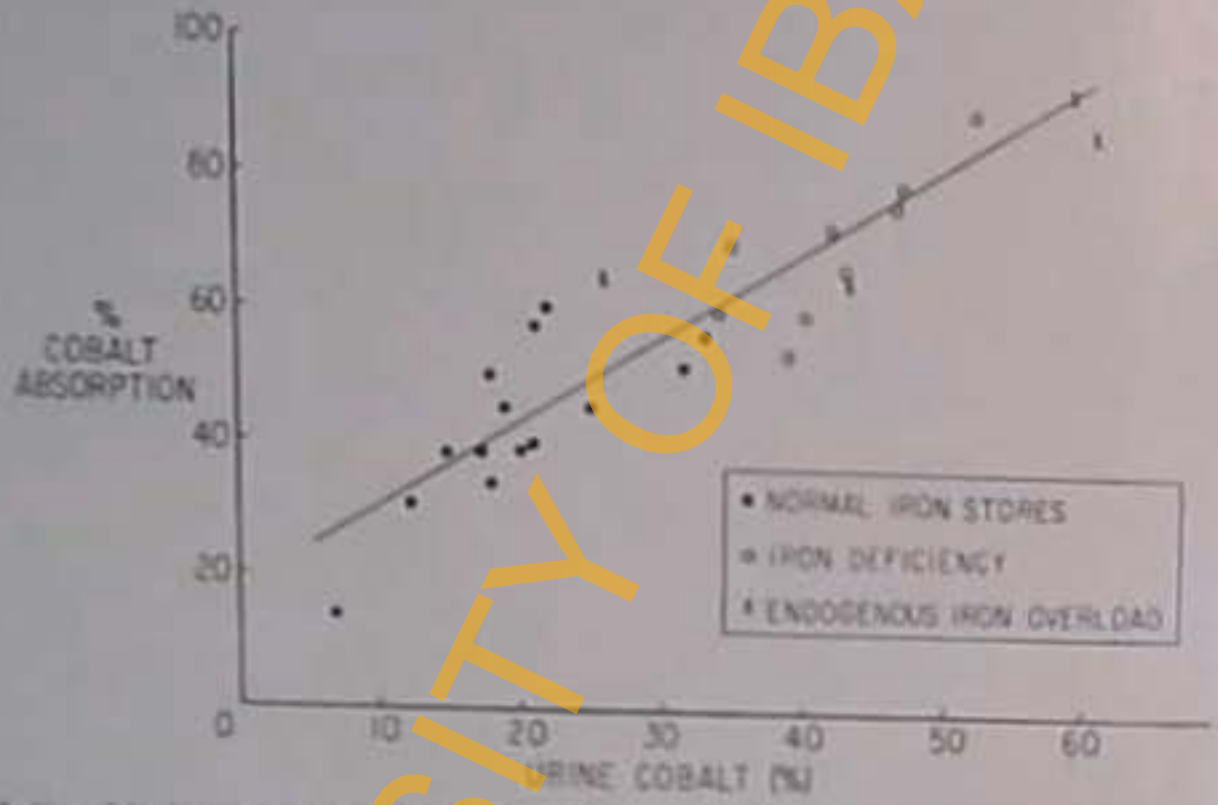

Fig. 2. Relationship between intestinal absorption and urinary excretion of cobalt.

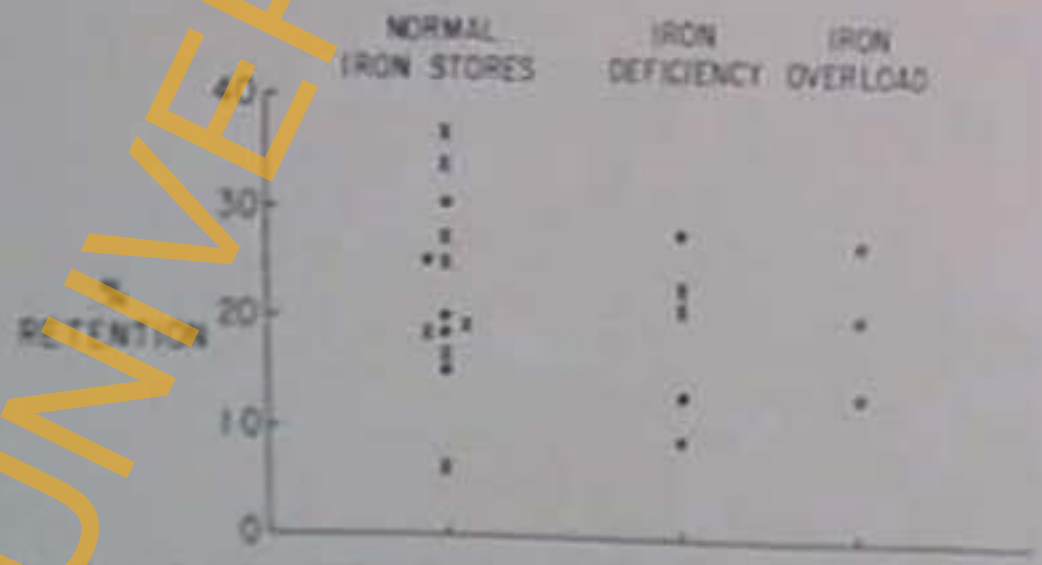

Fig. 3. Body retention of radioactivity 10 days following test dose of cobalt in subject with normal iron stores, iron deficiency, and iron overload. • Represents control subjects, and x represents the patients with liver disease.

Discussion

The absorption of iron was normal in patients with portal cirrhosis and alcoholic fatty liver associated with normal iron stores, and it was increased in patients with portal cirrhosis complicated by iron deficiency. The direct correlation between the absorption of iron and cobalt in these patients and in the control subjects (Fig. 1) suggests that the increase in cobalt absorption in the patients with liver disease was secondary to a modification in iron metabolism. The results of earlier studies[1, 2] suggest that these elements share at least part of the same intestinal absorptive pathway and that iron deficiency, which leads to an increased iron absorption, also enhances cobalt absorption.

Though the number of cases studied to date is limited, the consistent increase in cobalt absorption in patients with endogenous iron overload suggests that this test may be more useful than iron absorption for the detection of the underlying abnormality in intestinal absorption. Cobalt absorption was abnormal in all of our patients whereas iron absorption was normal in one of them, J. W. (Table II). It has been reported previously[6, 7] that iron absorption may be normal in idiopathic hemochromatosis prior to treatment, and, therefore, the absence of an increase in iron absorption in J. W. may be due to inhibition of absorption by the markedly expanded iron stores. The difference between the absorption of iron and cobalt in this patient may be explained by the absence of a similar mechanism for the inhibition of cobalt absorption. This is supported by the observation that cobalt absorption was not diminished in patients with increased body iron stores due to blood transfusions.[8]

The demonstration of an increase in cobalt absorption in idiopathic hemochromatosis shows that the abnormality in intestinal absorption in this disorder is not confined to iron. The report of a slight increase in manganese concentration in the liver of patients with idiopathic hemochromatosis[10] and an increase of manganese absorption in the experimental animal with iron deficiency[11] suggests that absorption of this element may be increased as well, but definitive studies of manganese absorption have not been reported in idiopathic hemochromatosis.

In common with all isotope techniques, the estimation of cobalt retention by the measurement of the excretion of Co^{57} fails to take into account the possibility of exchange between the absorbed isotope and endogenous cobalt. In the present study the size of the labeled test dose, 4 mg., was large in comparison to the pool of endogenous cobalt which is only of the order of one milligram.[12] Therefore, it is unlikely that there was sufficient variation in the magnitude of this exchange in 3 groups of patients studied to invalidate the results (Fig. 3).

The increased cobalt absorption in patients with iron deficiency and endogenous iron overload is unlikely to lead to cobalt overload because excess cobalt is excreted in the urine and the average daily intake of cobalt from food is only 25 μg per day.[13] The absence of an increase in body retention of the test dose of cobalt in patients with increased cobalt absorption (Fig. 3) and the report of normal cobalt levels in the liver of patients with portal cirrhosis lend further support to this view.

The discovery that cobalt absorption is increased in iron deficiency and endogenous iron overload and that cobalt balance is controlled by the excretion of

excess cobalt in the urine suggests that the measurement of the urinary excretion of an oral dose of cobalt might provide a means of detecting disorders of iron metabolism in which iron absorption is increased. This is currently under investigation.

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SIGNIFICANCE OF ALTERATIONS IN IRON ABSORPTION IN PATIENTS WITH PORTAL CIRRHOSIS

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Increased iron absorption has been a frequent finding in portal cirrhosis and liver disease has been implicated as a common cause of raised iron absorption. In most studies, however, stores have not been adequately assessed and iron deficiency has not been excluded as a cause of the increased iron absorption. The present investigation was designed primarily to assess the importance of iron deficiency and the rate of erythropoiesis in the production of increased iron absorption in cirrhosis. Iron stores were assessed by both bone marrow aspiration and liver biopsy. The absorption of a test dose of inorganic iron in 9 patients with portal cirrhosis and 6 with alcoholic fatty liver associated with normal iron stores was similar to the absorption in 12 control subjects with comparable iron stores. An appropriate increase in iron absorption was found in 5 patients with cirrhosis associated with depleted iron stores and an appropriate decrease was present in a patient with cirrhosis and increased iron stores due to blood transfusions. In contrast an inappropriate increase in absorption was detected in 2 patients with portal cirrhosis complicated by iron overload and in 2 patients with cirrhosis in whom it was not clear whether the liver disease or the iron overload was the primary event. No relationship was found between the level of iron absorption and the rate of erythropoiesis, the concentration of either serum folate or serum vitamin B_{12}, the severity of the liver disease, or the presence or absence of a portacaval shunt. The results indicate that increased iron absorption in portal cirrhosis is usually the result of decreased iron stores which in some patients is only evident upon examination of the bone marrow. In a small number of cirrhotic patients with normal or increased iron stores the normal system for excluding unwanted iron breaks down and an inappropriate increase in iron absorption occurs, but the cause of this remains obscure.

Although increased absorption of iron has been reported in 30 to 100% of patients with portal cirrhosis,[1-3] increased deposition of iron in the liver and other tissues has been found infrequently.[4-6] This discrepancy in results suggests that iron deficiency, which is a common finding in portal cirrhosis, may be responsible for the increased iron absorption in many of the patients. This is difficult to assess from most reports because definitive tests

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such as bone marrow aspiration were not carried out to evaluate body iron stores. The purpose of the present study was to determine the importance of iron deficiency as a cause of increased iron absorption in portal cirrhosis and to investigate the role of other factors such as folic acid and vitamin B_{12} deficiency, the rate of erythropoiesis, and liver disease per se in the production of increased iron absorption.

Materials and Methods

Selection of Patients

Control subjects with normal iron stores. Twelve control subjects were selected from patients in hospital employing criteria that have been described previously.[7] The amount of stainable iron in the bone marrow aspirate was within the range 1+ to 4+ found in normal subjects.[7]

Control subjects with iron depletion. Six subjects were selected from patients in the hospital on the basis of an absence of stainable iron in the bone marrow aspirate. Liver function tests were within normal limits. None of the patients was bleeding at the time of study.

Patients with alcoholic fatty liver and normal iron stores. The 5 patients in this group had drunk either wine or spirits in excessive amounts for many years. Liver biopsy in each showed fatty infiltration and alcoholic degenerative changes but no cirrhosis. Alcohol had been withdrawn on admission to hospital at least 5 days prior to the study. Iron was present in the reticulum cells in the bone marrow aspirate, but ringed sideroblasts were not present. Stainable iron in the liver biopsy specimens was 2+.[7]

Patients with portal cirrhosis and normal iron stores. Portal cirrhosis in the 9 patients in this group was established on the basis of liver biopsy.[10] Eight of them had alcoholic liver disease and in the other the etiology of the cirrhosis was unknown. Only 1 patient, N. S., gave a history of overt blood loss within 2 years and occult blood was absent from the stool at the time of study in all of them. The stainable iron in the bone marrow aspirate ranged from 1+ to 4+ and in the liver biopsy specimens from 0 to 3+.

Patients with portal cirrhosis and depleted iron stores. Eight patients with histologically proven portal cirrhosis were selected on the basis of the absence of stainable iron in both the bone marrow aspirate and liver biopsy specimen. In 7 of them the cirrhosis was due to chronic alcoholism, while in the other, P. E., the cause was unknown. Two patients, J. M. and P. E., had been treated previously by portacaval shunt for bleeding esophageal varices.

Patients with portal cirrhosis and iron overload. The 5 patients who were selected for this group had both portal cirrhosis and iron overload present during the course of their disease. Portal cirrhosis was established by the usual criteria[10] and iron overload by the finding of an increased (4+) stainable iron in the liver biopsy specimen. In 4 out of 5 patients, iron overload was confirmed by the removal of excessive amounts of iron by venesection, and in the other, R. W., extensive iron deposits were found in the liver and other organs at autopsy.

The patients fell into three groups. The first consisted of 2 patients, R. W. and V. D., with chronic alcoholism who developed increased iron stores following portacaval shunt for the control of bleeding from esophageal varices. The stainable iron in the liver biopsy specimens obtained at operation was not increased, but subsequently increased amounts of stainable iron were found in percutaneous biopsy specimens. The second group consisted of 2 patients, J. S. and R. B., who presented in the late stages of their disease with both advanced portal cirrhosis and iron overload. It was not clear whether the liver disease came before or after the iron overload. J. S. had been a chronic alcoholic but R. B. denied drinking excessively. The 1 patient, B. M., in the third group had portal cirrhosis due to chronic alcoholism. Eight months before the study he had been given iron orally and 2000 ml of blood for the treatment of anemia of unknown origin.

Patients with idiopathic hemochromatosis. The 4 patients in this group had marked iron overload as evidenced by the removal of iron stores in excess of 20 g from 3 of them, and in the other, J. W., by extensive deposition of iron in the liver and other tissues at autopsy, even though 8 g had been removed by venesection in the preceding year. In 3 of them portal

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cirrhosis was present in the first liver biopsy specimen, whereas in the other, J. W., the initial biopsy obtained at the time of operation for duodenal ulcer showed iron overload but no evidence of cirrhosis. One year later, at the time of this study, repeat biopsy showed cirrhosis and later a similar lesion was found at autopsy.

The assumption that the iron overload antedated the cirrhosis in the other 3 patients, V. C., E. G., and M. R., was based on the finding in the initial liver biopsy specimen of extensive iron deposits out of proportion to the degree of cirrhosis, return of liver function to normal following venesection therapy, and the absence of a history of chronic alcoholism. Patient M. R. had a positive family history of hemochromatosis.

Measurement of Iron Absorption

A test dose of 20 μmoles of ferrous ascorbate was given at 9 AM and 4 PM on 2 successive days as described previously.[10] The absorption of the test dose was calculated from the radioactivity excreted in the feces.

Other Methods

Serum iron and unsaturated iron-binding capacity were measured by the method described by Schade.[11] The plasma iron clearance rate (PICR) and the plasma iron turnover rate (PITR) were determined by the method of Bothwell et al.[12] Serum folate was measured by a microbiological assay, using *Lactobacillus casei* as described by Baker et al.[13] The normal values are 4 to 15 ng per ml. Folate concentrations between 3.1 and 4.0 ng per ml were considered borderline, whereas values of 3.0 ng or less were definitely low. Serum vitamin B_{12} was assayed by the microbiological method described by Ross.[14] Standard procedures were employed for the assessment of liver function with the bromosulphalein test, protein electrophoresis, prothrombin time, and serum bilirubin, alkaline phosphatase, and glutamic oxaloacetic transaminase.

Assessment of Iron Stores

The qualitative estimations of marrow and liver iron were carried out by one of us, W. E. C., without knowledge of the clinical details.

Bone marrow. Smears from bone marrow aspirated from the ilium were stained for iron with the Prussian blue reaction. The hemosiderin granules in the reticulum cells were examined by light microscopy and graded according to the following criteria: 0, absent; 1+, 1 to 25% of high power fields (× 500), contained stainable iron; 2+, 26 to 75% of high power fields; 3+, 76 to 100% of fields had granules in them; 4+, definite blue staining visible upon naked eye inspection of slides due to large clumps of hemosiderin in reticulum cells and in extracellular foci.

Liver. Histological sections of a liver biopsy specimen were stained for hemosiderin with the Prussian blue reaction and graded on an increasing 0 to 4+ scale using criteria similar to those described by Balcerzak et al.[15] In this system, 4+ is considered abnormal.

Statistical Methods

Student's *t*-test[16] was used to evaluate the difference between results. The equality of the variances was tested by the *F*-test[16] and whenever a significant difference was found between the variances the Cox Cochrane correction was applied.[16]

Results

Iron Absorption

Patients with alcoholic fatty liver and normal iron stores. The average iron absorption in the control subjects was 28%, with a range of 17 to 35%. No significant difference was found between the average absorption in patients with fatty liver and the control group (table 1).

Patients with portal cirrhosis and normal iron stores. The mean iron absorption in the patients with portal cirrhosis and normal iron stores was similar to that in the control group with comparable iron stores (table 1). The results in the 2 patients with a portacaval shunt were within the range in the control subjects.

Patients with portal cirrhosis and depleted iron stores. In comparison with the results in the control subjects with normal iron stores, the average absorption of iron was increased in both the controls with iron deficiency and the patients with cirrhosis complicated by iron deficiency (table 1). The increase was equal in both groups. Absorption in the 2 patients with portacaval shunt was higher than in the controls but within the lower part of the

TABLE 1. *Iron absorption in control subjects and patients with liver disease*

Subjects	No. of cases or initials of subject	Age	Haemoglobin	Marrow iron stain	Liver iron stain	Intestinal absorption*
		years	g/100 ml			% oral dose
Normal iron stores						
Controls	12	29–83	14.0 ± 0.9	1+–4+		28 ± 6.5 (17–35)
Fatty infiltration of liver	6	45–67	14.1 ± 0.9	3+	0–2+	23 ± 2.9 (21–26)
Portal cirrhosis	7	48–67	13.5 ± 1.3	1+–3+	0–2+	30 ± 8.0 (17–43)
Portal cirrhosis with porta-caval shunt	N. M	40	13.3	2+	1+	31
	N. S.	48	11.5	3+	0	26
Iron deficiency						
Controls	6	25–72	12.2 ± 3.6	0		62 ± 14† (51–84)
Portal cirrhosis	6	40–67	11.4 ± 2.0	0	0	63 ± 18† (40–85)
Portal cirrhosis with porta-caval shunt	J. M	50	12.2	0	0	40
	P. E.	69	13.2	0	0	46

* Mean values ± SD. Ranges in parentheses.

† Difference between mean value in this group and mean value in control group with normal iron stores statistically significant, $P < 0.01$.

‡ Outside range of results in controls with normal iron stores.

TABLE 2. *Serial measurements of iron absorption and iron stores in portal cirrhosis patients*

Patient age, sex	Diagnosis	Date	Treatment	Haemoglobin g/100 ml	Serum iron µg/100 ml	Serum UIBC† µg/100 ml	Marrow iron stain	Liver iron stain	Iron absorption % oral dose
R. H., 56, M	Portal cirrhosis	July 1967		13.9	83	240	2+	2+	20
	Bleeding haemorrhoids	Nov. 1967		13.0	82	253	0	0	72
		Jan. 1968	Parenteral iron, 1.5 g	12.4	88	104	3+		16
J. M., 57, M	Portal cirrhosis, portacaval shunt	June 1969		12.2	70	229	0	0	40
	Blood loss from trauma	Sept. 1969	Parenteral iron, 1.5 g	13.5	146	101	0	0	66
M. B., 32, M	Portal cirrhosis	May 1969		13.0	80	247	0		51
		June 1969		14.2			2+		32
P. E., 67, M	Portal cirrhosis, portacaval shunt	Nov. 1968		13.2	166	40	0		46
		Jan. 1969		11.5	218	0	1+	3+	
		Aug. 1969		13.9	173	0	1+		49
		Jan. 1970		13.1	90	134	0		58

range observed in the euthyroid patients who had not had a shunt.

Serial measurements of iron absorption in portal cirrhosis. Table 2 shows the results of serial studies in 4 patients. Iron absorption was within the normal range in R. H. when the iron stores were normal, but it was increased 4 months later when recurrent bleeding had led to depletion of iron stores; following restoration of his iron stores with parenteral iron, iron absorption returned to normal. In J. M. iron stores were depleted on both occasions that he was studied and iron absorption was increased both times. When patient M. B. was iron-depleted, his iron absorp-

TABLE 3. *Iron absorption in patients with iron overload*

Condition	Patient, age, sex	Date	History of chronic diarrhoea	Family history of iron overload	Iron removed by venesection	Haemoglobin	Serum iron	Serum UIBC	Bone marrow iron stain	Liver histology	Liver iron stain	Iron absorption	Comments
					g	g/100 ml	µg/100 ml	µg/100 ml				oral dose	
Normal subjects						F: 12–16 M: 14–18	55–185	185–335	1+–4+		0–3+	17–35	
Portal cirrhosis with secondary endogenous iron overload	R. W., 56, M	1967	Yes	No	0	10.4	150	0	1+	Advanced portal cirrhosis	4+	75	Portacaval shunt in 1965 for bleeding varices; extensive iron deposits in liver and other organs at autopsy
	V. D., 67, F	1967	Yes	No	2.2	12.4	166	50	1+	Advanced portal cirrhosis		48	Portacaval shunt in 1964; in 1966, serum iron 197 µg per 100 ml, marrow iron 4+, and liver iron 4+; venesection carried out and completed 6 months before study in 1967
		1969			2.2	12.1	291	0	1+	Same	3+	55	
Portal cirrhosis and endogenous iron overload; sequence of development uncertain	J. S., 59, M	1967	Yes	No	17.0	14.6	117	26	3+	Moderately advanced portal cirrhosis	4+	77	Iron overload discovered in 1963 at time portacaval shunt carried out for bleeding varices; blood loss from duodenal ulcer 3 months prior to study in 1969
		1969			18.5	15.4	108	0	0	Same		75	
	R. B., 63, M	1968	No	No	0	14.3	173	20	4+	Advanced portal cirrhosis	4+	42	Iron overload and cirrhosis discovered at cholecystectomy in 1968
		1969			4.5	13.5	251	0	3+	Same		70	
		1969			11.5	9.9	67	198	1+	Same		32	
Portal cirrhosis with exogenous iron overload	R. M., 61, M	1969	Yes	No	0	14.4	103	72	4+	Moderately advanced portal cirrhosis	4+	72	Anaemia treated with oral iron and 2000 ml of blood 1 year before first study
		1969			4.5	9.9	78	308	0	Same	0	69	

tion was raised; however, his iron absorption fell to normal 1 year later when his iron stores had been replenished. P. E.'s iron absorption was high and it remained relatively constant over a period of 2 years. During most of this time iron stores were depleted, but on one occasion small amounts of stainable iron were present in the bone marrow aspirate.

Patients with portal cirrhosis and iron overload. In portal cirrhosis with secondary endogenous iron overload, in comparison with the results in the control subjects with normal iron stores, the absorption of iron was increased in both patients (table 3). In portal cirrhosis with endogenous iron overload in which the sequence of events was uncertain, the absorption of iron was markedly increased in both patients (table 3). With portal cirrhosis and exogenous iron overload, the absorption of iron was decreased when iron stores were high, but depletion of iron stores by venesection produced an increase in absorption (table 3).

Patients with idiopathic hemochromatosis. The percentage of absorption of the test dose ranged from 55 to 85% in 3 of the 4 patients, and in the other, J. W., iron absorption fell within the normal range (37%).

Iron Kinetic Studies

Subjects with normal iron stores. There was no significant difference between the average PTTR in the control subjects and patients with either cirrhosis or fatty liver (table 4).

Subjects with depleted iron stores. The average PTTR in the patients with cir-

TABLE 4. *Iron kinetic studies in control subjects and patients with liver disease**

Subjects	No. of cases of subjects of subjects	Plasma iron	Total iron binding capacity	Plasma iron clearance rate	Plasma iron turnover rate
				$t_{1/2}$ in min	mg Fe/100 ml blood/day
Normal range of values		50–165	190–350	60–120†	0.80–0.80†
Normal iron stores					
Controls	17	94 ± 24	273 ± 43	80 ± 21	0.55 ± 0.16
		(55–141)	(209–334)	(49–113)	(0.33–0.86)
Portal cirrhosis	8	89 ± 30	224 ± 43	78 ± 12	0.73 ± 0.18
		(57–150)	(144–321)	(56–96)	(0.48–0.99)
Fatty infiltration of the liver	4	73 ± 35	260 ± 27	74 ± 19	0.55 ± 0.054
		(42–124)	(233–312)	(53–99)	(0.54–0.62)
Depleted iron stores					
Controls	7	61 ± 32‡	339 ± 39‡	73 ± 40	0.58 ± 0.33
		(10–96)	(276–380)	(32–210)	(0.29–1.34)
Portal cirrhosis	8	55 ± 24‡	292 ± 38‡	60 ± 28	0.65 ± 0.35
		(17–85)	(225–350)	(32–110)	(0.39–1.54)
Iron overload					
Portal cirrhosis with secondary endogenous iron overload	V. D.	160	216	172	0.85
Portal cirrhosis and suspected iron overload, sequence of events uncertain	J. L.	124	144	177	0.59
	M. D.	195	190	124	0.90
	B. M.	119	308	95	0.49
Portal cirrhosis with transfusion iron overload	V. C.	157	297	220	0.49
Idiopathic hemochromatosis	H. G.	183	300	102	0.68
	J. W.	300	254	102	1.18

*Mean values plus standard deviation. Range given in parentheses.

†Difference between mean value in this group and mean value in control group with normal iron stores statistically significant, $P < 0.05$.

rhosis was similar to the values in the control subjects with depleted iron stores. One of the results in this group was above normal and the other five fell within the normal range (table 4).

Subjects with iron overload. The PICR was prolonged in 5 out of the 7 patients and the PITR was increased in only 1, J. W. (table 4).

Correlation of PITR with iron absorption. No correlation was observed between the PITR and the absorption of iron in patients with normal iron stores, iron depletion, or iron overload.

Serum Vitamin B_{12} and Folate Levels

Subjects with normal iron stores. There was no significant difference in either the average serum vitamin B_{12} or serum folate levels between control subjects and patients with cirrhosis or fatty liver (table 5). In the control group selected from patients in hospital the serum folate was low in 5 and serum vitamin B_{12} decreased in 2, but no abnormality was present in the morphology of the bone marrow. In 2 of the patients with low serum folate and liver disease, hypersegmented polymorphs and giant metamyelocytes were found in the marrow, but frank megaloblastosis was absent.

Subjects with depleted iron stores. The average serum vitamin B_{12} and folate activity were similar to the values in the controls with normal iron stores (table 5). Serum vitamin B_{12} was greater than 1300 pg. per ml. in 2 uncompensated cirrhotics, presumably due to the release of vitamin B_{12} from necrotic liver cells.

Subjects with iron overload. The serum vitamin B_{12} level was within the normal range in 6 subjects, reduced in 2, and

TABLE 5. *Serum vitamin B_{12} and folate activity in control subjects and patients with liver disease*

Subjects	No. of cases or serum folic acid date	Serum vitamin B_{12}	Serum folate
		pg./ml.	μg./ml.
Normal range of values		200–1000	4–15
Normal iron stores			
Controls	12	400 (100–1017)	3.8 ± 1.5 (2.0–6.0)
Portal cirrhosis	5	384 (200–750)	5.8 ± 2.7 (2.1–8.7)
Fatty infiltration of the liver	3	409 (376–442)	4.7 ± 1.0 (3.5–6.0)
Depleted iron stores			
Controls	6	330 (95–1004)	3.4 ± 2.1 (2.0–7.0)
Portal cirrhosis	6	650 (262–2230)	4.4 ± 2.2 (2.0–7.6)
Iron overload			
Portal cirrhosis with secondary endogenous iron overload	V. D., 1967	236	2.0
	1969		>20*
	R. W., 1967	2400	4.6
Portal cirrhosis and endogenous iron overload; sequence of development uncertain	J. S., 1967	346	3.0
	1969		5.0
	R. B., 1968	222	3.7
	1969		4.0
Portal cirrhosis with exogenous iron overload	B. M., 1969	177	7.5
Idiopathic hemochromatosis	V. C., 1967	230	2.5
	E. G., 1967	275	2.3
	1969		4.2
	M. R., 1969	170	6.9
	J. W., 1967	314	>20*

* On folic acid therapy.

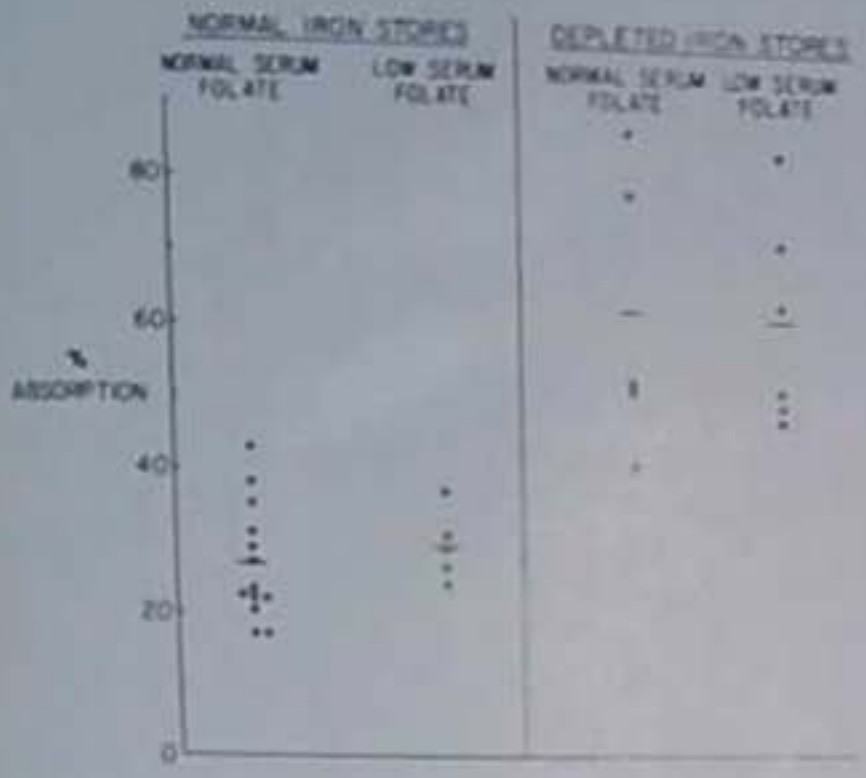

FIG. 1. Relationship of serum folate activity to iron absorption.

markedly increased in 1 patient with decompensated cirrhosis (table 5). Serum folate activity was variable and it bore no relation to the level of iron absorption.

Relationship between serum folate, serum vitamin B_{12}, and iron absorption in patients with normal or deficient iron stores. Patients were divided into two groups, one with normal iron stores and the other with iron depletion (fig. 1). Each group was subdivided into one which was definitely folate deficient on the basis of a serum folate less than 3 ng per ml and one which was folate-sufficient with serum folate concentration greater than 4 ng per ml. No relationship was observed between the serum folate level and the percentage of iron absorbed in patients with either normal or depleted iron stores.

No relationship was found between serum vitamin B_{12} concentration and iron absorption, but there were too few observations in patients with low levels to determine whether vitamin B_{12} deficiency affected iron absorption.

Discussion

Under normal circumstances iron absorption is regulated by the size of body iron stores. When there is a need for iron, as in iron deficiency, the intestinal epithelium is instructed in some unknown manner to enhance iron absorption and, when iron stores are replete, iron absorption declines to values less than normal. The results of the present study indicate that iron deficiency is a common cause of increased iron absorption in cirrhosis. This probably explains the discrepancy between the common finding of increased iron absorption in this disorder[1-7] and the infrequent occurrence or iron overload in cirrhosis.[8, 9] In some of our patients the iron deficiency was occult and only became evident when iron stores in the bone marrow were assessed.

Although iron absorption was regulated in a physiological manner by the size of body iron stores in most of the patients with portal cirrhosis that were studied, there were 4 patients with an inappropriate increase in absorption (table 3). The results of serum iron, unsaturated iron-binding capacity, PTTR, and iron absorption in these patients were indistinguishable from those in patients with idiopathic hemochromatosis. The only difference found between the two conditions was the comparatively small increase in iron absorption in 1 of the patients, V. D., with secondary iron overload.

The frequency of iron overload secondary to portal cirrhosis is uncertain, but a review of the literature suggests that it is uncommon (table 6). There appear to be only 11 patients reported in the English literature[8-18] in whom portal cirrhosis was proved to antedate the development of iron overload and in 3 of these cases the administration of iron and blood transfusions makes it difficult to assess the importance of increased iron absorption in the development of the condition. In many of the reports[1, 2, 19, 20, 22] of iron overload complicating cirrhosis, it is not possible to determine whether the liver disease or the iron overload was the initial disorder because the sequence of events is not clear.

Although the results of this study do not provide a definitive explanation for the inappropriate increase in iron absorption that occurs in some patients with cirrhosis, they do help to exclude a number

TABLE 6. *Review of cases in the literature of iron overload preceded by portal cirrhosis*

Author	Age of patient	Sex of patient	Chronic alcohol ingestion	Portal cirrhosis present	Magnitude of iron excess	Hemoglobin	Serum iron	Total iron binding capacity	Remarks
						g/100 ml	ug/100 ml		
Case records, Mass. General Hospital[23]	38	M	Yes	No	Unknown	15.0	330	430	Portal cirrhosis with excess stainable iron in initial biopsy; 4.5 years later at autopsy, extensive iron deposition in the liver
Tuttle et al.[10]	50	M	Yes	Yes	Unknown	14.2			Portal cirrhosis with no stainable iron at time of portacaval shunt; died 3 years later with excess iron in liver, heart, pancreas and other organs
Hoffbauer et al.[10]	55	F	Not given	Yes	Unknown				Eleven years after onset of jaundice a portacaval shunt carried out for bleeding varices associated with postnecrotic cirrhosis; no stainable iron in liver biopsy specimen; at autopsy 10 months later excessive iron deposits in liver
Tisdale[10]	27	F	No	Yes	Unknown	11.4			No stainable iron in liver at time of splenorenal shunt; died 9.5 years later; dense clumps of hemosiderin in liver and pancreas; administration of 3.5 g iron given in the form of blood transfusion for anemia contributed to the iron overload
	53	F	No	Yes	Unknown				Stainable iron absent in the liver at the time of portacaval shunt; 3 years later abundant iron deposits in pancreas and liver; administration of 5000 ml blood in the 3 weeks prior to death makes it difficult to assess whether an underlying abnormality was present in iron absorption; no stainable iron in liver biopsy specimen when portacaval shunt made; at autopsy 3 years later excess iron in liver and pancreas
Schaefer et al.[10]	37	F	Yes	Yes	Unknown	14.0			
Sabesin and Thomas[10]	20	M	No	No*	Unknown		160	160	Small quantities of hemosiderin in initial liver biopsy specimen; died 2 years later with marked iron deposition in liver, pancreas, and other organs

* Esophageal varices present.

of factors that have been proposed by previous workers.[12] The finding of normal iron absorption and normal iron stores in 6 patients with alcohol-induced fatty infiltration and degeneration of the liver suggests that neither chronic alcoholism nor fatty liver are important. This is also supported by the recent observation of Landvall and coworkers[14] that the iron stores in alcohol abusers were not increased as compared with controls.

Liver damage itself does not appear to have an important effect on iron absorption. Normal iron absorption has been found in acute liver disease[15, 16] and absorption was normal in our patients with cirrhosis or fatty liver associated with normal iron stores.

The finding of normal iron absorption in 3 of our patients with portacaval shunt confirms two previous studies[6, 7] in which no difference in iron absorption was found between cirrhotic patients with and without shunts. These observations and the absence of increased iron absorption in dogs with experimental portacaval shunts[17] suggest that the shunt per se does not enhance iron absorption. However, it might contribute to the development of iron overload indirectly by diminishing blood loss and lengthening the life span of patients with enhanced iron absorption due to other causes.

It has been proposed that pancreatic insufficiency may be the causative factor in increased iron absorption in liver cirrhosis,[6, 7] but recent studies indicate that pancreatic insufficiency is not associated with increased iron absorption in the absence of iron deficiency.[18, 19] Pancreatic function was not assessed in our patients.

Murray and Stein postulated that increased erythropoiesis associated with hemolysis might lead to increased iron absorption in liver disease.[7] However, this is not supported by the results of the present study in which the rate of erythropoiesis, as measured by the FTTR, was within normal limits in the patient, V. D., with iron overload. Furthermore, no correlation was present in either the control

subjects or the patients with cirrhosis between the absorption of iron and the PITR.

The finding of low serum folate in some of the control subjects was not surprising because many of them, like the patients with liver disease, gave a history of poor dietary intake of folate-containing foods prior to hospitalization. Mild folate deficiency does not appear to affect iron absorption because similar levels of absorption were found in control subjects with normal serum folate and a comparable group with low serum folate (fig. 1). Therefore, it is unlikely that folic acid deficiency is an important factor in the raised iron absorption in patients with cirrhosis and iron overload.

The present study of the factors that have been postulated[24] to affect iron absorption in liver disease has not uncovered the cause of the inappropriate increase in iron absorption that occurs in some patients with cirrhosis, and further investigation of other factors is warranted.

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