ABSORPTION OF IRON FROM THE SMALL BOWEL

Herbert L. Duthie.

September 1961

M. B., Ch. M. (Glasgow)
F. R. C. S. (Edinburgh)
F. R. C. S. (England)

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Though at the present time the value of iron in cases of chlorosis is everywhere recognised, still the earlier scepticism as to how it could act is fully justified by the modern discussion as to the manner of absorption, physiological action and excretion of iron when given medicinally - questions not yet definitely settled.

Christian (1903).

CONTENTS.

OUTLINE.

This thesis presents a study of the absorption of iron from isolated loops of small bowel in animals and in man.

Methods of estimating absorption of iron from such isolated loops have been established in anaesthetised dogs and then used to study the effect of factors within the lumen of the bowel on this absorption, as well as to compare the absorption of iron from the duodenum and from the ileum. The importance of the duodenum in the absorption of iron is then investigated in a series of experiments in dogs and in rats. A modification of the above methods is applied to the measurement of absorption of iron from isolated loops of bowel in man. The results of these studies are discussed in relation to the possible mechanism of absorption of iron.

In the main body of the Thesis a description of the methods used and a statement and a discussion of the results are presented. The Appendix to the main Thesis contains details of the various studies.

ACKNOWLEDGEMENTS.

This work has been carried out in the Mayo Foundation, Rochester, Minnesota, during the tenure of a Rockefeller Travelling Fellowship in Medicine and in the Professorial Surgical Unit at the Western Infirmary, Glasgow. I am deeply indebted to Doctor Charles Code and Doctor Charles Owen for their guidance and criticism of my work in U.S.A. and to Professor Sir Charles Illingworth for his encouragement and for making facilities available for this research.

I gratefully acknowledge the assistance of several colleagues in parts of the study. Dr. Silvey of the Mathematics Department of the University of Glasgow advised on the statistical analysis. Technical assistance came from Miss E. Graf, Mr. E. van Hook on the work on rats, and Mr. R. McAllister in the human studies. The figures were the work of the Medical Illustration Department at the Western Infirmary - Mr. Gabriel Donald (Director), Miss K.J. Graham, Miss R. Henderson and Miss N. Broadfoot: the final manuscript was executed by Miss A.R. Hamilton.

INTRODUCTION

REVIEW OF THE LITERATURE.

INTRODUCTION

REVIEW OF THE LITERATURE

Iron has been known to man since time immemorial. Proof of its early use comes from Egyptian inscriptions (3000 B. C.) and old Chinese writings claim that iron was discovered in China about 2900 B. C. (Goldwater, 1935). The therapeutic virtues of iron were extolled in about 2000 B. C. by Apollodorus who reported a wonderful cure of impotence by Melampus of Argos who gave rust dissolved in wine. This and many other of the first uses of iron in medicine were coloured by its connection with the god Mars. Indeed the metal was given his name and was supposed to give strength and potency (Wootton, 1910). Initially administered in all states of weakness it was gradually noted that it was efficacious in anaemia. Sydenham (1681) was amongst the first to recommend its routine use in the anaemic state of chlorosis. A more rational basis for iron therapy was established by the discovery of iron in the ash of blood by Lemery and Geoffrey in 1713 (Christian, 1903).

Despite centuries of proven clinical value iron still remains something of an enigma. Even relatively recently /

/recently its efficacy in the treatment of anaemia was doubted by some. The arguments advanced are reviewed by Robscheit-Robbins (1929). The divergent views of its place in therapy arise from the difficulty of estimating the small amounts of iron taken into and excreted from the body. Most of the early studies on the absorption of iron were clinical and dealt with the response of anaemic patients to different iron salts. Absorption was at first measured by the rise in haemoglobin produced, then by the rise in the serum iron and later by balance studies using chemical methods. The advent of readily available radioactive isotopes of iron has allowed accurate iron determinations to be made with relative ease. A critical review of these methods was made by Josephs (1958). He underlined the value of careful chemical balance studies and felt that their importance had been unfairly minimised. Although agreeing with the general opinion that radioisotopes have made concise measurements possible with physiological doses, he noted that there were some limitations in the system, as a single dose had to be used and any day-to-day variation would not be accounted for.

The continuing interest in the problems of the /

/the absorption of iron is emphasised by the number of recent reviews on the subject (Gubler, 1956; Bothwell and Finch, 1957; Josephs, 1958; Callender, 1959; Smith, 1960).

FACTORS INFLUENCING THE ABSORPTION OF IRON.

The various factors which have been thought to influence the absorption of iron will be discussed under the following headings:-

LOCAL FACTORS IN THE BOWEL LUMEN AND THE BOWEL WALL.

Ascorbic acid
Acidity
Valency of iron
Ferritin
Others

GENERAL FACTORS

Body stores of iron
Erythropoiesis
Serum iron and transferrin
Anaemia
Others

LOCAL FACTORS.

Ascorbic acid: It is generally agreed that ascorbic acid enhances the gastrointestinal absorption of iron. This has been frequently shown both in animals and in man by chemical methods (Moore et al., 1939) and with radioactive irons as inorganic salts and incorporated into food (Moore /

/ (Moore and Dubach, 1951; Bothwell et al., 1958; Bonnet, 1958; Bonnet et al., 1960). This promoting action of ascorbic acid is usually thought to be a result of its reducing power. It has also been suggested to be due to slowing down of the return of duodenal pH towards neutrality (Groen et al., 1947). These authors found that other organic reducing agents and organic acids possessed the same action. That cysteine also augmented absorption of iron was found by Moore (1955) although he could not confirm that organic acids had a similar effect. It has also been suggested that the action of ascorbic acid in promoting absorption of iron may be due to formation of a readily absorbed chelate with the iron (Finch and Finch, 1955).

Acidity: In contrast with the agreement on the action of ascorbic acid, the part played by gastric acid secretion in the absorption of iron is ill-defined. The effect of variations of pH in the bowel lumen on absorption of iron has been studied mainly by indirect means. Early studies utilised the haematological response as an index of iron absorption. Incubation of a dose of iron with pepsin and hydrochloric acid before giving it to patients at a pH of 3 or at a pH of 7 to 8 resulted in a slightly greater reticulocyte response at pH 3 (Mettier and Minot, 1931). Achlorhydrics were shown to /

/to have a smaller haematological response to iron than comparable patients with free hydrochloric acid in their stomach (Minot and Heath, 1932) and to have a better response when iron was incubated with pepsin and hydrochloric acid before administration than when given untreated iron (Mettier et al., 1933). Observing haemoglobin levels Kellog and Mettier (1936) found that alkaline therapy reduced the utilisation of iron in patients with peptic ulcer. However, there was an increase in serum iron following oral iron therapy irrespective of the presence of free acid in the stomach (Moore et al., 1939) and a study of haemoglobin levels in a group of 100 women of whom 20 were achlorhydric, led Brummer (1950) to the conclusion that achlorhydria is a result and not a cause of anaemia. Grace and Doig (1953) found no difference in serum iron curves when iron was fed to normal patients, with or without sodium bicarbonate, or to achlorhydric patients. The main criticism of these methods is that the utilisation of iron in the formation of haemoglobin does not necessarily reflect the amount of iron absorbed (Brock and Hunter, 1937; Dubach et al., 1948; Bothwell et al., 1955) and levels of serum iron are of doubtful value in measuring absorption of iron (Laurell, 1952).

Chemical determination of iron in balance studies /

/ studies demonstrated that achlorhydrics retained less dietary iron than normals but when large supplementary doses of iron were given (500 mg. per day) no difference was seen between the two groups (Barer and Fowler, 1937).

More recently radioactive iron has been used to study this effect. When it was given with a meal, Pirzio-Biroli and others (1958) found no significant deviation of absorption of iron from the normal in four patients who had a histamine-fast achlorhydria. Later work by Williams (1959) with radioactive iron baked into bread revealed a significantly smaller absorption in four achlorhydrics than in normal subjects. This result in turn was not confirmed by Goldberg and Lochhead (1960) who could find no difference between normals and patients with histamine-fast achlorhydria. Furthermore, giving hydrochloric acid with radioactive iron did not increase absorption (Moore, 1955; Callender et al., 1957; Smith and Mallett, 1957).

The role of achlorhydria in the pathogenesis of anaemia is still uncertain and recent evidence comes from gastric biopsy techniques. Correlating the results of gastric biopsy with the gastric secretory response to an augmented histamine test in both anaemic and normal patients Davidson and Markson, (1955) held that achlorhydria was not a cause but a result of anaemia, although only two out of five achlorhydric patients had /

/had hydrochloric acid in their gastric juice after treatment. The above conclusion was supported by Badenoch and co-workers (1957) in a study of 50 patients with iron-deficiency anaemia. The opposite opinion was put forward by Lees and Rosenthal (1958) who investigated 19 anaemic patients before and after treatment. Despite good haematological responses and cure of epithelial lesions, no improvement was found in the appearance of the gastric mucosa on biopsy and no increase in the output of gastric acid.

Many of the studies which bear on the question of acid secretion in iron absorption have been done on patients after partial gastrectomy when the gastric acid production is greatly reduced. It was believed by Bruusgaard (1946) that postgastrectomy anaemia was due to this diminution in gastric acid, but Watson (1947) in a study of gastric test meals after gastrectomy could find no relationship between postoperative achlorhydria and anaemia. Work with radio-active iron salts also did not show any difference in absorption before and after gastrectomy (Smith and Mallett, 1957; Baird, Podmore and Wilson, 1957). When radioactive iron was incorporated into food no alteration in absorption occurred following partial gastrectomy (Baird and Wilson, 1959) unless postgastrectomy anaemia were present when /

/when the normal increase in iron absorption in response to diminished iron stores did not occur. Similar studies using radioactive iron given with a meal did reveal a slightly smaller absorption in patients after gastric resection and again there was no response to anaemia (Stevens et al., 1959). This was confirmed by Williams (1959) using radioactive iron baked into bread. Several factors other than acidity are involved in these human studies and such evidence must be regarded as circumstantial.

In summary, despite the theoretical advantage of having iron presented to the intestinal mucosa at an acid pH, there is no overall agreement amongst the experimental work on this subject.

Valency of Iron: Clinical and experimental experience in the treatment of anaemia in man has shown that effective therapeutic responses can be obtained with smaller doses of ferrous than of ferric iron (Witts, 1935; Wintrobe, 1956). Determinations of the serum iron have revealed that this increases to a greater extent after ferrous iron than after ferric iron (Heilmeyer and Plötner, 1936). The best evidence that iron is absorbed to a greater extent in the /

/the ferrous form is found in the work of Lintzel (1933). He gave α-α- dipyridyl to rats along with a diet adequate in iron content. The dipyridyl combines with ferrous iron to form a red compound which is not altered by gastric or intestinal secretions. No reaction occurs with ferric iron. The rats to whom the dipyridyl was given failed to gain weight normally and had less iron in their tissues than a control group. Lintzel suggested that iron was absorbed from the gastrointestinal tract only in the ferrous state. These results were confirmed by Lucas and Summerfeldt (1939).

Although the above statements would appear to have left little doubt that ferrous iron would be absorbed to a greater extent than ferric iron, the findings in studies of the absorption of iron are not uniform. In favour of the superiority of ferrous iron are the results in vitro dialysis experiments of Tompsett (1940). He believed that ferric iron was less likely to be absorbed from the gut as it more readily forms insoluble complexes. This belief was supported by Moore and co-workers (1944) who demonstrated that more ferrous than ferric iron was absorbed from equal doses in man, a similar finding to that of Hahn and colleagues (1943; 1945) working with the dog. /

/dog.

The contrary view, that is, that both forms of iron are absorbed equally in man was held by several groups of workers (Brock and Hunter, 1937; Steinkamp et al., 1955; Bonnet et al., 1960). This suggestion is in keeping with the results of studies in experimental animals which indicate that both forms of iron are equally absorbed (Whipple and Robscheit-Robbins, 1930, 1936; Underwood, 1938; Austoni and Greenberg, 1940; Nakamura and Mitchell, 1943; Street, 1943; Moore et al., 1944, Freeman and Burill, 1945).

Some of the divergence of the opinions can be explained by the use of ferrous iron made from ferric iron by the addition of ascorbic acid. Any excess of the latter reagent would give a falsely high absorption of iron. Thus, the prevalent opinion is that ferrous iron is absorbed to a greater extent than ferric iron but some of the experimental backing for this opinion is not without doubt.

Ferritin: The iron-containing protein Ferritin came into prominence in connection with iron absorption as a result of histochemical studies and was thought to be the physical basis of the "mucosal block" theory of the absorption of iron (Granick, 1946). This theory was /

/was founded on data from iron absorption experiments in man (McCance and Widdowson, 1937, 1938) and the dog (Hahn et al., 1943). Later work on iron absorption has thrown some doubt on the importance of the block and the histochemical findings have been interpreted in different ways.

It has long been known that only a small amount of iron is absorbed from the diet and that a very small amount of endogenous iron is excreted in the stools (McCance and Widdowson, 1938; Dubach et al., 1949, 1955; Moore, 1955). Working on dogs Hahn and his colleagues (1943) showed that the absorption of a dose of iron was diminished if another dose had been given soon before. It was felt that this indicated some mechanism which could be saturated with iron and which regulated the amount of iron absorbed. When Granick (1946) noted increased ferritin in the duodenal mucosa of the guinea pig following oral administration of iron he thought this substance might be the controlling mechanism (Granick, 1946b). However, Endicott and his co-workers (1949) found that the histochemical iron in the duodenum did not coincide with the radioactive iron in the mucosa after a dose of the latter. This made it seem that ferritin might not /

/not necessarily mediate iron transport across the intestinal mucosa.

Other workers have expressed doubts about the importance of ferritin on the basis of histochemical studies (Heilmeyer et al., 1957; Wohler et al., 1957). On the other hand similar reports from France and Spain have supported it as a factor of note (Mouriquand and Edel, 1958; Mouriquand and Muller, 1958; de Vinals and de Campo, 1957). Electron microscopic investigations have revealed ferritin and its iron-free precursor, apoferritin, in the duodenal mucosa of animals overloaded with iron (Bessis and Breton-Gorious, 1957; Richter, 1957; Bessis et al., 1958) but no evidence was presented to ascribe a function to these crystals.

Several groups of investigators have shown that no absolute mucosal block to the absorption of iron exists and that any block present probably does not function at physiological levels (Dubach et al., 1948; Kinney et al., 1949; Bothwell et al., 1953; Smith and Pannicculi, 1958; Chodos et al., 1957; Brown et al., 1958; Charley and Saltmann, 1960).

In short, it would seem that while there may be some /

/some mucosal control of the absorption of iron from the gastrointestinal tract, it is not the only influence and it is not established that ferritin is the compound through which it acts.

Other Local Factors. The amount of phsophate in the diet affects the absorption of iron, probably by forming insoluble iron precipitates (McCance et al., 1943) and the amount of iron absorbed shows an inverse relation to the amount of phosphate in the diet (Kinney et al., 1949).

Absorption of iron increases when the pancreatic secretion is diminished or absent, either due to ligation (Taylor et al., 1935) or section (Gillman et al., 1947) of the pancreatic duct or to pancreatic damage from administration of ethionine (Kinney et al., 1955) or from a low protein high fat diet (Kaufmann et al., 1958).

Other substances which augment absorption of iron are sorbitol or sorbitol derivatives (Wissler et al., 1954: Mori et al., 1957; Herndon et al., 1958). Despite contrary views on the vitamin-sparing properties of sorbitol (Morgan and Yudkin, 1957; Mehnert et al., 1958) it was believed to act as a coenzyme or an activator /

/activator in absorption of iron (Herndon et al., 1958) although Mori and co-workers (1957) felt that a change in bowel motility was the more likely explanation of the augmenting action of Tween 20 (a sorbitol derivative) on absorption of iron. Inosine given intravenously prior to the oral dose of iron increased absorption, possibly due to its action as an anti-xanthine oxydase (Cheney and Finch, 1960).

Opposing views have been held on the effect of gastric mucin. It has been said to retard absorption of iron (Heath et al., 1938) and to have no effect (Moore et al., 1939) both groups using the levels of serum iron to assess absorption.

Chelation of iron with either sodium versenate (Will and Vilter, 1954) or with calcium citrate (Bothwell et al., 1958) gave absorption equivalent to an equal dose of ferrous iron.

GENERAL FACTORS.

Body stores of iron: It has been shown frequently both by chemical balance and by radioactive absorption techniques that iron-deficiency increases the amount of iron absorbed from the alimentary canal (Hahn et al., 1943; Moore and Dubach, 1951; Chodos et al., 1957; Bothwell /

/Bothwell et al., 1958). An increase in the body stores of iron by parenteral injection depresses absorption of iron (Bothwell et al., 1958; Krantz et al., 1959; Pirzio-Biroli and Finch, 1960). It is thus surprising that the increase in iron in the body in the disease of haemochromatosis does not prevent further absorption (Finch and Finch, 1955; Chodos et al., 1957).

Erythropoiesis: Increased production of red blood cells has been shown to increase absorption of iron both in humans and rats (Bothwell et al., 1958). Conversely decreased erythropoiesis following overtransfusion has a depressing effect on absorption of iron in rats. Similar work in mice confirmed these findings and a plasma factor has been isolated from sheep which is capable of transferring this enhancing effect to mice (Gurney and Filmanowicz, 1960). This humoral factor has been called Erythropoietin and its nature was fully reviewed by Gordon (1959) with more up-to-date details in the Ciba Symposium on Haemopoiesis (Gordon, 1960; Linman and Bethell, 1960; van Dyke, 1960). Other workers have been unable to isolate any humoral factor in mice with increased erythropoiesis (Beutler and Buttenweiser, 1960). Thus, the exact mechanism of the increased absorption of iron with increased erythropoiesis /

/erythropoiesis is undecided although the effect itself is quite clear.

Serum Iron and Transferrin: The levels of iron in the serum give an impression of the state of the body stores of iron but are not directly correlated with them (Beutler et al., 1958). In general, the absorption of iron is high when serum is low and vice versa. However, the size of the increase in the serum iron level after a dose of iron gives no good indication of the amount of iron absorbed. This aspect has been the subject of much research and debate and is well reviewed by Laurell (1952).

Transferrin is the plasma protein which carries iron. Opinions differ on its part in the regulation of the absorption of iron. In dogs Yuile and his collaborators (1950) found that saturating the plasma iron-binding capacity, a measure of the amount of transferrin not attached to iron, with intravenous iron did not appreciably affect the absorption of iron. Confirmatory results were obtained in similar experiments in man (Bothwell et al., 1958). On the other hand, Hallberg and colleagues (1959, 1960) showed a notable increase in the absorption of iron after intravenous injection of transferrin and Hyde (1957) obtained a definite though smaller increase in /

/in rats.

Anaemia: When the effect of anaemia is separated from that of iron deficiency it has no effect per se on the absorption of iron (Hahn et al., 1943; Dubach et al., 1948; Finch, Haskins and Finch, 1950; Pirzio-Biroli et al., 1958); although it has been noted that iron absorption can return towards normal in a patient suffering from iron-deficiency anaemia when the haemoglobin level is normal but before the body stores are repleted (Finch, Haskins and Finch, 1950).

Other General Factors: Inflammation in the form of sterile turpentine abscesses was said to impair the absorption of iron measured by incorporation into haemoglobin (Hahn et al., 1946). Giving intravenous iron Wintrobe and his colleagues (1947) found that infection retarded the increased uptake of iron in iron-deficiency. The presence of turpentine abscesses gave a temporary fall in serum iron and haemoglobin and a delay in iron incorporation into haemoglobin (Yuile et al., 1949). Other workers did not find a decrease in iron absorption when the stool collection method was used in the presence of fever (Dubach et al., 1948). It would appear that the original impression of a fall in the absorption of iron with inflammation was due /

/due to diminished utilisation of iron. At that time utilisation was used to measure absorption.

The effect of environmental oxygen tension has been studied in several centres. In 1945, Hurtado and co-workers found that prolonged or intermittent anoxaemia, associated with altitudes up to 16,000 feet, caused an increase in red blood cell production. The corollary was shown by Tinsley and others (1949) by giving continuous oxygen therapy to patients and noting a diminution of the red blood cell production. The change in serum iron level was used by Smith and collaborators (1957) to assess the absorption of iron when oxygen tension was altered: an increase in oxygen tension produced a decrease in absorption and vice versa. In a study on humans Reynafarje and others (1959) demonstrated that iron absorption, measured by utilisation in the blood, was increased up to three times within the first 48 hours of entering the hypoxia of high altitudes (over 10,000 feet in the Andes). Mice had an increase in absorption of iron when exposed to hypoxia (Krantz et al., 1959) and a similar result was found within six to eight hours of exposure in rats (Greenberg et al., 1960). These authors found a rise in the turnover of serum iron and suggested that this might be the mechanism of the /

/the increase in absorption of iron. Thus, relative anoxia increases the red blood cell production, the serum iron and the absorption of iron. It is not clear which, if any, of these changes is the primary factor.

The influence of the endocrine glands on absorption of iron has been recently reviewed by Demulder (1958), the most obvious effect being the general acceleration of iron metabolism by thyroid hormone.

THE SITE OF ABSORPTION OF IRON FROM THE GASTROINTESTINAL TRACT.

It is generally believed that iron is absorbed principally in the duodenum and that absorption progressively diminishes distally in the jejunum and ileum.

Few direct experimental studies have been made in man to compare the capacity of different areas of the bowel to absorb iron. Evidence that the duodenum is principally responsible for this absorption has come from patients who have had a gastric resection. When the anastomosis after partial gastrectomy is of the Polya type in which the duodenum is by-passed, there is a higher incidence of anaemia than when the duodenum is still in continuity, as in the Billroth I operation (Bohmansson, 1950; Henley, /

/Henley, 1952; Wallensten, 1954). The amount of acid secreting gastric mucosa removed in each operation should be approximately the same. Thus it is inferred that when the duodenum is by-passed the risk of anaemia increases. Direct studies of iron absorption in patients who have had both types of operation have given conflicting results. Using serum iron as a measure of absorption Wallensten (1958) felt that patients after Billroth I anastomosis had a superior absorption. This was not confirmed by Duthie (1959) using radioactive iron and estimating blood and faecal levels; he found no appreciable difference between the two types of anastomosis. Comparison of groups of patients with these operations has shown no difference in absorption of iron when inorganic salts were used (Smith and Mallett, 1957).

In animals most of the evidence for the site of absorption of iron is histological or histochemical. In guinea pigs McCallum (1894) found that when a small dose of iron was given, iron was present in the bowel mucosa only near the pylorus but after a large dose it is found along most of the rest of the bowel. In 1946 Granick drew attention to the occurrence of a maximal /

/maximal concentration of the iron-containing protein Ferritin in the mucosa of the pyloric region and duodenum of the guinea pig after a dose of iron. The amount of ferritin became less distally and was absent by the mid-jejunum. Other workers confirmed this (Gillman and Ivy, 1947). The mucosal block theory of the control of the absorption of iron put forward by Hahn and his co-workers (1943) was supported by Granick (1946) who ascribed the block to ferritin and suggested that ferritin gave an indication of the site of absorption of iron. As previously mentioned, Endicott and his colleagues (1949) confirmed that histochemical iron was maximal in the duodenal mucosa of the guinea pig, and they showed that, after the ingestion of radioactive iron, the radioactivity in the mucosa did not coincide with the histochemical iron. This threw some doubt upon the validity of the histochemical findings in relation to the site of absorption of iron.

In the rat autoradiographs of the whole length of the bowel after intragastric administration of radioactive iron demonstrated radioactivity only at the pylorus and in the duodenum. When the rat was anaemic the radioactivity extended to the caecum (Wack and Wyatt, 1957). This work /

/work showed the area of intestine most impregnated with iron but this, while suggestive, did not necessarily mean that most iron was absorbed at that point. In vitro experiments using everted loops of rat intestine showed that an active transport of iron took place across the bowel wall from mucosa to serosa. A gradient of this transport existed greatest at the pylorus and diminishing in the jejunum and ileum (Dowdle et al., 1960). Previous work on the same preparation with slightly different experimental details did not point to any active transport (Brown and Justus, 1957).

Other evidence of the site of absorption of iron from the alimentary canal was presented by Hahn and his co-workers (1943) studying anaemic dogs using the incorporation of radioactive iron into the blood as the index of iron absorption. They found some absorption from a total gastric pouch. In one dog absorption of iron was 23% when the dose was given orally and only 6% when the same dose was given into a jejunal fistula, so by-passing the stomach and the duodenum. Further work by Stewart and colleagues (1950) confirmed the latter finding in another animal and also showed that less than 1% of iron introduced into the colon via fine catheter was /

/was absorbed. However, Reismann and others (1955) were able to produce acute iron intoxication in dogs and rabbits when the dose was large (50 to 750 mg/kg) whether the iron was given via a stomach tube or as an enema.

In brief, while fairly strong indications exist that the duodenum is the site of maximal absorption of iron this is not definitely proven and the relative importance of this area in the total intake of iron is not well established.

From the review of the literature it can be seen that no general agreement exists on the possible local mechanisms in the bowel lumen and in the bowel wall which may influence the absorption of iron and that the relative importance of the duodenum in the absorption of iron has not been completely defined. These are the main questions explored in the studies to be reported. The general factors noted in the review have been kept constant as far as possible throughout the investigations.

ABSORPTION OF IRON FROM ISOLATED LOOPS OF THE SMALL BOWEL OF DOGS.

ABSORPTION OF IRON FROM ISOLATED LOOPS OF THE SMALL BOWEL OF DOGS.

In the great majority of studies of iron absorption by the alimentary tract the iron has been given orally or by intragastric infusion, with the result that the absorptive capacity of the tract as a whole has been measured. Since the iron in solution in such experiments is diluted and absorbed as it passes along the bowel, the contribution of each segment of bowel cannot be assessed separately. For this purpose some form of isolation of areas of the bowel would be advantageous. Previous work along these lines has been done using both in vivo and in vitro techniques. The first such studies in man involved the injection of iron in solution through a triple lumen intestinal tube (Miller and Abbott, 1936) or behind the bulb of a double lumen intestinal tube (Groen and Taylor, 1937). Both groups studied the small bowel and estimated by chemical means the amount of iron remaining in the solution withdrawn from the bowel lumen. When ferric ammonium citrate was the test substance all the dose could be /

/be recovered by aspiration one half to one hour after its injection. Only 50% of ferrous iron similarly tested was recovered by Miller and Abbott although Groen and Taylor recovered all of it by repeated washing. The washings apparently released iron which had been adsorbed onto the bowel mucosa. Thus no obvious iron absorption was shown in the upper small bowel of man by these methods.

Similar findings were obtained by Groen and his colleagues (1947) in the rat. Six hours after a dose of iron had been injected into a loop of intestine just distal to the duodenojejunal flexure, the animal's abdomen was reopened, the loop excised and chemically analysed for iron. No iron seemed to be absorbed unless ascorbic acid or some other organic reducing agent or organic acid was added to the injection. Using radioactive ferric iron with ascorbic acid Hyde (1957) perfused segments of rat duodenum in vivo. The amount of iron retained by the rat was measured by tissue analysis at autopsy. This was proportional to the weight of the bowel segment expressed as a percentage of body weight but not to the amount of iron in the perfusate. Iron was absorbed by the rat throughout the perfusion (maximum duration 125 min). Active transport of iron across the wall of /

/of everted sacs of rat intestine in vitro could not be demonstrated by Brown and Justus (1954) but Dowdle and co-workers (1960), with a modified technique, did show such an active transport.

It can be seen that some difficulty has arisen in defining iron absorption by using loops of intestine in man and in animals. In the present study use has been made of isolated loops in the dog, and three different methods of measuring the absorption of radioactive iron have been used simultaneously. In this way the amount and duration of absorption of a single dose of iron has been examined. The effect of change of pH and of the addition of ascorbic acid on iron absorption has also been studied.

MATERIALS AND METHODS.

Sixty healthy mongrel dogs weighing 14 to 22 Kg. were used in acute experiments. Loops of the small intestine were formed so that the lumen was excluded from the contents of the rest of the alimentary tract. Solutions of radioactive iron of varying composition were put into these loops and the absorption of iron measured. Dogs were admitted to the study only if the haemoglobin level (measured as cyanmethaemoglobin) was more than 13 gm.% and the /

/the serum iron (measured by the method of Ramsay 1957) was more than 120 micrograms %. They were fasted for the 24 hours prior to the experiment, having been fed previously on standard kennel ration.

Preparation of the isolated loops.

The method was essentially similar to that of Code and his co-workers (1960). With the dog under pentobarbitone anaesthesia (25 mg./Kg.) a midline epigastric incision was made. Aseptic precautions were used in order to prevent any alteration in absorption which might result from early changes of peritonitis. A loop of duodenum and one of ileum were closed off from the contents of the rest of the alimentary canal by means of tape ligatures tied over plastic cannulae. Tape was used as it could be tied firmly and yet not cut into the bowel wall. The plastic cannulae each consisted of two Perspex spools, joined by a polythene catheter, which passed through one spool and was blocked at the end by the other. (Fig. 1). The spools on the duodenal cannula were 1.6 cm. in diameter and were 16 cm. apart. In the case of the ileal cannula these measurements were 1.1 cm. and 21 cm. respectively. These dimensions resulted in loops /

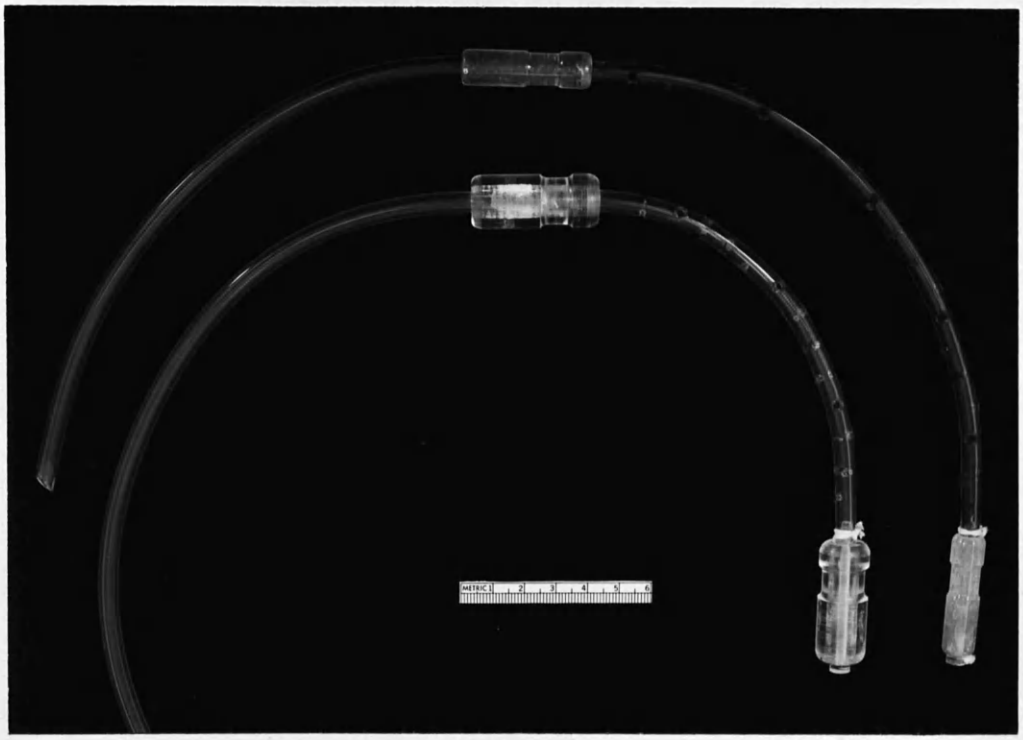


FIG. 1. (from Code et al., 1960)

Perspex spools and attached catheter used to isolate loops of small bowel: inner - for duodenal loop: outer - for ileal loop.

/loops approximately 100 sq. cm. in serosal area (95 to 115). An accurate measurement of the area was made at autopsy. The portion of the catheter between the two spools was perforated to allow filling and emptying of the isolated loop. The perforations were 0.3 cm. in diameter. In 29 dogs both a duodenal and an ileal loop were made; in 31 dogs only a duodenal loop was formed. The cannulae were introduced into the bowel through an incision in the antemesenteric border and passed in a caudad direction. After the tapes were tied a soft rubber catheter was put in to drain the blind end of bowel lumen proximal to the cannula, and so avoid any distension of the bowel due to accumulation of intestinal contents. The incision in the bowel was closed round these two catheters with atraumatic catgut sutures. The proximal end of the duodenal loop was at the level of the main pancreatic duct. The ileal loop ended 12 to 15 cm. from the ileocaecal valve. The ends of the tape ligatures were left long and were used to fix the extremities of the loops to the anterior abdominal wall. The attachment of the loops to the parietes allowed only a minimum of movement and facilitated direct scintillation counting of the radioactivity in the bowel. Care was /

/was taken throughout to avoid damage to the blood supply of the intestine.

A fine polythene catheter (internal diameter 1.0 mm.) was introduced into the portal vein through a jejunal radicle of the superior mesenteric vein in the 20 experiments in which portal blood was sampled. The abdominal wall was closed in layers around the catheters and cannulae. Thus any local cooling or drying of the loops was avoided and as physiological conditions as possible were maintained throughout the test period. The right femoral artery was also cannulated with fine polythene tubing. Both these catheters for blood sampling were kept patent with heparinised saline (1 i.u./ml.). The total operating time was about 45 min.

Test Procedure.

Light anaesthesia, short of spontaneous movements, was maintained throughout with small doses of pentobarbitone, usually about 10 to 20 mg./hour. The dog lay on its back and its temperature (measured on a rectal thermometer) was kept at 37 to 39 C. with an electric blanket under the animal, as it has been shown that a fall in body temperature can influence absorption from the /

/the small bowel (Lind, 1960). In fact, if the towels from the operation were kept draped over the dog additional heat was rarely needed.

The loops were washed clean of intestinal content with modified Tyrode's solution soon after their preparation. An interval of 30 min. was then allowed for the bowel to recover from the handling at operation and the distension of the cleansing irrigation and to absorb any residual irrigating fluid. Then 50 ml. of test solution was introduced into the lumen and serial samples of it were taken, usually at 15, 30, 45, 60, 90 and 120 min. The volume and pH of the loop contents were determined at each interval. Portal venous and systemic arterial blood specimens were obtained every 30 min. At the end of the test period, usually 5 hours, the animal was exsanguinated through a carotid cannula after full heparinisation. At autopsy the loops were excised intact. Samples were taken of liver, spleen, kidney and thoracic vertebral bone marrow and samples of lung, myocardium, skeletal muscle, bile and urine on occasion.

Test Solutions.

The test solution consisted of 50 ml. modified Tyrode's solution (Code and McIntire, 1956) containing /

/containing amounts of iron varying from 0.5 to 10 micrograms labelled with 5 to 10 microcuries of radioactive iron (Fe 59). The modified Tyrode's solution provided the following concentrations which are close to those in dog plasma:

Sodium	149.0 mEq./L	Bicarbonate	12.0 mEq./L
Potassium	3.3 "	Chloride	143.0 "
Calcium	2.7 "	Phosphate	0.7 "
Magnesium	0.2 "		

This solution avoided any disturbance of bowel content due to osmotic gradients. Ferric chloride or ferrous sulphate was used and in all experiments the carrier and the radioactive iron were in the same chemical form. The chelating agent Fe-3- specific versene was added in 3 tests. Deuterium oxide (0.4 ml.) was added as a label for water. The solution was at a temperature of 37 C and a pH of either 2 or 7. In some experiments the test solution contained 100 mg. ascorbic acid.

DETERMINATION OF THE AMOUNT OF IRON ABSORBED.

The amount of iron absorbed from the loops was estimated in the following 3 ways:-

A. Direct in vivo scintillation counting over the loop /

/loop.

B. Analysis of the loop and its contents after the 5 hours of the test.

C. Analysis of plasma and tissue samples.

A. Direct Scintillation Counting.

A portable thallium-activated sodium iodide crystal was firmly clamped about 15 cm. above the area of the anterior abdominal wall to which the loop was attached (Fig. 2). The lead shield around the crystal projected 1 cm. beyond its extremity giving a degree of collimation. A graphic record of the disintegrations observed was obtained on a direct-writing rectilinear ammeter (Fig. 38b). In general only one such counter was available and it was placed over the duodenal loop. When a second counter was used it was fixed over the ileal loop.

A background reading was obtained and a standard was placed 15 cm. in front of the crystal before and after the test to check that no change in sensitivity had occurred in the interim. The reading when the counter had stabilised immediately after the introduction of the radioactive iron into the loop was taken as 100%. The change in radioactivity was related to this level. /

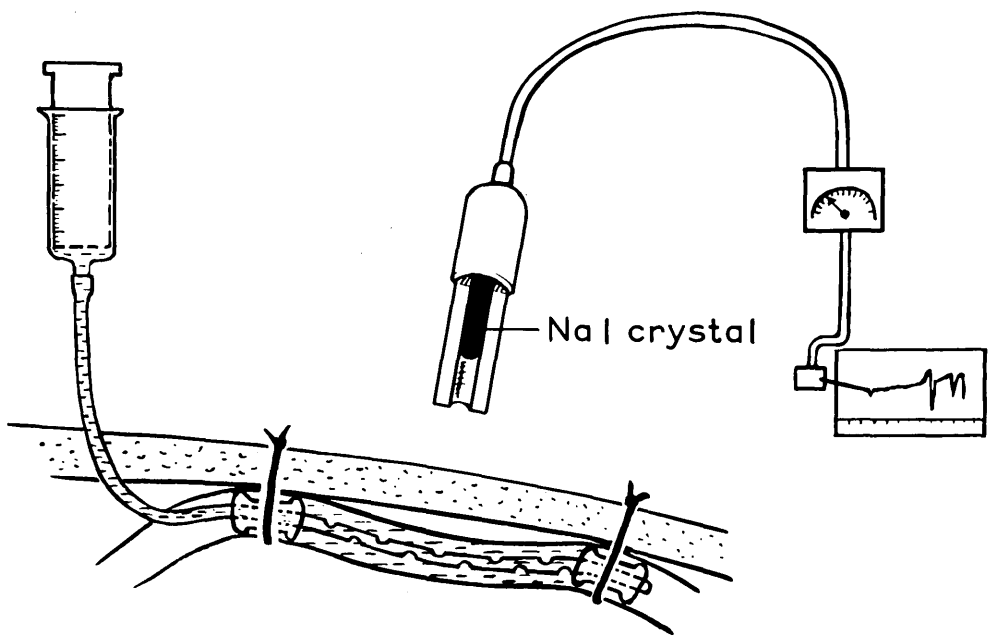


FIG. 2.

Diagram of arrangement of scintillation crystal placed over isolated loop of intestine containing radioactive iron in solution which can be sampled via the cannula. The attachment of the loop to the abdominal wall is shown.

/level.

Possible Errors.

It was hoped to record the decrease in the radioactivity in the loop resulting from absorption of iron. Any change of position of the loop relative to the crystal would give a false reading. This was avoided as far as possible by the firm fixation of the crystal and by the attachment of the ends of the loops to the abdominal wall.

A change in reading on the counter might indicate a change in concentration rather than a change in the radioactivity of the loop. This was shown not to be the case by the rapid addition of 40 ml. Tyrode's solution to a loop containing 5 ml. of radioactive fluid. No appreciable change in radioactivity recorded occurred. It is worthy of note that the maximum volume used, 50 ml. was not sufficient to distend the segments. Further evidence along these lines came from the several tests in which the radioactivity reading remained constant while the volume in the loop decreased rapidly. It would appear that the slight changes in position attendant on changes of volume of fluid in the loop did not cause artefacts in the recording of the radioactivity.

The uptake of iron in some tissues, especially /

/especially the liver or vertebral bone marrow, might influence the counter at the same time as the disappearance from the loop. The crystal was directed away from the liver when the duodenal loop was being monitored and away from the vertebrae in case of both duodenal and ileal loops. In addition, the collimation of the crystal helped to obviate such errors. Furthermore, the amount of radioactivity in the other organs was dispersed widely.

It is well known that electrolytes have a bi-directional flux across the intestinal mucosa (Visscher et al., 1944). If the bowel handled iron in a similar manner, a falsely high reading would result in the direct counter indicating a smaller absorption than had occurred. In the case of iron, there is known to be a very small daily excretion into the gastrointestinal tract (McCance and Widdowson, 1937; Dubach et al., 1949; Ingalls and Johnston, 1954; Finch and Loden, 1959). The extent of such bi-directional flux was estimated in this preparation by intravenous infusion of 10 micrograms ferric iron labelled with 15 microcuries Fe 59 in 2 dogs. The test procedure was as outlined above except that no iron /

/iron was put into the loops. Even at the end of 5 hours no excretion of radioactive iron was detected in either the duodenal or the ileal loops.

The apparent fall in radioactivity of the loop could have been due to a leak of the Fe 59 past the cannulae and into other parts of the bowel. Careful examination of the adjacent segments of gut failed to reveal any radioactivity. Radioactive iron did not leak through the bowel wall into the peritoneal cavity.

Selection of records for detailed analysis.

Forty three records were available for 5 hour tests and 36 of these were deemed suitable for detailed analysis. This analysis was to estimate the rate of absorption in consecutive periods of 15 min. throughout the experiment. Before a record was considered suitable there had to be a smooth curve of disappearance between all of the 15 min. points of reference and no artefacts. While this was achieved during most of the time of all tests, it failed occasionally and records wherein this occurred were discarded in their entirety as they could not be analysed accurately in these periods. They were still valid as an estimate of the overall absorption of iron. /

/iron.

B. Analysis of the Loop and its Contents.

At the end of the 5 hour test period the loop was emptied through the cannula prior to the exsanguination of the animal. Then 100 ml. Tyrode was used to irrigate the loop. Both these quantities of bowel content were found to contain particles of mucus and their radioactivity was counted as a whole in plastic cups above a well type Geiger-Muller counter and compared with the radioactivity of a sample of the test solution diluted to an equal volume. Even with this arrangement the mucus which contained the greater part of the radioactivity tended to give a falsely high reading when it slowly settled out. This was obviated by interrupting the counting period of 100 seconds on three occasions to mix thoroughly the contents of the cup, and so approximate to a homogeneous distribution of radioactivity.

At autopsy the loop was excised entire with the cannula in situ. The tape ligatures were cut and the loop was allowed to slide off the cannula into a boiling tube. A plug of cotton wool was used to compress the loop into the bottom of the tube. The radioactivity was counted by placing the tube just above a well type Geiger-Muller counter (Texas Instr.) and was compared with a control sample of the test solution diluted to the same volume. /

/volume. The cannula itself was similarly examined.

The amount of radioactive iron remaining in the loop and attached to the cannula was subtracted from the amount given. Allowance was made for the Fe 59 in the serial samples of the intestinal fluid taken during the course of the test and for the radioactivity on the glassware, 50 ml. syringes and measuring cylinders, which was counted by the Geiger-Muller well. Up to 3% of the dose adhered to them.

Possible Errors.

The geometry of the specimens and the control samples were matched as nearly as possible to obviate errors in counting on this score. However, the cannulae, the loops and the glassware would not have the homogeneous distribution of the radioactive iron of the control specimens. The possible effect of this uneven distribution was lessened by having the specimens at some distance from the Geiger-Muller tubes.

C. Analysis of Tissue and Plasma Specimens.

At necropsy two samples of approximately 4 gm. were taken of each tissue, the precise wet weight noted, and the wet weight of the whole organ determined. The /

/The radioactivity of the samples was estimated immediately in a well type thallium-activated sodium iodide crystal and was compared with that of control samples of equal volume. Bone marrow was taken as 2% of body weight (Fairman and Whipple, 1933). Two specimens of plasma each 2 ml. were assayed and plasma volume taken as 4% of body weight (Von Porat, 1951). The radioactive iron in the body was calculated from the amount in the liver, the spleen, the kidneys, the bone marrow and the plasma.

Possible Errors.

The presence of blood in the tissue samples might give a falsely high reading of radioactivity. This was mostly avoided by exsanguinating the animal. In addition, the concentration of radioactivity in the plasma was almost always at very low levels at 5 hours so that any residual blood in the tissue would have a small effect. Two samples of each organ were taken from separate sites to balance the error arising from the lack of homogenising. Close results of the duplicates was found, the maximal difference being less than 2 counts per sec./gm.

The assumption of a normal value for any measurement in the body inevitably causes some error in the individual case. Two such assumptions have been made: one /

/one for bone marrow and the other for plasma volume. Four per cent of body weight is at the lower limit of normal for plasma volume in the dog. This was to allow for the slight blood loss during the operation and for the samples which amounted to about 90 to 150 ml.

The assay of the radioactive iron was limited to the organs listed since control studies in four animals showed barely detectable concentrations of Fe 59 in the other tissues of the body. In addition, 2 dogs were given intraportal infusion of 10 micrograms Ferric iron labelled with 15 microcuries Fe 59 over a period of 1 hour. Duodenal and ileal loops were isolated and the procedure was the same as described above except that there was no iron put into the loops. The recovery using the above method of tissue and plasma analysis was 92 and 94 % respectively.

Comparison of the Three Methods of Estimating Iron Absorption.

In 43 readings in 36 experiments, methods A and B bore a close relationship to one another ($r = 0.86$ $P < 0.001$ Fig. 3). In the results section of this report the figures for 5 hour iron absorption are given as the means of methods A and B. When this mean value was compared with the results of method C the relationship was again close. In 18 experiments using method C in which a duodenal loop alone was formed $r = 0.83$ $P < 0.001$ and on 21 occasions when both duodenal and ileal loops were used in the one animal /

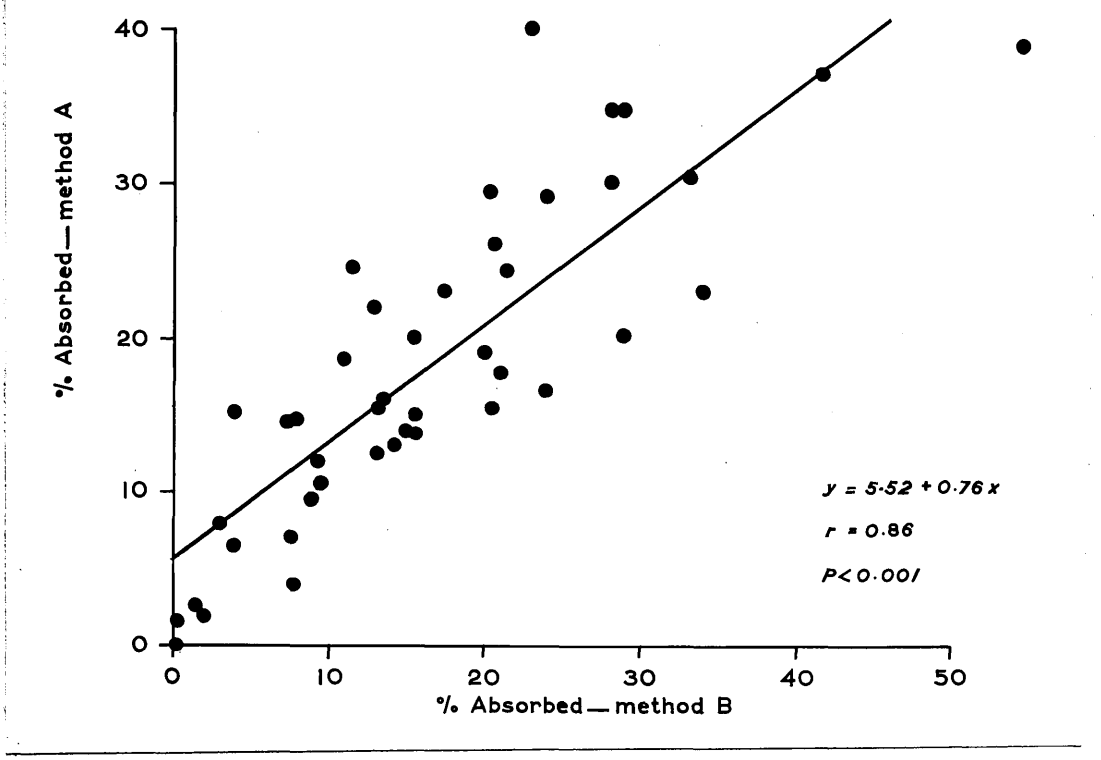


FIG. 3.

Correlation between in vivo scintillation counting (Method A) and estimation of intestinal loop and contents (Method B) as measures of absorption of radioactive iron from 43 isolated loops of small intestine in the dog.

/animal $r = 0.89$ $P < 0.001$. In the latter group this gave confirmation of the total amount absorbed but did not indicate the part played by each loop.

Counting of Radioactivity.

In all experiments a minimum of 4,000 counts were recorded for each sample or counting was continued for 1,000 seconds.

In the sodium-iodide (thallium activated) well type scintillation counter, 1 microcurie of Fe 59 gave about 10,000 counts per second over a background of about 2 counts per second. Corresponding figures for the Geiger-Muller well type counter used with the plastic cups were 1,000 counts per second and 7 counts per second.

Other Determinations.

The concentration of dueterium oxide in the test solution and in the sample of intestinal fluid obtained at 15 min. was estimated on the mass spectrophotometer (Solomon et al., 1950; Code et al., 1954). The bi-directional rates of flux were calculated using the formula of Visscher and colleagues (1944). The nomenclature used in reporting the results of movement of water is that recommended by Code (1960) in which movement of water from bowel lumen to the blood is styled insorption; movement /

/movement from the blood into the bowel lumen is called exsorption. Absorption is said to occur when insorption exceeds exsorption, so that there is a gain to the blood. Enterosorption is when a net loss to the blood takes place, that is, when exsorption is greater than insorption. A Beckman model G pH meter was used to estimate the pH of the intestinal fluid.

In 6 dogs autoradiograms of histological sections of the duodenal and the ileal loops were made using standard X-ray film (Eastman Kodak) exposed from 1 to 4 weeks.

In 3 dogs samples of the intestinal fluid were examined to assess the radioactivity attached to protein at the various time intervals. The radioactivity of each sample was measured before and after centrifugation. The supernatant was then treated with 10% trichloracetic acid and the radioactivity again estimated. The results were compared with aliquots of the test solution treated in the same manner.

Statistics.

Standard statistical methods were employed (Fisher, 1930; Moroney, 1953) using the distributions of r and students "t" as measures of significance (Fisher and Yates, 1948). /

/ .. 1948).

The following symbols have been used in the text and illustrations.

S. D.	=	Standard deviation
S. E.	=	Standard error
S. E. M.	=	Standard error of the mean
S. E. M. Diff.	=	Standard error of the mean difference
P	=	Probability
d. f.	=	Degrees of freedom
b	=	Regression coefficient (slope of regression line).
r	=	Correlation coefficient
t	=	Students "t"

RESULTS.

Iron absorption is expressed per 100 sq. cm. serosal area of the loop and is the mean of methods A and B unless otherwise stated. The results of the plasma samples are reported separately.

Comparison of duodenum and ileum.

In 18 experiments identical test conditions were maintained in both duodenal and ileal loops. Duodenal absorption of iron was up to 14 times greater per 100 sq. cm. serosal surface than that of the ileum in 12 tests. The ratio of duodenal to ileal absorption was greatest when the pH of the test solution was 2 and ascorbic acid was added. Under these conditions the ratio was significantly greater than unity (Table I). /

TABLE I.

COMPARISON OF THE RATIOS OF THE AMOUNT OF IRON ABSORBED BY DUODENAL AND ILEAL LOOPS SIMULTANEOUSLY IN THE SAME ANIMAL.

Test Conditions	No. of tests	Mean Ratio Duod/Ileum	Diff. from Unity P
pH 2 Vit C	9	3.57	< 0.01
pH 2	6	1.48	< 0.2
pH 7 Vit C	3	0.88	< 0.5

In view of the tendency to skewness of distribution the logs of the ratios were used to assess significance ($t = 3.63$ $p < 0.01$).

/ (Table I).

Factors influencing Iron Absorption.

a) Dose: Duodenum. There was a linear relationship on a log/log scale between the dose of iron and the amount absorbed in the 39 tests reviewed ($r = 0.508$ $P < 0.01$ Table II). Further analysis revealed that this relationship did not hold equally under different test conditions. In 17 experiments when 100 mg. ascorbic acid were added to the test solution of pH 2 the linear relationship was most significant ($r = 0.90$ $P < 0.001$ Fig. 4). It was less significant in the 13 experiments where the initial pH was 2 and no ascorbic acid was present ($r = 0.66$ $P < 0.01$ Fig. 5). The relationship did not hold when the initial pH of the test solution was 7, whether the ascorbic acid was present or not (9 tests).

Ileum. In 20 experiments with varying test solutions the relationship noted in the case of the duodenum was again present but less significant ($r = 0.54$ $P < 0.02$ Table II). Subdivision of the tests revealed a correlation only when the initial pH was 2 and ascorbic acid was present ($r = 0.8722$ $P < 0.001$ Fig. 6).

The amount of iron absorbed, expressed as a percentage of the dose, increased, as the dose was reduced in duodenal (Fig. 7) and ileal (Fig. 8) loops. This was /

Isolated Loop	Test Conditions	No. of tests	Correlation of logarithm of amount of iron given and logarithm of amount absorbed		
			Regression Coeff. b	Correlation Coeff. r	P
Duodenum	pH2 + Vit C	17	0.967	0.901	< 0.001
	pH2	13	0.285	0.663	< 0.01
	pH7 with or without Vit C	9	1.601	0.398	< 0.1
	All tests	39	0.807	0.508	< 0.01
Ileum	pH2 Vit C	10	1.149	0.872	< 0.001
	pH2	6	0.491	0.507	< 0.1
	pH7 with or without Vit C	4	0.956	0.340	< 0.1
	All tests	20	0.848	0.544	< 0.02

TABLE II.

Correlation of the logarithm of the amount of iron given and the logarithm of the amount of iron absorbed from 39 isolated ileal loops under various test conditions.

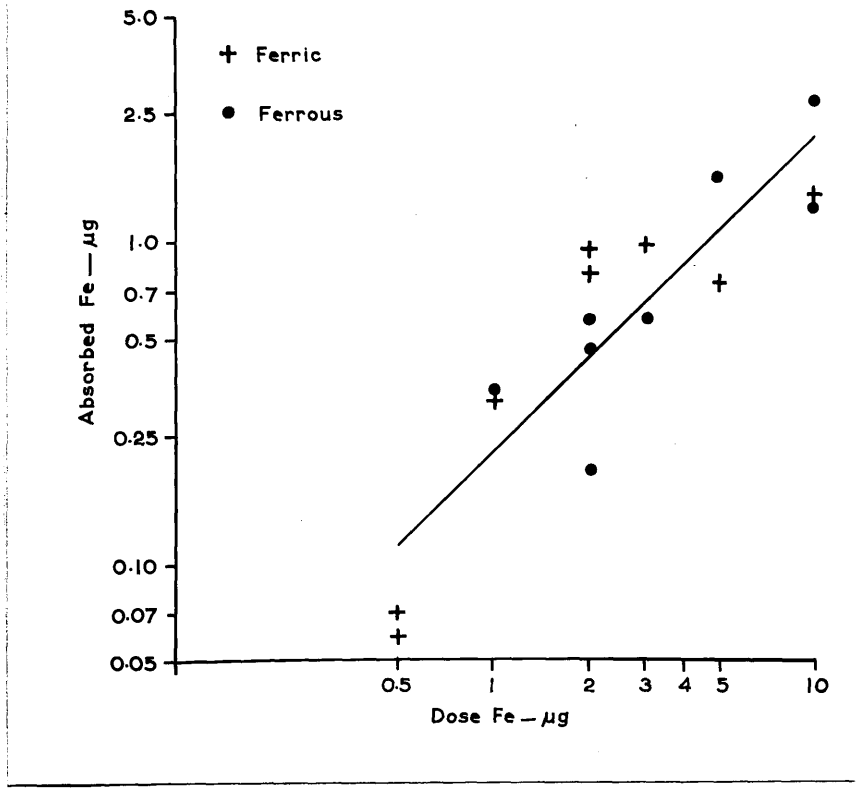


FIG. 4.

Correlation of the logarithm of the amount of iron given at pH 2 and with ascorbic acid and the logarithm of the amount of iron absorbed from 17 isolated duodenal loops.

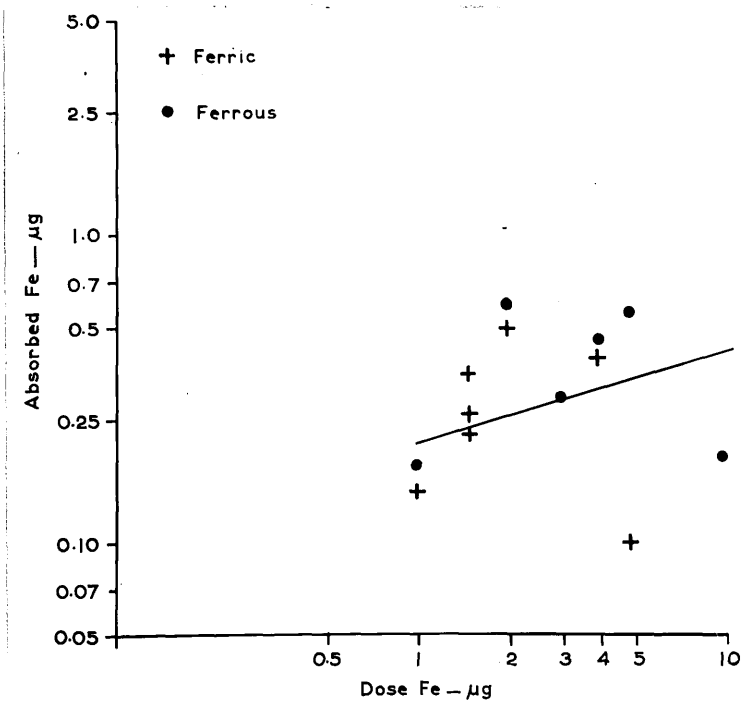


FIG. 5.

Correlation between the logarithm of the amount of iron given at pH 2 without ascorbic acid and the logarithm of the amount of iron absorbed from 13 isolated duodenal loops.

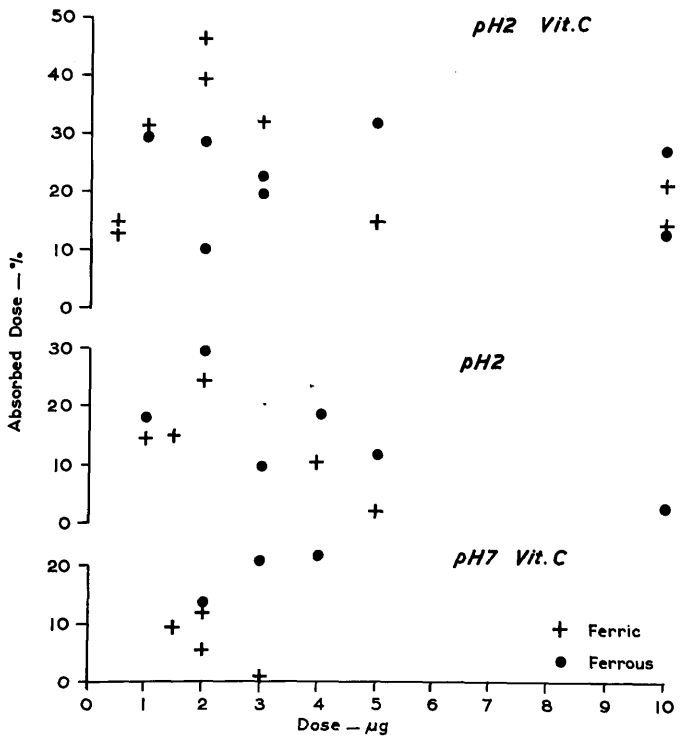


FIG. 7.

Percentage absorption of various doses of radioactive iron in isolated duodenal loops under varying test conditions; pH 2 with ascorbic acid: pH 2 without ascorbic acid: pH 7 with ascorbic acid.

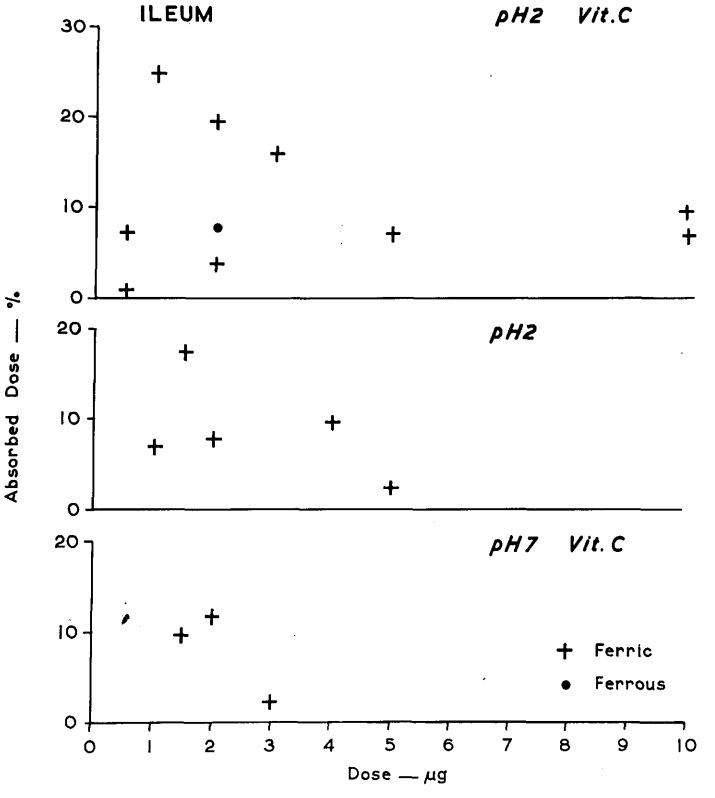


FIG. 8.

Percentage absorption of various doses radioactive iron in isolated ileal loops under varying test conditions: pH 2 with ascorbic acid: pH2 without ascorbic acid: pH7 with ascorbic acid.

/was true until small doses (0.5 to 1 micrograms) were reached. At this level the percentage absorption was reduced below the value for a dose of 2 micrograms.

b) Acidity and Ascorbic Acid.

No appreciable iron absorption was observed either in duodenum or ileum from test solutions of pH 7 containing no ascorbic acid (2 tests). Giving the iron at a pH of 2 or adding ascorbic acid and keeping the pH at 7 both gave the same order of absorption (Figs. 7 & 8). When both these factors were present together the percentage absorption was approximately doubled (Figs. 7 & 8).

c) Ferric and Ferrous Iron.

Ferrous iron was absorbed to a greater extent than equivalent doses of ferric iron only in test solutions of pH 7 to which ascorbic acid had been added (Fig. 7).

d) Chelation.

In 3 tests Fe-3-specific versene, an iron-chelating agent was added to the test solution which was given at pH 7 to 8. The absorption was equivalent to when the same dose had been given with ascorbic acid at pH 7 (Table III).

Duration and Rate of Iron Absorption.

Duration: The direct recordings from the scintillation /

Dog	Dose of Iron micrograms	Absorption of Iron % Dose	
		With Vit C	With Versene
S390	2.0		10.7
P815	2.0	11.8	
S933	2.0	8.8	
S.365	3.0		4.3
P808	3.0	0.1	
S52	10.0		9.0
S73	10.0*	12.3	

* at pH 2

TABLE III

Percentage absorption from isolated duodenal loops of iron given in solution at pH 7 with ascorbic acid or with versene.

/ scintillation counter fixed over the loops showed that absorption was rapid in the first 60 min. and had stopped or slowed to insignificant levels by 120 min. in most cases (Fig. 9). In others in duodenal loops there was a second small absorption at about 3 hours (Fig. 10). The mean amount of iron remaining in duodenal loops (31 tests) at each time interval is shown in Fig. 11. The virtual cessation of absorption by 120 min. occurred with all variations of the test solution. A similar pattern of absorption was found in the 5 suitable ileal recordings.

RATE:-

The mean amount of iron remaining in the isolated duodenal loops diminished in an exponential fashion in the first two hours at a rate of 1.98 % min. ($T\frac{1}{2}$ 35 min.). Fig. 12. When individual tracings were studied this exponential curve was found in only 10 of 31 tests. The mean rate of this group was 1.73% min. (Fig. 13). In another 7 tests there were two components, an initial slow one, mean 0.76 % min. in the first 15 to 30 min. and a later one, mean 2.04% min. similar to the first group.

In view of the lack of a uniform exponential disappearance curve the following convention was adopted in order to compare all tests. It was assumed that the rate /

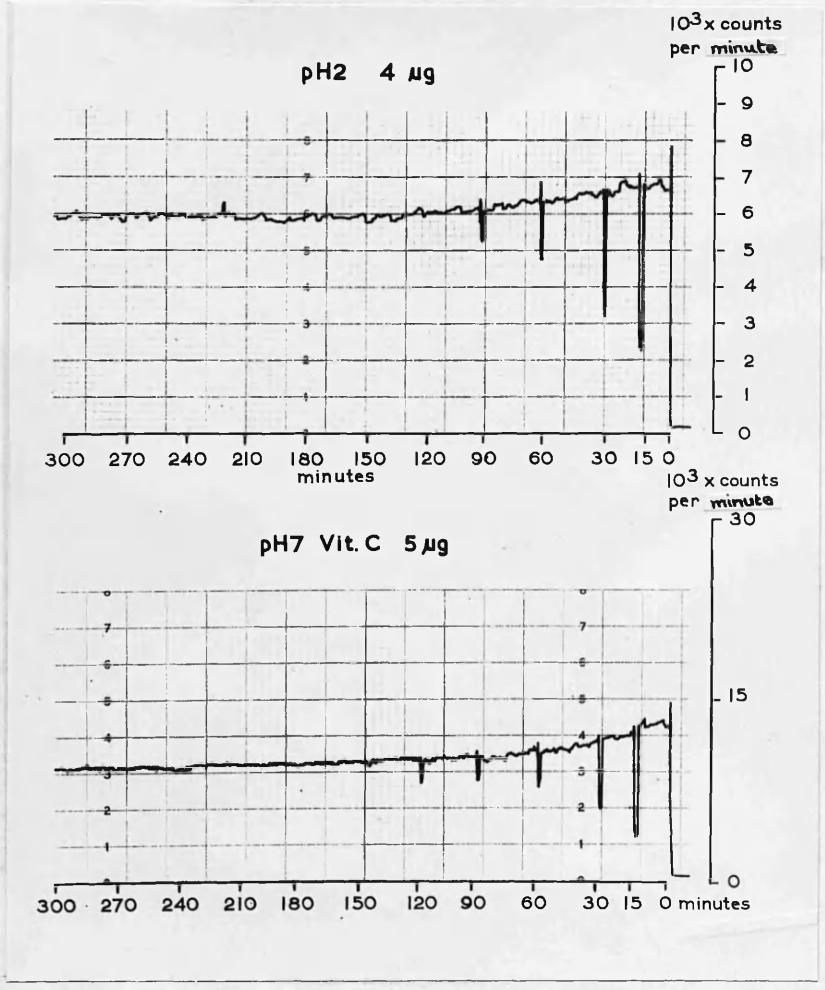


FIG. 9.

Recordings, reading from right to left, from the direct writing in vivo scintillation counter showing flattening out at 120 mins.

Upper tracing - Dog T52 4 micrograms ferrous iron at pH 2

Lower tracing - Dog T53 4 micrograms ferrous iron at pH 7 with 100 mg. ascorbic acid.

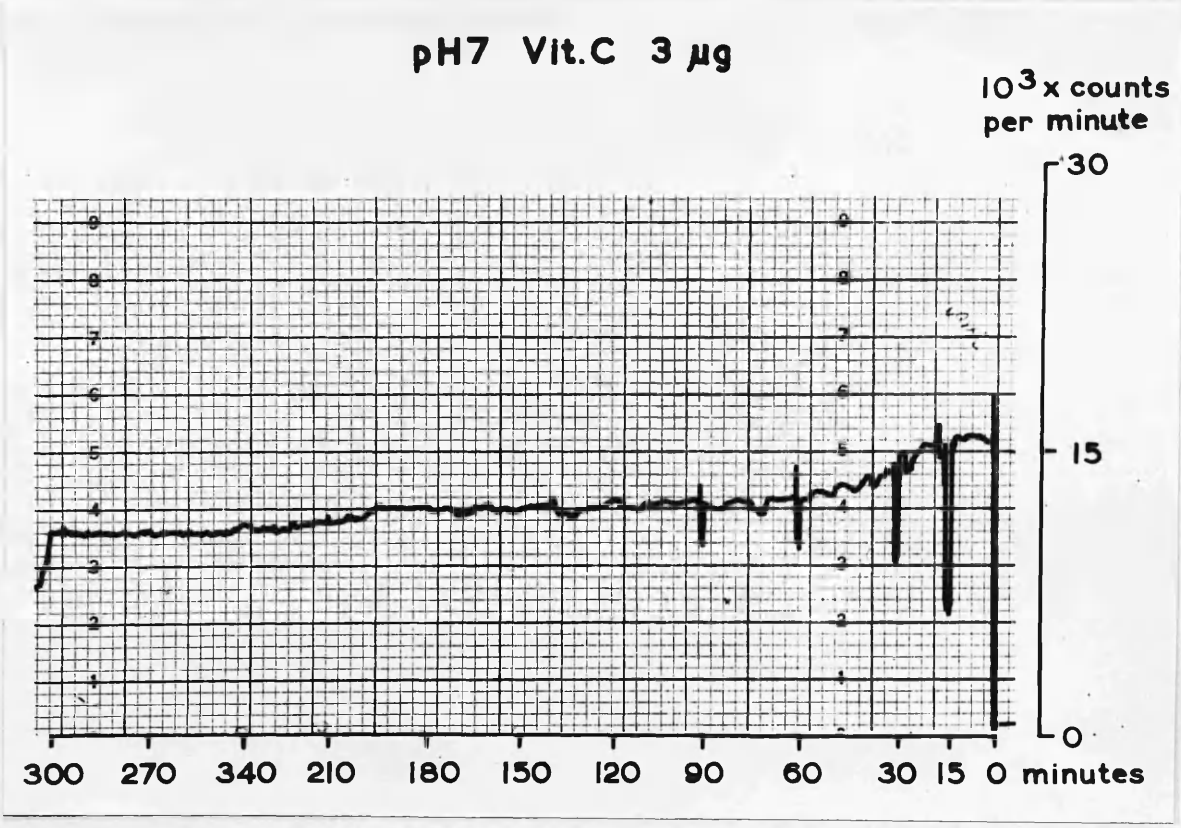


FIG. 10. Dog T15

Recording, reading from right to left from direct writing in vivo scintillation counter showing second phase of absorption at $3\frac{1}{2}$ hours from test solution containing 3 micrograms of ferrous iron at pH 7 with 100 mg. ascorbic acid.

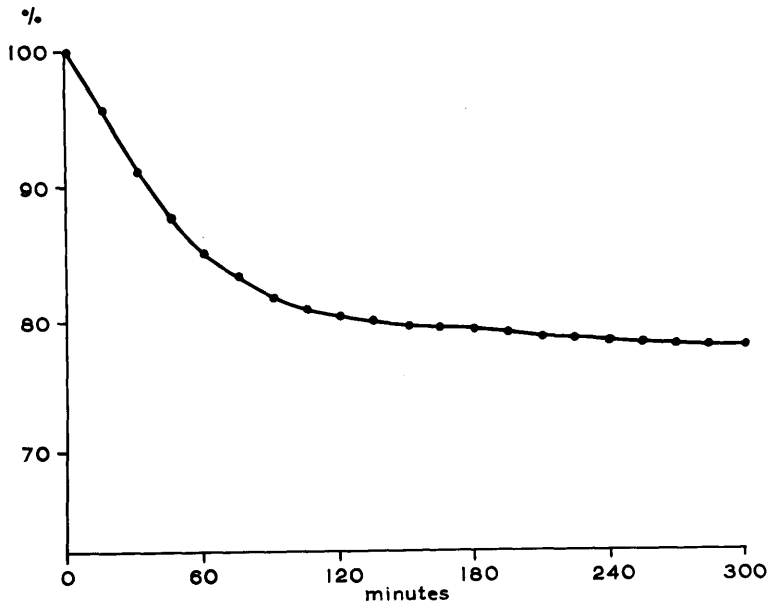


FIG. 11.

Amount of radioactive iron remaining in the isolated duodenal loops as recorded by the in vivo scintillation counter - expressed as per cent of dose of iron. Mean of 31 tests.

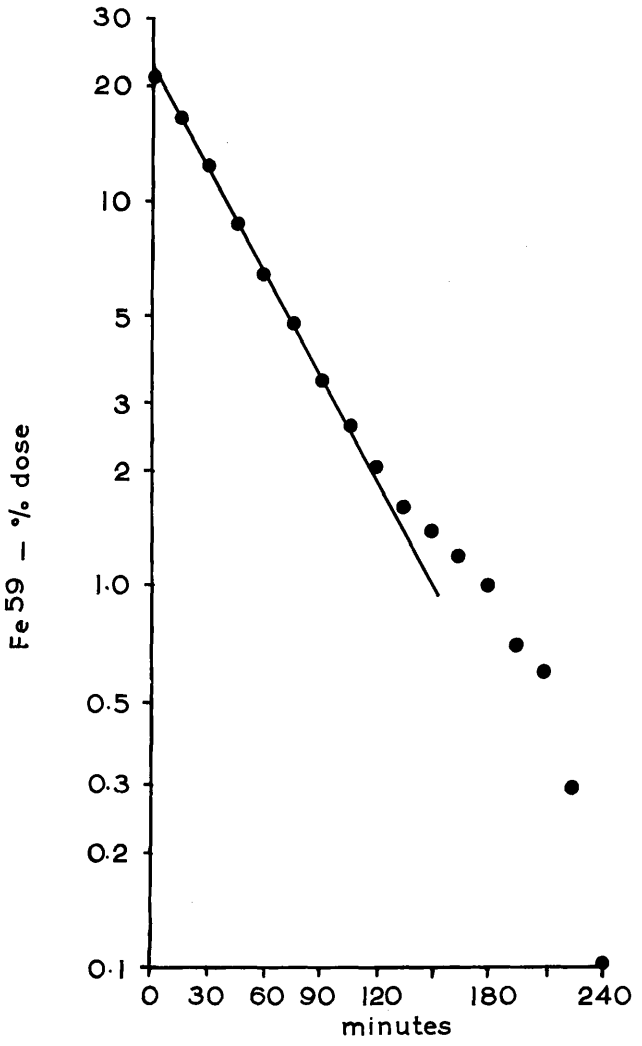


FIG. 12.

Mean values for radioactive iron remaining in 31 isolated duodenal loops plotted semi-logarithmically showing exponential rate of change in first 2 hours.

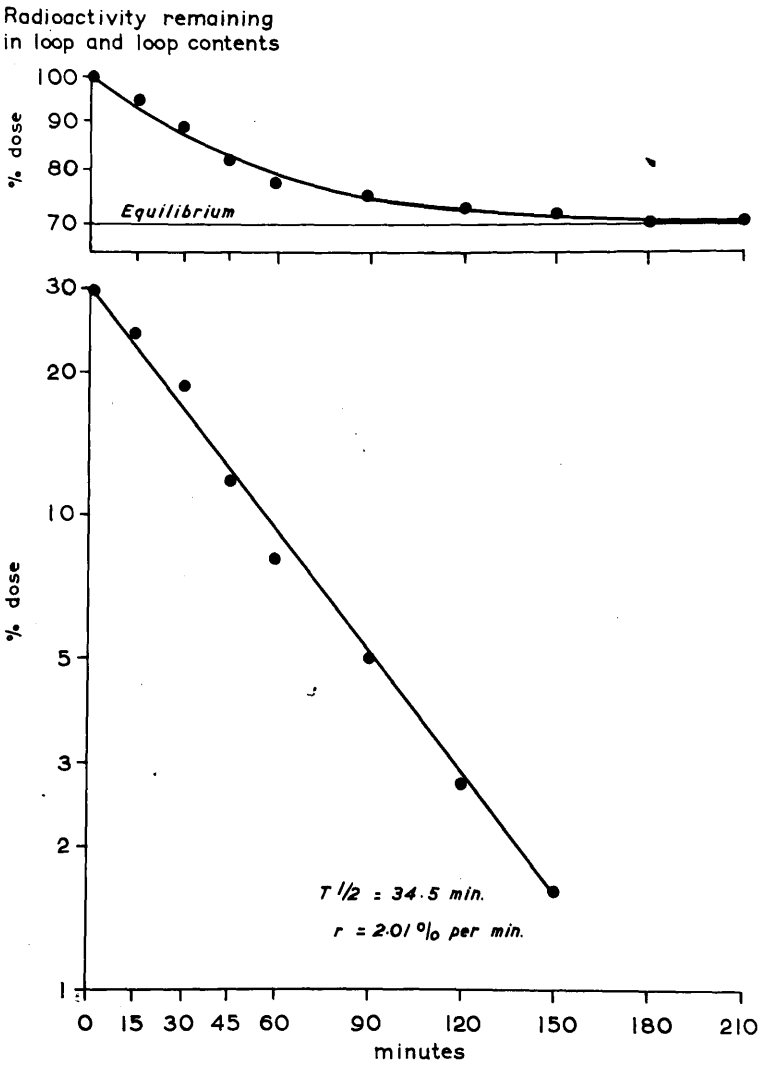


FIG. 13.

Upper Chart - Percentage of dose remaining in isolated loop and contents as recorded by in vivo scintillation counter in dog T53.

Lower Chart - Exponential rate derived from equilibrium value in upper chart.

/rate of absorption would be constant over a period of 15 min. and consecutive 15 min. periods were studied. The rate was expressed as per cent of the amount of radioactive iron remaining in the loop per 100 sq. cm. of serosal area per min.: it ranged up to 0.7 per cent per min. The mean rates for the duodenum (31 tests) are illustrated in Fig. 14. The maximum rate was in the period from 15 to 30 min. after the administration of the dose in 18 of the 31 tests; of the remaining 13, 6 were maximal in the first 15 min., and 7 from 30 to 45 min. In the case of the 5 ileal recordings 2 were fastest in the first 15 min. period, 2 in the second 15 min. period and 1 showed only minimal absorption at 120 min. The presence of ascorbic acid or an initial pH of 2 made no significant difference to the maximal rates of iron absorption.

The amount of iron absorbed in the 5 hour test was significantly correlated with the duration of absorption in the duodenal loops ($r = 0.48$ $P < 0.01$ Fig. 15) but not with the maximal rate of absorption ($r = 0.10$ $P > 0.1$ Fig. 16).

Another measurement was possible from the direct recordings. When the fluid was withdrawn from the bowel /

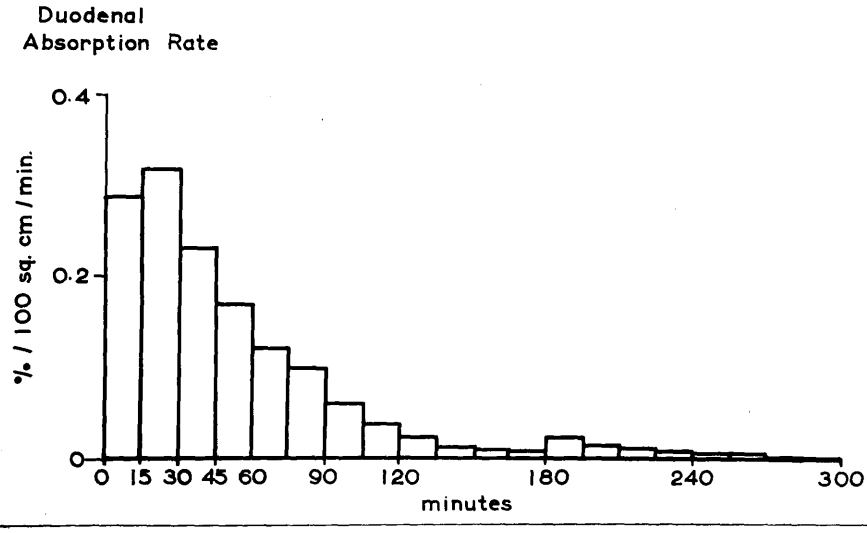


FIG. 14.

Mean rates of absorption of iron from 31 isolated duodenal loops expressed as percentage of amount of iron given per minute per 100 sq. cm. serosal surface of the loop during the 5 hour test period.

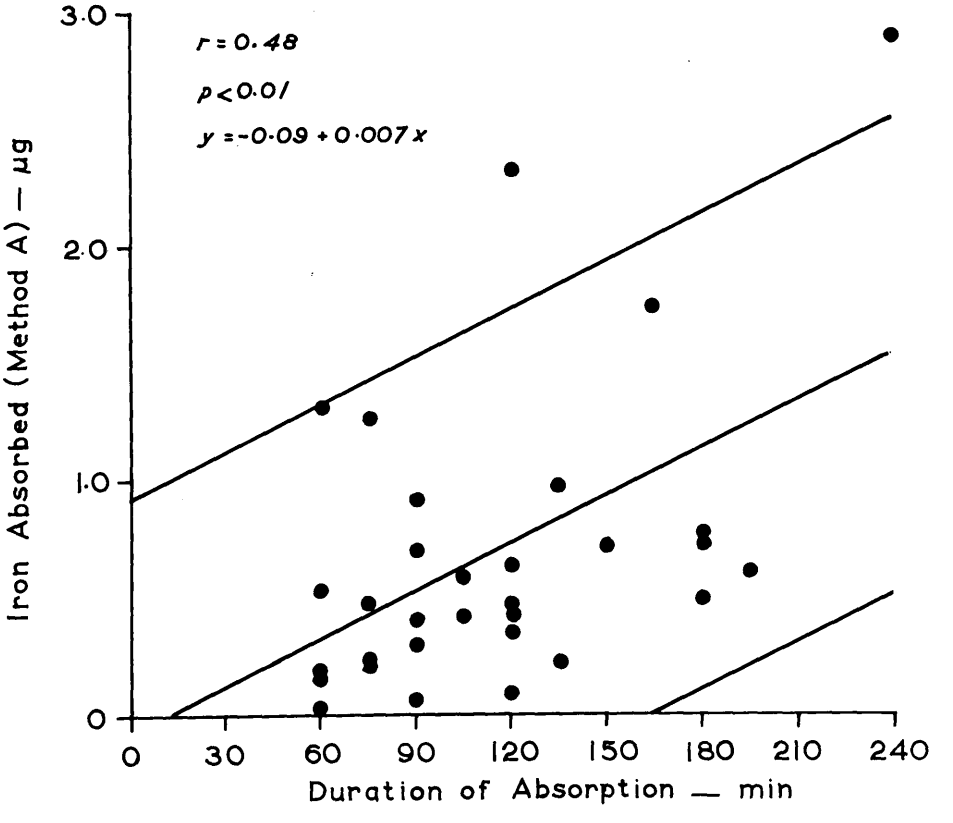


FIG. 15.

Correlation between amount of iron absorbed from 31 isolated duodenal loops and duration of absorption. Any slight further absorption at 3 hours and over is not included. The 95% confidence limits are shown.

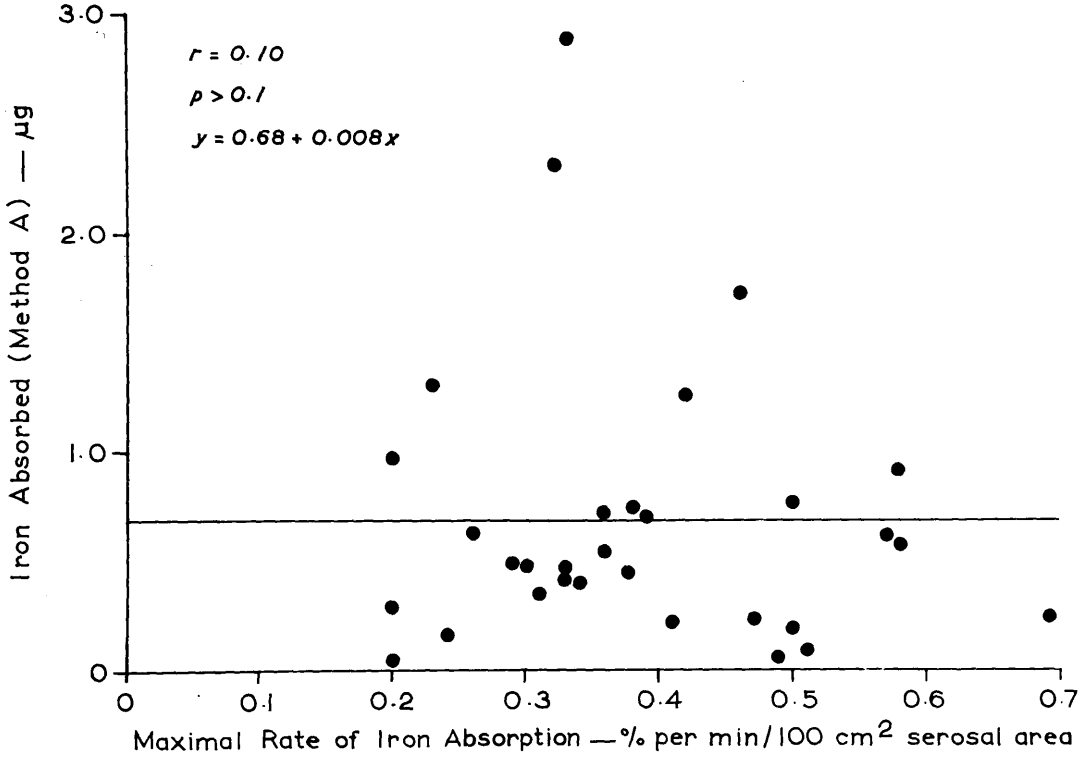


FIG. 16.

Correlation between amount of iron absorbed from 31 isolated duodenal loops and the maximal rate of absorption calculated for 15 minute periods. Correlation is not significant.

/bowel at intervals for sampling, the radioactivity fell to a level which indicated the Fe 59 remaining in the loop (Figs. 9 & 10). This radioactive iron which could not be aspirated increased in exponential fashion in 26 of 31 tests (Fig. 17). It reached equilibrium at 120 mins. after the iron was placed in the segment of bowel. The mean rate was 4.69% per min. Furthermore when the pH of the solution was 2, a significantly slower rate followed the addition of ascorbic acid (Table IV).

Possible Mechanisms for Cessation of Iron Absorption.

1. Mucosal block.

The effect of repeated doses of iron into the same duodenal loop was studied. After each dose the test lasted 120 min. with a 30 min. interval between tests. Three doses were given to each animal. The direct writing scintillation counter fixed over the loop was used to estimate the rate and amount of absorption (Fig. 18). When the composition of the test solution was the same in each of the three consecutive tests (3 dogs) a similar absorption occurred in all three tests in each dog with only a slight diminution in the amount absorbed by the end of the third test (Table V). The rate of absorption followed the same pattern in all three /

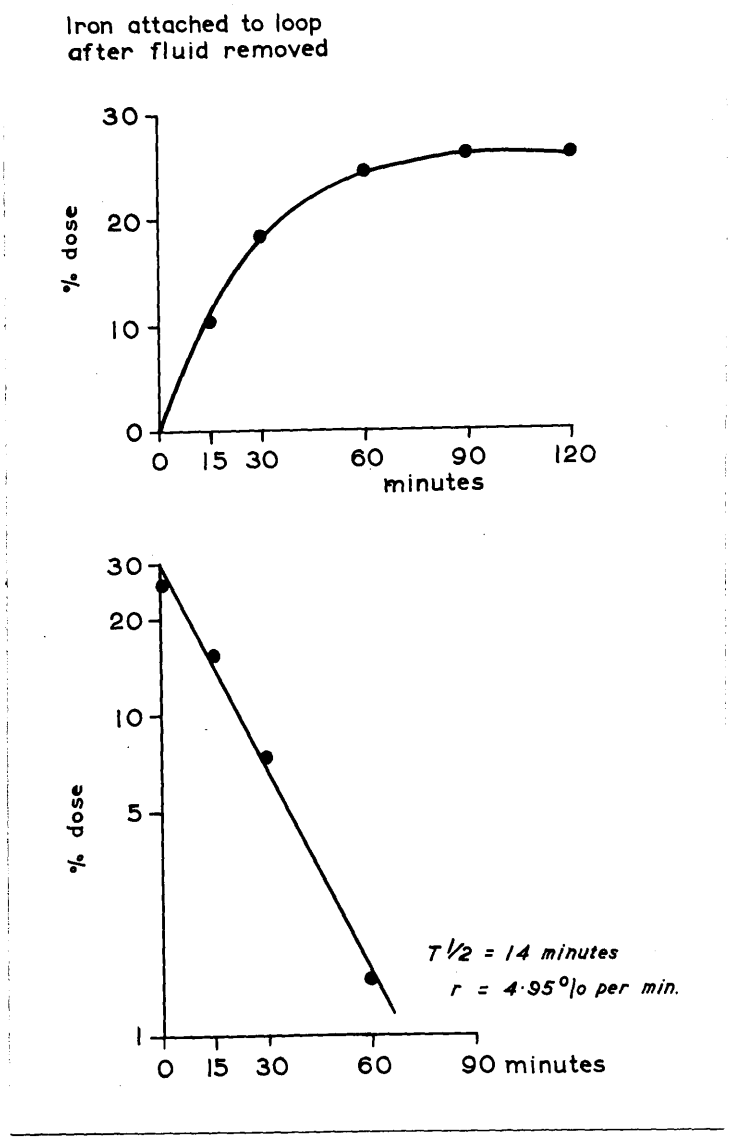


FIG. 17. Dog T53.

Upper Chart - Radioactive iron remaining attached to isolated duodenal loops when intestinal contents are withdrawn: values from in vivo scintillation counter.

Lower Chart - Semilogarithmic plot from upper graph showing exponential rate of change.

Test Conditions	No. of tests	Rate of iron in loop becoming unavailable for aspiration through cannula			
		Mean Half Time min.	Mean Diff.	t	P
pH2 Vit C	14	17.18 ± 1.33*	5.01 ± 1.92**	2.61	<0.02
pH2 No Vit C	9	12.17 ± 1.20*			
All tests	26	14.77 ± 0.68*			

* S. E. M.

** S. E. Diff. Means

TABLE IV.

Mean half times for rate at which iron is isolated duodenal loops became unavailable for aspiration through cannula showing the prolonging effect of ascorbic acid.

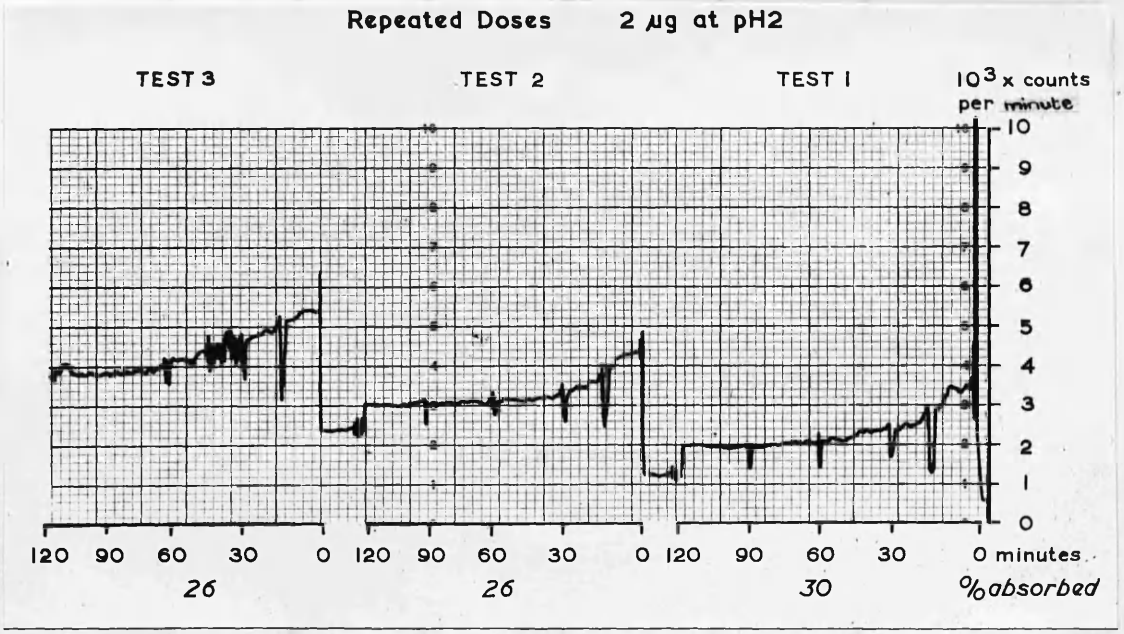


FIG. 18. Dog S942.

Recording, from right to left, from direct writing in vivo scintillation counter showing three tests of absorption of iron from 2 micrograms ferric iron at pH 2 placed in an isolated duodenal loop.

Dog	Test	Iron Dose ug	Initial pH	Vit C mg.	Iron Absorption %
S554	1	2	2	-	32
	2	2	2	-	28
	3	2	2	-	20
S942	1	2	2	-	30
	2	2	2	-	26
	3	2	2	-	26
S608	1	10	2	100	19
	2	10	2	100	17
	3	10	2	100	17

TABLE V.

Absorption of iron from repeated doses given into the same isolated segment of duodenum in 3 dogs.

/three tests in each animal, being greatest in the 15 to 30 min. period in 2 dogs and in the first 15 min. in the third.

2. Change of pH.

The curve of diminution of radioactivity during the test was similar to that of the change of pH of the loop contents (Fig. 19). This was shown not to be a causative relationship by maintaining the intestinal pH at less than 3.5 throughout the 5 hour test with small additions of hydrochloric acid in 2 dogs. Iron absorption was not significantly prolonged (Fig. 19). Conversely, when the pH of the loop contents was rapidly brought to neutrality by the addition of sodium hydroxide at 30 min. there was no sudden cessation of iron absorption (Fig. 20). Further evidence came from repeated doses given to one dog. After a control test of 2 micrograms ferric iron at an initial pH of 2, an identical solution was used for the second test and the pH of the bowel contents was kept below 3.5 by small additions of hydrochloric acid. (Fig. 21). The absorption of this second dose was slightly increased (28% as compared with 24% control) but no prolongation of absorption occurred. The third test identical to the first gave 26% absorption. (Fig. 22). /

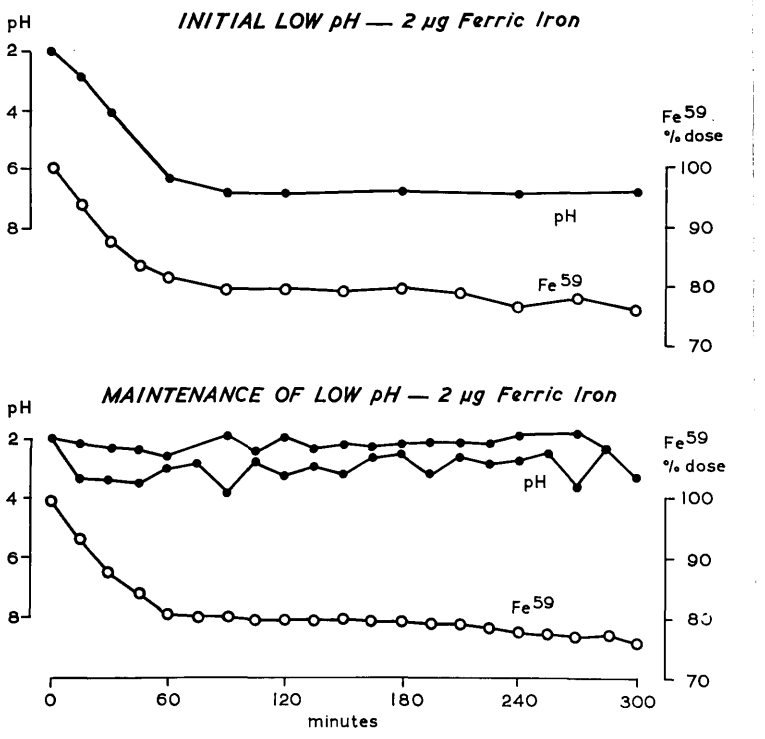


FIG. 19.

Curves of radioactive iron recorded in vivo by scintillation counter over isolated duodenal loop and pH of loop contents during tests of absorption from 2 micrograms of ferric iron placed in isolated duodenal loops.

Upper Chart - Test solution at initial pH of 2.

Lower Chart - pH of intestinal fluid maintained at less than 3.5 throughout 5 hour test period with no change in absorption of iron.

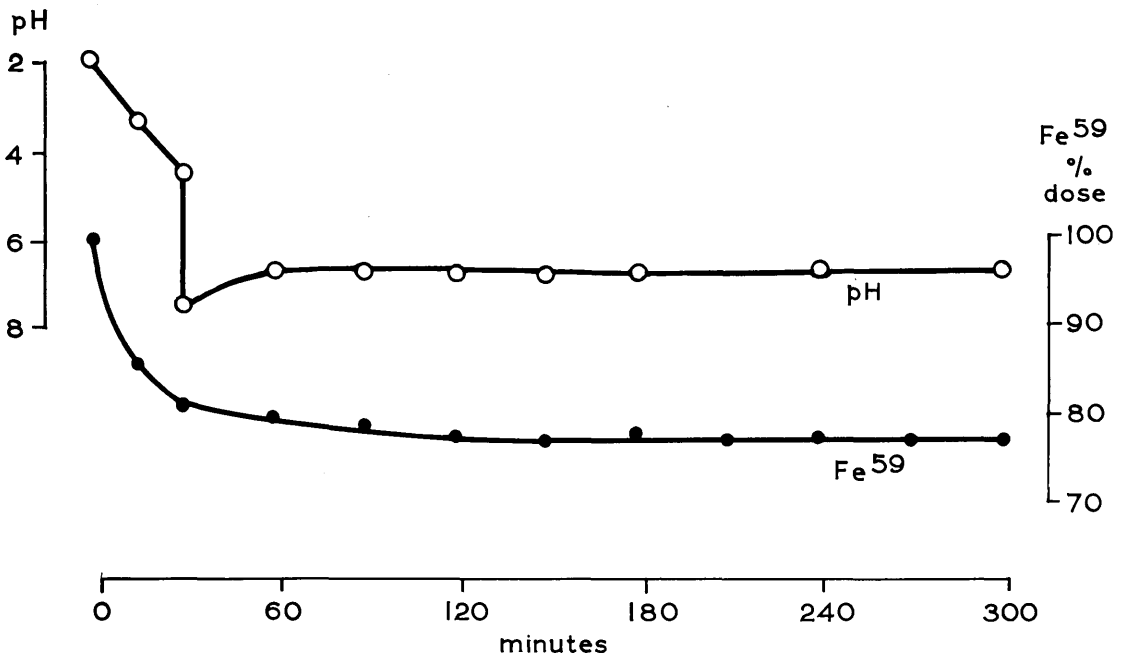


FIG. 20.

Curves of radioactive iron recorded by in vivo scintillation counter placed over isolated duodenal loop and pH of loop contents showing lack of change of absorption of iron when contents were neutralised with sodium hydroxide at 30 minutes.

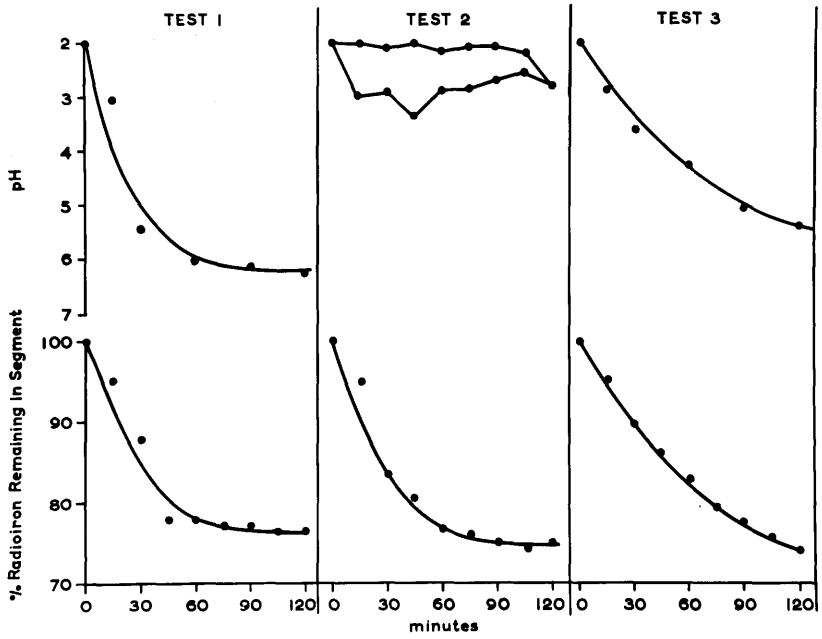


FIG. 21.

Curves of radioactive iron recorded by in vivo scintillation counter over isolated duodenal loop and pH of loop contents in repeated tests of absorption of 2 micrograms of ferric iron from the same loop. In test 2 the pH is kept less than 3.5 with hydrochloric acid and no change in the curve of radioactive iron is noted.

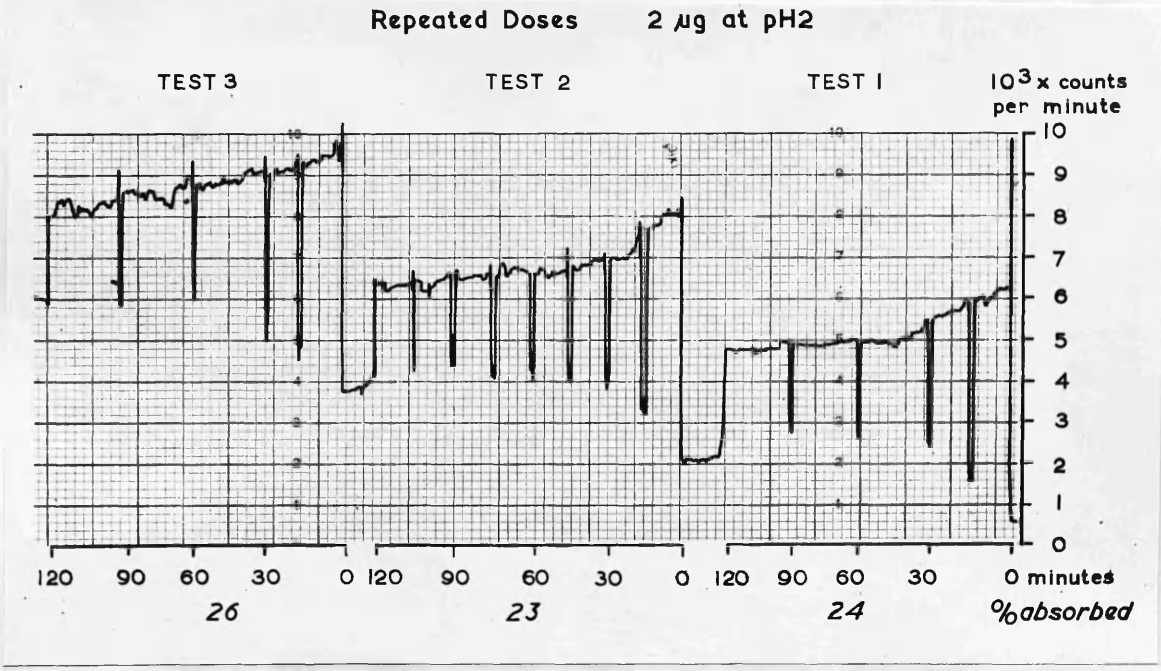


FIG. 22. Dog T37

Recording, from right to left, from direct writing in vivo scintillation counter showing 3 tests of absorption of 2 micrograms ferric iron placed in an isolated duodenal loop in solution at pH 2. In test 2, the pH of the loop content was maintained at less than 3.5 with only a slight increase in absorption of iron.

/(Fig. 22).

3. Utilisation of Ascorbic Acid.

When an absorption test with 2 micrograms ferric iron at a pH throughout of less than 3.5 had been proceeding for 120 min. and the absorption had virtually stopped, 100 mg. ascorbic acid were added to the loop. (Fig. 23). Only slight further absorption of iron occurred.

4. Intraluminal Change in Iron.

In 5 dogs the test solution was removed from a duodenal loop after absorption had been proceeding for varying times (15, 30, 60 or 120 min.). The loop was then washed thoroughly with 500 ml. Tyrode's solution. The direct recording scintillation counter over the loop estimated the absorption before and after the withdrawal of the solution (Fig. 24). The test lasted 5 hours. Radioactivity was seen to leave the loop even after the withdrawal of the iron-containing solution in all cases except when the removal was at 120 min. (Fig. 25). The total amount of iron absorbed in these experiments was within the range of the usual tests in which the iron solution was not removed until the 5 hours had elapsed, with the following exceptions. When the iron was taken out of the loop at 15 and at 30 min. a /

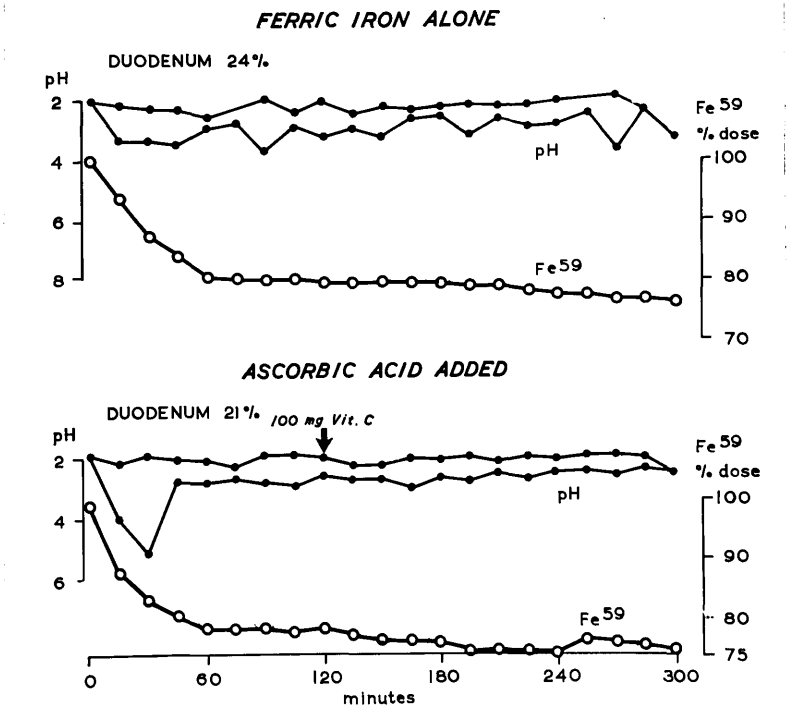


FIG. 23.

Curves of radioactive iron recorded by in vivo scintillation counter over isolated duodenal loops and range of pH of loop contents showing no effect from addition of 100 mg. ascorbic acid to the loop contents at 130 minutes.

Upper Chart - Dog S38 2 micrograms ferric iron with pH kept less than 3.5.

Lower Chart - Dog S151 2 micrograms ferric iron with pH kept less than 3.5 except at 15 and 30 minutes.

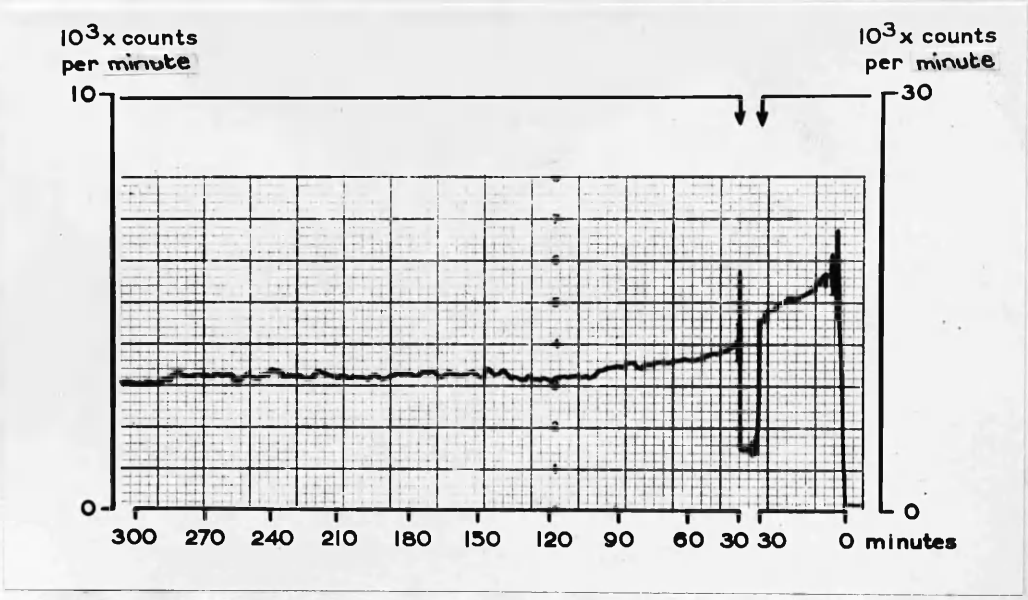


FIG. 24. Dog S874.

Recording, from right to left, from direct writing in vivo scintillation counter placed over an isolated duodenal loop showing the effect of removing the iron containing fluid from the loop at 30 min. At this time the sensitivity of the recorder was changed. Further absorption of iron occurred until about 120 min.

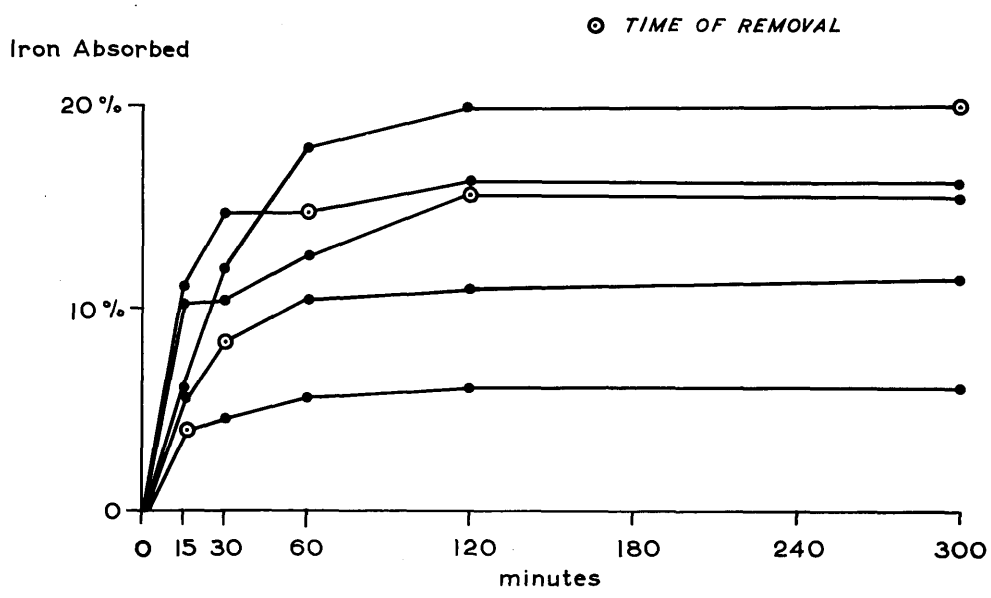


FIG. 25.

Percentage absorption of 2 micrograms of iron in solution at pH 2 placed in 5 isolated duodenal loops the iron-containing content of the loop being removed at various times.

/a decrease in overall absorption occurred although absorption lasted until 120 min. as in the other tests.

In 3 tests the contents of a duodenal loop were removed at 120 min. when absorption had virtually ceased and they were introduced into a recently prepared duodenal loop in another animal. A small amount (1 to 4%) was then absorbed in 5 hours but no more than might have been absorbed in the original preparation if the iron had not been removed.

As has been noted already when considering the results of the in vivo scintillation counter a diminishing amount of radioactive material was available for aspiration from the isolated loops during the first two hours of the test. Part of this radioactivity was found to be attached to particulate matter which could be separated by centrifugation. This part amounted to only 5% of the radioactivity aspirated at 15 min. but had increased to 50 to 66% by 120 min. (Table VI). A further small fraction of the radioactivity aspirated from the loop could be precipitated by trichloracetic acid. Together these two procedures removed 60 to 70% of the radioactive iron which was aspirated from the loop at 120 min. It must be remembered that by 120 min. the aspirate only /

Dog	Time	Fe 59 remaining in intestinal fluid - % dose		
		Untreated (A)	After centrifuging	After T. C. A.
	0	100	100	100
S608	30	74	69 (93)*	62 (84)*
pH 2	60	41	34 (83)*	19 (46)*
Vit C	120	20	13 (66)*	7 (35)*
S620	15	76	72 (95)*	69 (91)*
pH 2	30	47	27 (57)*	24 (51)*
	60	25	13 (52)*	11 (44)*
S619	15	83	79 (95)*	76 (92)*
pH2	30	55	53 (96)*	27 (49)*
	60	33	11 (30)*	10 (33)*
	90	24	10 (42)*	9 (37)*
	120	29	10 (35)*	9 (31)*

* % of values in column A

TABLE VI.

Percentage of dose of radioactive iron remaining in fluid in isolated duodenal loops in 3 dogs at various intervals and after centrifuging and treatment with trichloracetic acid (T. C. A.).

/only contained 20 to 30% of the original dose so that a mere 7 to 9% of the iron given was not attached to protein or particulate matter. No reaction was obtained from the residue on addition of potassium ferrocyanide or potassium ferricyanide or of α-α-dipyridyl but the amounts of iron involved were so small that it is doubtful if any colour reaction would have been detectable.

Site of Action of Ascorbic Acid.

The promoting action of ascorbic acid on iron absorption noted in comparing one animal with another was confirmed in repeated tests in one animal. After a control test of 2 micrograms ferric iron at an initial pH of 2, 100 mg. ascorbic acid were added to the second dose which was otherwise identical to the first. The absorption was doubled (24% as compared with 10% in the control). After thorough washing of the loop with 500 ml. Tyrode's solution and the usual 30 min. rest period, a third dose of 2 micrograms ferric iron at an initial pH of 2 showed an absorption of 20% indicating some persistence of the effect of the ascorbic acid. (Fig. 26).

A similar series of experiments was done on another dog. In this instance the test solution was again 2 micrograms ferric iron at an initial pH of 2. During the 30 min. /

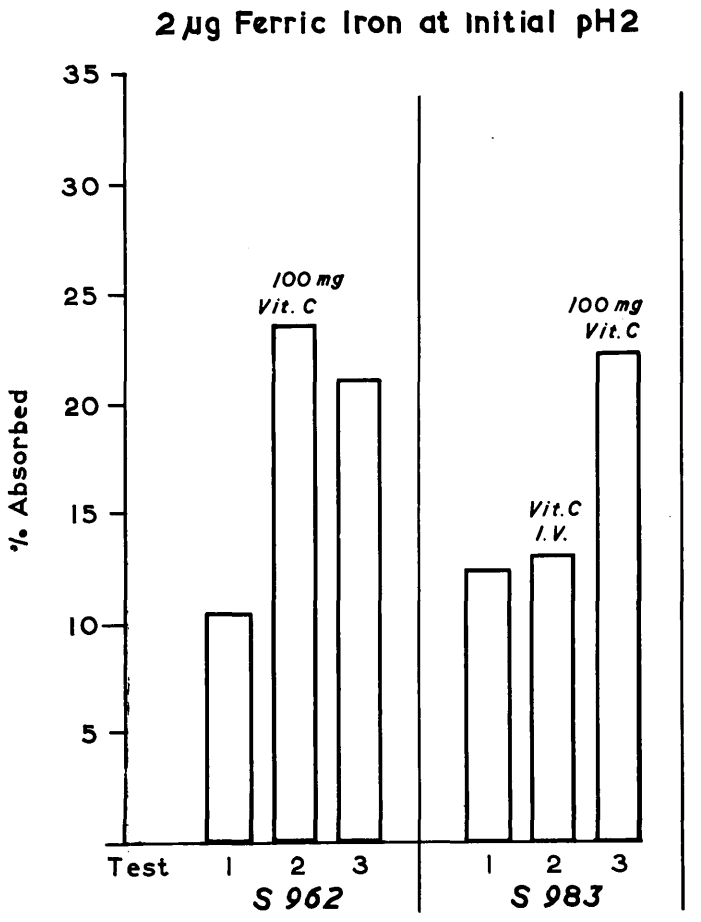


FIG. 26.

Percentage absorption in repeated tests with 2 micrograms ferric iron in solution at pH 2 placed in an isolated duodenal loop.

Dog S962 - In test 2, 100 mg. ascorbic acid was added to the test solution.

Dog S983 - In the $\frac{1}{2}$ hour prior to test 2, 20 mg. ascorbic acid (per Kg body weight) was given by intravenous infusion. In test 3, 100 mg. ascorbic acid was added to the test solution.

/min. rest period between tests 1 and 2, 20 mg. ascorbic acid per Kg. body weight was given intravenously in 250 ml. physiological saline. No increase in iron absorption resulted. Yet when 100 mg. ascorbic acid were added to the test solution for the third test on the same dog, there was a considerable increase (22% as compared with 12% in the control) (Fig. 26).

When ascorbic acid was present in the test solution the return of pH towards neutrality from an initial level of 2 was slower than when no ascorbic acid was in the solution (Fig. 27). The differences were significant in the first hour in the ileum and the first 3 hours in the duodenum (Details in Appendix).

Distribution of Radioactive Iron in the Tissues at the end of a 5 Hour Test.

A. Wall of Isolated Loops: In two tests radioactive iron was given by intraportal infusion. Five hours later the whole small bowel was excised and the radioactivity measured. The small bowel contained 1 and 2 % respectively of the amount of iron given. In the routine procedure the radioactivity of the excised loops was estimated and then they were opened and washed in a stream of warm saline with gentle scraping of the mucosa to dislodge any loosely adherent particles. /

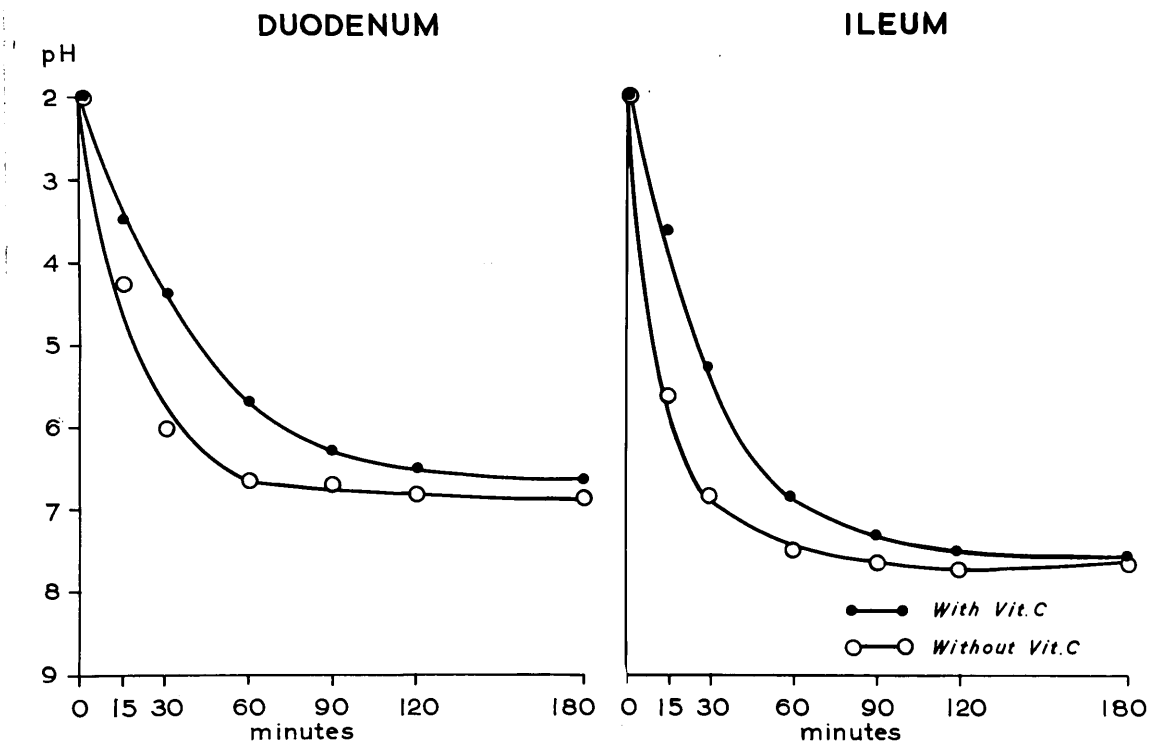


FIG. 27.

Mean pH of contents of isolated duodenal and ileal loops during tests of absorption of iron from solution with an initial pH of 2, showing the effect of ascorbic acid in delaying the return of pH towards neutrality.

/particles. Radioactivity was again measured in the well type Geiger-Muller counter. They contained 2 to 18% (mean 4.9% S.E.M. $\pm$ 0.47%: gemetric mean 3.92%) of the original dose, with the exception of the cases in which no absorption occurred, the maximum then being 1.5%. The actual amount in the gut wall was correlated with the amount of iron absorbed ($r = 0.58$ $P < 0.001$ Fig. 28) in the 38 duodenal loops studied in this manner. It did not hold in the 20 ileal loops ($r = 0.30$ $P > 0.1$).

Autoradiographs demonstrated that the radioactive iron in the wall of the loops was concentrated in the mucosa (Figs. 29 & 30). Some particles of mucosa broken during preparation allowed accurate orientation of the histology and autoradiography. The radioactivity was at no time deeper than the mucosal layer. As the high energy particles of Fe 59 scatter somewhat in the film emulsion, it may be that some of the radioactivity lay on the surface of and not in the mucosal cells.

B. Other Organs: The amount of iron in the various tissues at the end of the 5 hour test was expressed as a percentage of the total radioactive iron in the body as measured by method C. This percentage distribution was remarkably constant in the 39 animals fully studied, no /

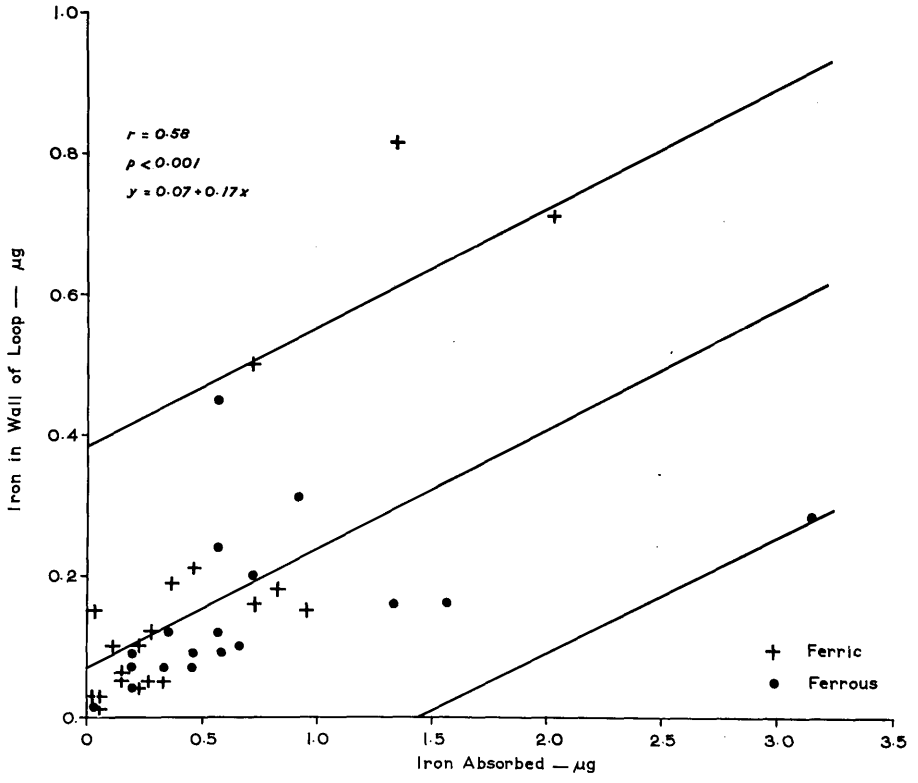


FIG. 28.

Correlation between amount of iron absorbed from and amount of iron in the wall of 38 isolated duodenal loops. The 95% confidence limits are shown.

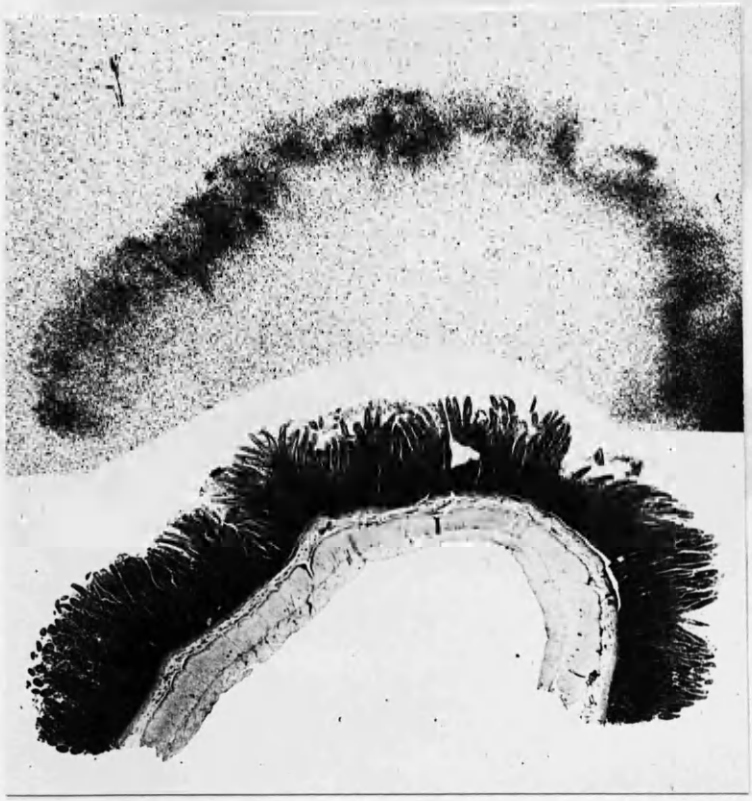


FIG. 29.

Autoradiogram (above) and corresponding histological section (below) from isolated duodenal loop after test of absorption of radioactive iron.

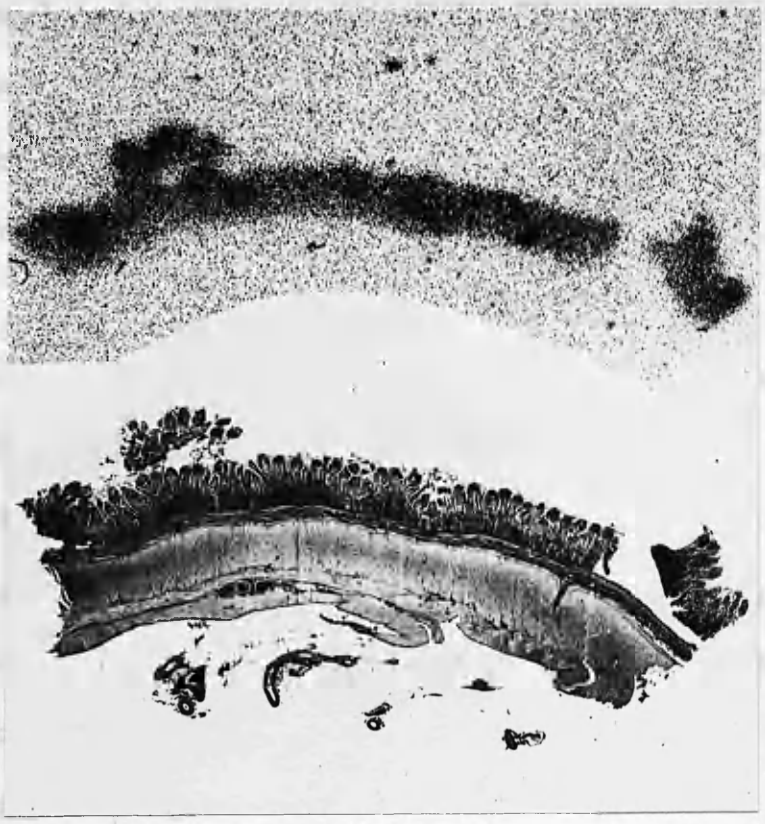


FIG. 30.

Autoradiogram (above) and corresponding histological section (below) from isolated ileal loop after test of absorption of radioactive iron.

/no matter the composition of the test solution (Fig. 31, Table VII). The largest amount was in the bone marrow, about 2/3 of the amount absorbed. The only other notable deposits were in the plasma and the liver. Spleen and kidney each contained up to 2.5%. On 20 occasions heart and lung were sampled and contained negligible radioactivity.

In other three tests in which versene was put into the solution containing iron, the tissue distribution of absorbed iron differed from above. Less iron was taken up by the bone marrow, and more remained in the plasma (Fig. 31). Proportionately more of the radioactive iron leaving the plasma was found in the liver although when expressed as a percentage of the total dose absorbed the difference was not statistically significant.

MOVEMENT OF WATER:

Duodenal Segments. In four tests in which the pH of the test solution was 7, and no ascorbic acid was present, mean insorption was 30.6 ml. per 15 min. per 100 sq. cm. serosal area, mean exsorption 30.23 ml. and mean net movement 0.42 ml. No significant change was found in these rates either when the test solution was acidified to a pH of 2 or when ascorbic acid was present, although insorption was diminished under these circumstances. (Table VIII). /

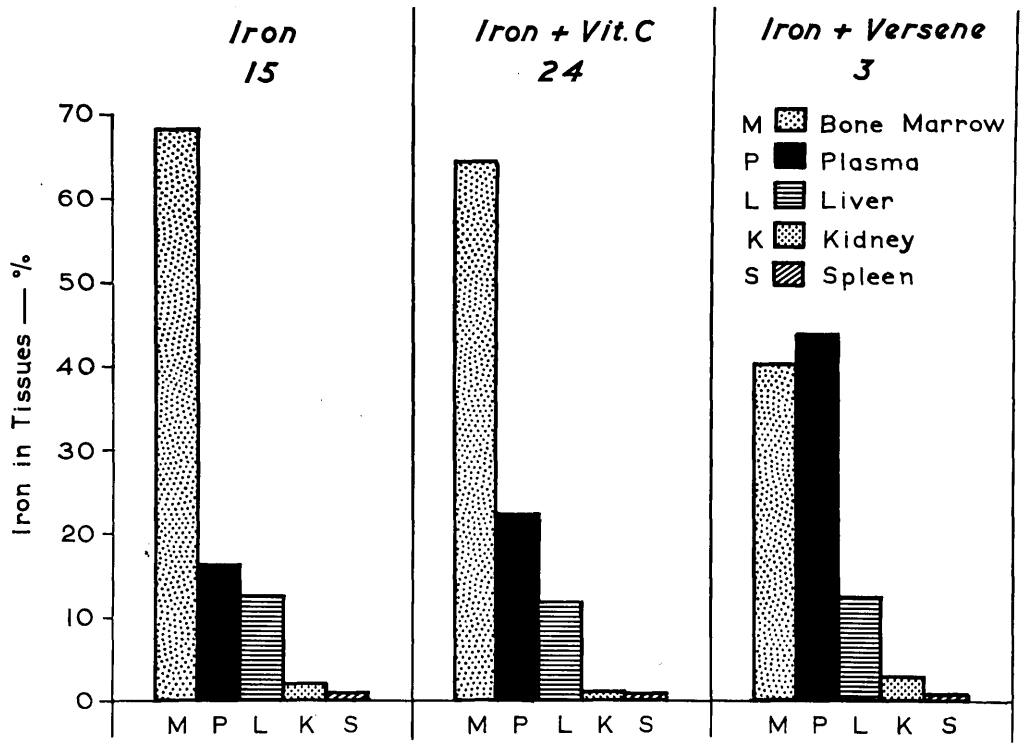


FIG. 31.

Percent distribution in bone marrow, plasma, liver, spleen, and kidneys of iron absorbed from test solutions with iron alone, with iron plus ascorbic acid and with iron plus versene.

Comparison	Iron alone (15) / Iron & Vit C (24) t	P	Iron & Vit C (29) / Iron & Versene (3) t	P
Marrow	0.40	< 0.7	2.34	< 0.05
Plasma	1.02	< 0.4	1.89	> 0.05
Liver	0.12	> 0.9	0.41	< 0.6
Kidney	0.32	< 0.9	3.17	< 0.01
Spleen	0.10	> 0.9	0.16	< 0.9
Comparison	**Ferric Iron (21) / Ferrous Iron (18) t**	**P**	**pH 2 (30) / pH 7 (9) t**	**P**
Marrow	0.30	< 0.9	1.28	< 0.3
Plasma	0.85	< 0.5	2.02	< 0.1
Liver	1.60	< 0.2	1.74	< 0.1
Kidney	1.29	< 0.3	2.58	< 0.02
Spleen	0.24	< 0.9	0.01	> 0.9

TABLE VII

Significance of comparison percentage tissue distribution of absorbed iron under various test conditions (logarithmic values used).

Test Conditions	No. of tests	Mean Movement of Water ML/15 min.		
		Insorption	Exsorption	Net
Duodenum				
pH 7: No Vit C	4	30.65 ± 3.56*	30.23 ± 4.68*	+ 0.42 ± 1.41*
pH 2: with or without Vit C	40	28.34 ± 3.47*	31.29 ± 2.58*	- 2.85 ± 2.82*
Diff		2.31 ± 2.04**	1.06 ± 2.21**	3.27 ± 2.84**
t		1.130	0.480	1.151
P		>0.2	>0.6	>0.2

* S. E. M. ** S. E. M. Diff.

TABLE VIII.

Movement of water (ML. per 15 minutes per 100 sq. cm. serosal surface) from intestine (insorption) and to intestinal contents (exsorption) in isolated duodenal loops.

Test Conditions	No. of tests	Mean Movement of Water		
		Insorption	Exsorption	ML/15 min. Net
Ileum				
pH 7: No Vit C	4	28.93 ± 1.81*	19.43 ± 3.70*	+ 9.50 ± 3.37*
pH 2: with or without Vit C	26	34.93 ± 1.56*	26.83 ± 1.48*	+ 8.10 ± 1.56*
Diff		6.00 ± 4.02**	7.40 ± 4.00**	1.4 ± 2.24**
t		1.494	1.849	0.625
P		>0.1	>0.05	>0.5

* S. E. M. ** S. E. M. Diff.

TABLE VIII.

Movements of water (ML. per 15 minutes per 100 sq. cm. serosal surface) from intestine (insorption) and to intestinal contents (exsorption) in isolated ileal loops.

/ (Table VIII).

Ileal Segments. The bidirectional movement of water in the four ileal tests with a solution of pH 7 and no ascorbic acid differed from that in the duodenal segments, mean insorption being 28.93 ml. per 15 min. per 100 sq. cm. Serosal area, mean exsorption 19.43 ml. and mean net movement 9.50 ml. absorbed.

An increase of both insorption and exsorption was noted in the 25 tests at an initial pH of 2 with or without ascorbic acid, the latter being relatively greater: but these differences were not statistically significant (Table VIII).

Repeated Tests. The movement of water was measured during the first 15 min. of each of the three tests on the same isolated duodenal segment in 6 dogs. Thus, measurements were made at zero time, $2\frac{1}{2}$ hours and 5 hours. All tests were done with a solution of initial pH 2. The mean values for the first test at zero time were insorption 28.4 ml., exsorption 32.5 ml. and net movement 4.1 ml. enterosorbed.

In three animals both insorption and exsorption gradually diminished as time passed, the maximal diminution being 33% of the value at zero time. In the other three animals both insorption and exsorption increased in the /

/the second and third tests: the greatest increase being 18% for insorption and 35% for exsorption.

While the bidirectional rates of movement of water were measured only in the first 15 min. period of each test, the net movement from the loop was monitored throughout the test. The initial tendency to enterosorption shown in experiments in the duodenal segments when pH was 2, passed off in 30 to 60 min. and a net absorption then followed, so that about 5 to 10 ml. of intestinal content remained at 5 hours.

DISCUSSION.

The duodenum and ileum have shown marked similarities in their handling of iron in the forms and amounts used in the study. The duration of absorption and the range of rates of absorption were similar, although the few recordings (5) available for the ileum reduce the validity of this particular comparison. The absorption of iron was compared for equal serosal areas of duodenal and ileal loops. The correct comparison would have been of equal mucosal surfaces but estimation of this surface depends upon assumptions of the height and number of villi and of mucosal folds. Warren (1939) /

/ (1939) reviewed previous work on this subject and gave his results on one dog. He found that the estimated mucosal surface per unit serosal area of the duodenum was less than twice that of the ileum. Such a disparity would not account for all the difference between the absorption of iron from the duodenum and the ileum in our results. A further difficulty in assessing absorbing surface has been revealed by the discovery of micro villi on the surface of intestinal mucosa cells with the aid of electron microscopy. (Palay and Karlin, 1959).

The total amount of iron absorbed per 100 sq. cm. serosal surface in the five hour tests was up to 14 times greater in the duodenum than in the ileum. However, this difference was only significant when a large percentage of the dose was absorbed i. e., when the pH of the test solution was 2 and ascorbic acid was present. This might have been due to a small part of the iron-absorbing capacity of the bowel being tested by the small doses used in this study. If the gut were tested to greater capacity differences might be more marked. The finding that the duodenum absorbed more iron than an equivalent area of ileum falls in with the general opinion that the duodenum is more active in this respect, and agrees with /

/with the in vitro work of Dowdle and colleagues (1960). In the present studies the duodenum has absorbed more iron even though an equal opportunity for absorption has been afforded to the ileum; conditions which do not occur in the intact animal.

Confirmation has been obtained of the increase in the amount of iron absorbed with an increase in amount given and also of the increase in percentage absorption as the amount of iron given diminished. (Hahn et al., 1940; Moore et al., 1944; Smith and Pannacculi, 1958; Bonnet et al., 1960). This latter finding did not hold in the present study at a dose of 0.5 micrograms. This may be due either to the rapid attachment of iron to mucus or to its combination with the phosphate in the Tyrode's solution so that it was not available for absorption. A linear relationship on a log/log scale existed in both duodenum and ileum between the amount of iron absorbed and the amount given. Again it was noted that when absorption was greater this relationship was more significant being most significant when the iron was given at a pH of 2 with ascorbic acid. Re-examination of the data of Bonnet and colleagues (1960) on humans also /

/also reveals a similar correlation. While such a "double log" relationship has no obvious biological significance, it permits a dose to be selected by simple interpolation to give any desired absorption. It will be shown to have a similar form in the rat. This similarity may condone comparison of data obtained in the dog and in the rat.

The present findings confirm the action of ascorbic acid in augmenting absorption of iron. However, to our knowledge this is the first report that an initial pH of the test solution of 2 enhanced the absorption of iron to the same extent as the addition of 100 mg. ascorbic acid when the pH was 7. A possible theoretical explanation was the maintenance of the ferrous state in both instances. However, this would hardly be compatible with the finding that, when both factors were present simultaneously, the augmentation of iron absorption was equivalent to the sum of the separate effect of ascorbic acid and of pH 2.

The addition of ascorbic acid to the test solution of pH 2 slowed the return of pH towards neutrality. This action has been said to be the reason for the increase in iron absorption caused by ascorbic acid (Groen et al., 1947). In the present study, only a slight increase in iron absorption resulted when a similar and more long lasting /

/lasting maintenance of low pH was achieved by the addition from time to time of hydrochloric acid to the solution in the bowel lumen. It appeared from these results that acidity and the addition of ascorbic acid acted via two different mechanisms whose effects seem to have been additive when the two factors were present in combination.

Intravenous administration of ascorbic acid produced very little increase in iron absorption compared with the increase resulting from its presence in the solution in the bowel lumen. The amount of ascorbic acid given in the intravenous test was sufficient to saturate the tissues and, in fact, a little might have been excreted into the bowel (Sebrell and Harris, 1954). Thus the promoting action of ascorbic acid apparently took place in the lumen and not in the bowel wall.

The suggestion has been made that ascorbic acid acts to increase absorption of iron by forming a chelate with iron. (Finch and Finch, 1955). To test this hypothesis a known chelating agent, versene, was given with the doses of iron. The promoting action was of a similar magnitude to that of ascorbic acid given at the same pH. However, examination of the distribution of the iron absorbed in the tissues revealed that the versenate /

/versenate remained to a significantly greater extent in the plasma, and of the remainder less was in the bone marrow and proportionately more in the liver. No difference in tissue distribution was noted between iron given with or without ascorbic acid. Thus, while it is possible that an iron chelate is formed with ascorbic acid, it would seem to break down during the absorption process so that the iron once in the plasma follows the normal metabolic pattern, in contrast to the chelate with versene which appears more stable and either stays in the plasma or goes to the liver, probably to the reticulo endothelial cells.

The lack of differentiation between ferrous and ferric iron in the dog (Moore et al., 1944) was confirmed, although a tendency appeared for more ferrous iron to be absorbed at a pH of 7 when ascorbic acid was present. This may have been fortuitous and shown only by the small numbers available for comparison. The ascorbic acid used (100 milligrams) would have converted all the iron into the ferrous state. Or again it may be that some slightly different forms of chelate are formed from ferrous and ferric iron.

The retention of iron within the isolated loop /

/loop increased rapidly in exponential fashion up to 60 to 120 min. when equilibrium was reached. Rarely was any of this iron absorbed. It is an open question whether the exponential rate characterised one or more than one simultaneous processes. The delaying action of ascorbic acid on the rate of change of iron so that it cannot be aspirated from the loop gives an indication that its action in promoting iron absorption is due, as has previously been suggested, to maintenance of iron in an available form for a longer time. At the end of the test the loop was opened and much of the radioactivity, which could not be aspirated through the cannula, was easily washed off the mucosa. This was in keeping with previous work (Miller and Abbott, 1936; Groen and Taylor, 1937; Groen et al., 1947) which suggested that much of the iron which could not be aspirated from segments of bowel was merely adsorbed onto the mucosa and not absorbed. In the present study the scintillation counting over the loop showed that the iron, while not able to be aspirated had not in fact left the segments. Despite repeated washing a small residue of radioactive iron could not be released from the bowel wall. Autoradiography showed that this was in or attached to the mucosa, and the /

/the amount was proportional to the amount of iron absorbed from test solution. It may be that this iron indicates a step in the absorption procedure and that the later absorption, at 3 hours and over, noted in some tests, is from this depôt.

The slowing and virtual cessation of iron absorption after two hours confirmed the opinion of Stewart and Gambino (1954) from work on radioactive iron levels in the plasma of dogs. This stopping of absorption seemed, at first, to be due to the change of pH towards neutrality. Further work showed that when the pH was kept below 3.5 throughout the test, iron absorption was not prolonged, so that rise in pH was not the only factor halting iron absorption. The giving of repeated doses of iron into the same segment of bowel caused only slight reduction in absorption in the later doses and demonstrated that the bowel had not exhausted its capacity for iron absorption even though it ceased at 120 min. in each individual test. The "mucosal block" did not function at these low dose levels. When the iron absorption had been progressing for 120 min. and was thus almost finished the bowel contents were transferred into another freshly-prepared loop in another dog. Minimal absorption occurred indicating that the /

/the iron remaining in the intestinal fluid was no longer available for absorption. It would seem, therefore, that further absorption was prevented by some intra-luminal change in the nature of the iron. This change apparently occurred in the first 30 to 60 minutes, for the removal of the test solution after this time did not cause much diminution in the total amount of iron absorbed in a 5 hour test period. The results of testing the fluid itself show that by 60 to 120 min. very little of the original dose is not attached to particulate matter or to material precipitable with trichloracetic acid. Thus, attachment to the mucosa would appear to have been accomplished in the first hour and the iron remaining in the bowel contents had little or no influence on the process of absorption. The change in iron making it unavailable for absorption could be delayed by the addition of ascorbic acid but ascorbic acid could not reverse the change.

The absorption of iron from the loops of bowel did not regularly follow an exponential pattern. This agrees with the finding of Halberg and Sölvell (1960). There was no significant difference between the mean maximal absorption rates whether the pH was initially 2 or 7, whether there was ascorbic acid present or not, or whether the iron /

/iron was initially in the ferric or ferrous form. This uniformity in overall rate despite change in test solution and in the amount of iron absorbed suggests that a uniform process is involved in absorption of iron. The correlation of duration of absorption rather than rate of absorption, with the total amount of iron absorbed would indicate that this process increases absorption by acting for a longer time.

The bidirectional movement of water from the bowel segments in the present study was similar both in the initial rates and the effect of acid pH to results previously obtained (Code et al., 1960). In addition these authors found that the rates of water movement did not differ greatly in the dog whether segments were prepared in the anaesthetised animal or whether chronic loops were tested without the dog being anaesthetised. Thus we have an indication that the segments we have used have some physiological value. Repeated tests of movement of water have been done on isolated segments of ileum using neutral solutions (Shields and Code, 1961). Insorption of water declined up to 20% and by 4 hours this was at its maximum remaining virtually unchanged to 8 hours. Exsorption was variable but maximal increases up to 20% had occurred by 4 hours. The present results of repeated tests on the duodenum show /

/ show no constant pattern but the size of the changes are slightly greater than Shields and Code (1961) found in the ileum. This may be due in part to the test solution being acidic, as well as to the different region of the bowel used. That there was still transport of water in both directions across the duodenal mucosa at 5 hours in our results confirms that the physiological integrity of the segment was being maintained despite the passage of time.

CONCLUSIONS.

1. Methods for study of absorption of iron from isolated loops of canine intestine in vivo have been established.

2. Comparison of duodenal and ileal loops revealed a parallelism in response to increasing dose, to ascorbic acid to change of pH and in the duration and maximal rate of absorption. Despite this parallelism the duodenum absorbed up to 14 times as much as a corresponding area of ileum.

3. In these isolated loops of intestine the amount of iron absorbed in a 5 hour test was directly proportional on a log/log scale to the amount of iron given. Absorption of iron from the test solution was increased to a similar /

/similar extent by addition of 100 mg. of ascorbic acid or by making the initial pH of the solution 2 instead of 7. When both factors were present they had an additive effect.

4. The action of ascorbic acid in augmenting the absorption of iron took place in the bowel lumen and did not appear to be due to formation of a stable iron chelate.

5. Iron in the test solution became attached in an exponential fashion to the isolated loop so that it could not be aspirated through the cannula placed in the loop. The addition of ascorbic acid slowed the rate at which the exponential change occurred.

6. Absorption of iron from a single dose continued for up to two hours in most tests. After the first 60 min. the iron in the bowel lumen had no further effect on the total amount of iron absorbed, which presumably then occurred from iron in or attached to the bowel wall. The maximal rate of absorption of iron was in the period 15 to 30 minutes after placing the dose in the loop.

7. Absorption of iron was not blocked by three doses of up to 10 micrograms of iron at $2\frac{1}{2}$ hour intervals. Cessation of absorption of iron after 2 hours was due to some intraluminal change in the iron making it unavailable for absorption.

PLASMA LEVELS OF RADIOACTIVE IRON AND THEIR RELATIONSHIP TO THE ABSORPTION OF IRON.

PLASMA LEVELS OF RADIOACTIVE IRON AND THEIR RELATIONSHIP TO THE ABSORPTION OF IRON.

Much difference of opinion exists on the value of changes in serum levels as a measure of the absorption of iron from the gastrointestinal tract. This section reports a study to clarify this dispute.

MATERIAL AND METHODS.

The dogs used in this study were prepared as indicated on page 27, loops of small bowel being isolated. The portal vein and femoral artery were cannulated in 20 animals and the femoral artery alone in 39. The procedure has been described whereby a test solution containing radioactive iron was placed in these isolated loops and the absorption of iron measured over 5 hours (page 29). Blood samples were taken at 30 minute intervals during this time. The first 3 ml. of blood withdrawn were discarded. The next 6 ml. were taken with a dry disposable plastic syringe and clotting was prevented with dried heparin. The radioactivity of 2 ml. aliquots of plasma was measured in a thallium-activated sodium iodide scintillation counter. /

/counter. Plasma was used instead of whole blood to avoid the possible error of altered haemocrit due to the dog lying immobile on its back through the test and to eliminate the difference in haemocrit between the portal and systemic arterial samples when comparison was made of the levels of radioactivity in these systems. It was permissible to use plasma as the small amounts of iron absorbed would all be bound to the iron-carrying plasma protein, transferrin. The dogs were exsanguinated at the end of the test and the liver analysed for radioactive iron. In 20 cases specimens of lungs and heart were similarly examined.

In one dog a cannula was inserted into the inferior vena cava via the left femoral vein and placed so that its open end was at the level of the hepatic veins. In addition, the portal vein and the right femoral artery were cannulated along with the isolation of a duodenal loop in the usual manner. Just prior to closing the abdominal cavity the hepatic artery was ligated at the porta hepatis. Any more proximal site of ligation would not have occluded all the arterial supply to the liver (Grindlay et al., 1951). Two micrograms of ferric (Fe59) iron with 100 mg. ascorbic acid in 50 ml. Tyrode's solution at an initial pH of 2 were placed in the loop. Blood samples were taken every 15 minutes for 90 minutes. At the /

/the end of this time the dog was exsanguinated through a carotid cannula and specimens of the liver obtained at necropsy.

Hepatic blood flow in the anaesthetised dog was assumed as 25 ml. per Kg body weight per minute and 15 ml. per Kg body weight per minute after ligation of the hepatic artery (Grindlay et al., 1941). These figures are lower than in the unanaesthetised dog although the hepatic arterial circulation makes a similar contribution to the total hepatic bloodflow which is about 39 ml. per min. per Kg body weight and after ligation of the hepatic artery falls to about 32 ml. per Kg body weight per min. (Bollman et al., 1953).

Plasma volume was taken as 4% of the body weight (von Porat, 1951). This value is at the lower limit of normal to allow for the slight blood loss at operation and for the volume of the samples obtained in the course of the test.

RESULTS.

The amount of radioactive iron in the plasma increased rapidly to reach a maximum 90 minutes after the iron was placed in the bowel. (Fig. 32). The maximal level occurred within 120 minutes in 54 of 57 experiments. A significant correlation was found between this peak plasma radioactivity and the amount of iron /

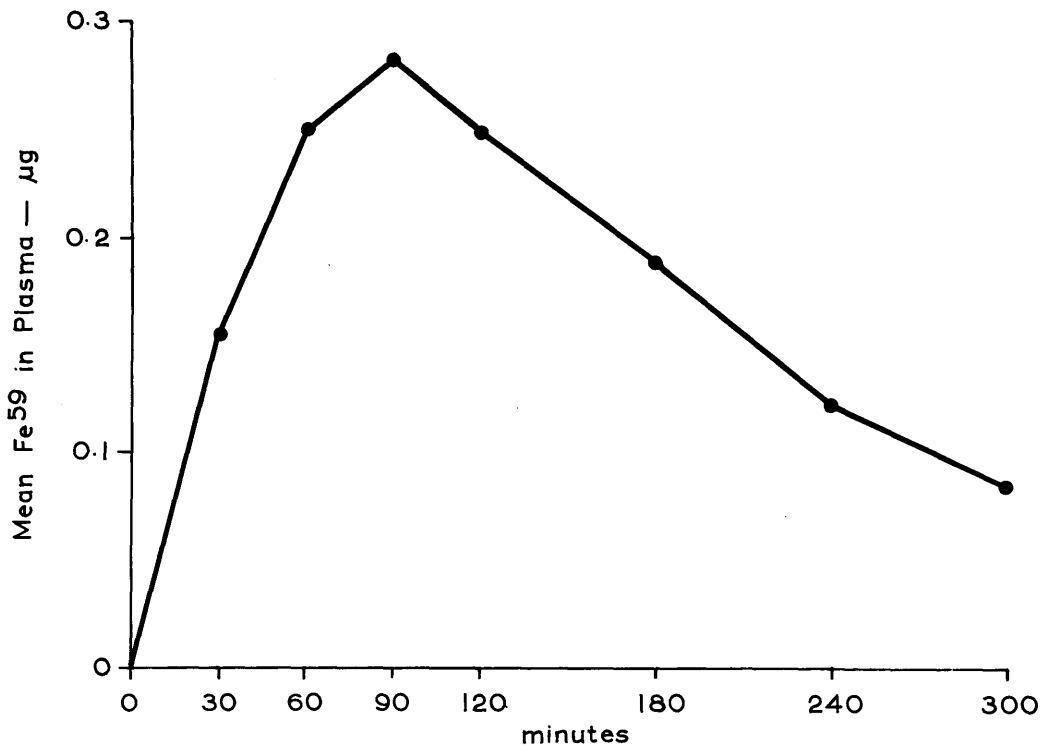


FIG. 32.

Radioactive iron (Fe 59) in plasma during absorption of iron from isolated duodenal loops (mean of 23 dogs) showing maximal level 90 minutes after placing iron in the loops.

/iron absorbed in 23 tests with single duodenal loops ($r = 0.89$ $P < 0.001$ Fig. 33). The regression coefficient was 0.73 ($\pm$ 0.083 S.E.) indicating that about 3/4 of the iron absorbed over a five hour period was in the plasma about 90 minutes after placing the radioactive iron in the bowel. Close analysis of individual results revealed that the amount of radioactive iron in the plasma would not have been a reliable index of absorption of iron in 9 out of the 23 tests. This is best seen by examination of the results expressed as a percentage of the amount of iron given (Fig. 34). When less than 4% of the dose appeared in the blood the range of absorption of iron was from 0.2 to 15.7%.

An attempt was made to estimate the disappearance rate of radioactive iron from the plasma using the part of the curve after absorption of iron from the loop as measured by in vivo scintillation counting had stopped, that is, around 120 minutes. Although the mean levels of radioactive iron in the plasma showed an exponential rate of 0.587% per min., (Fig. 35) the individual curves were very variable, only 13 of the 23 approximating to exponential rates. Evidence of the accuracy of the exponential disappearance of the /

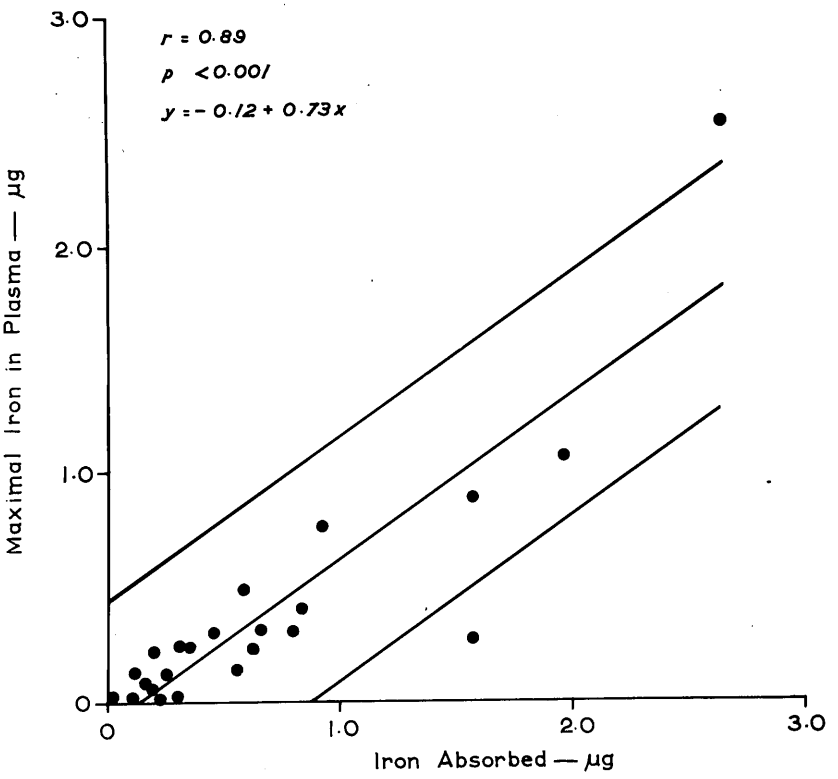


FIG. 33.

Correlation between the maximal amount of radioactive iron in the plasma and the amount of iron absorbed from isolated duodenal loops in 23 dogs. The 95% confidence limits are shown.

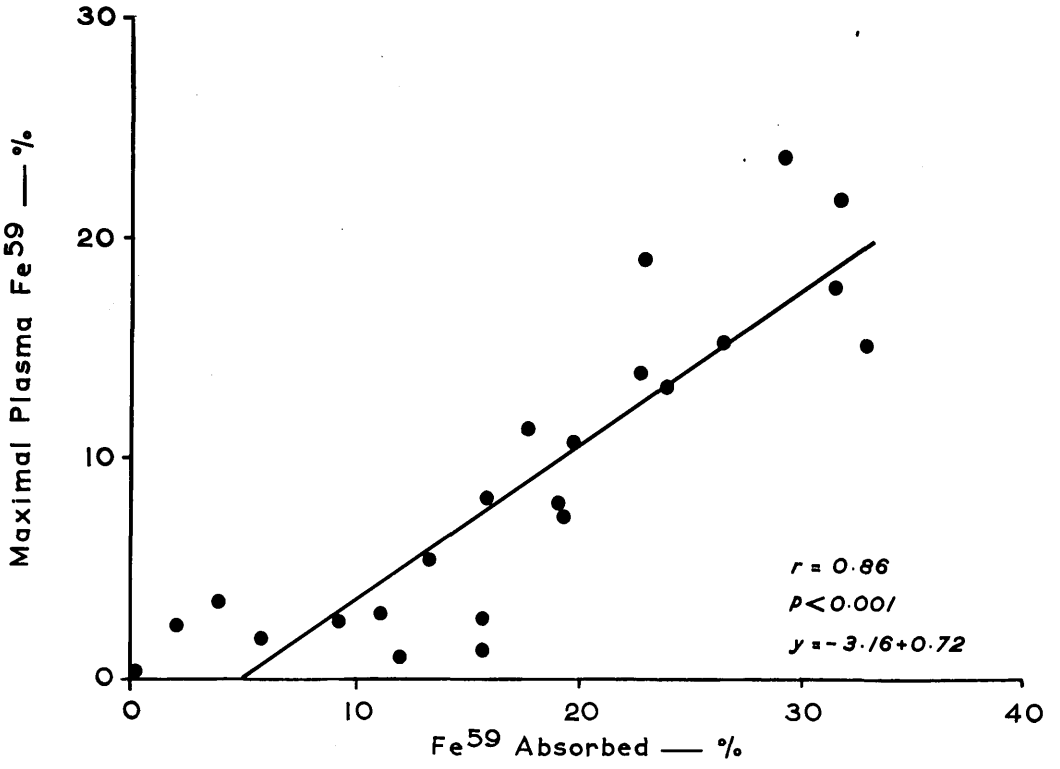


FIG. 34.

Correlation of maximal amount of radioactive iron in the plasma and the amount of iron absorbed, from 23 isolated duodenal loops, both amounts expressed as a percentage of the dose.

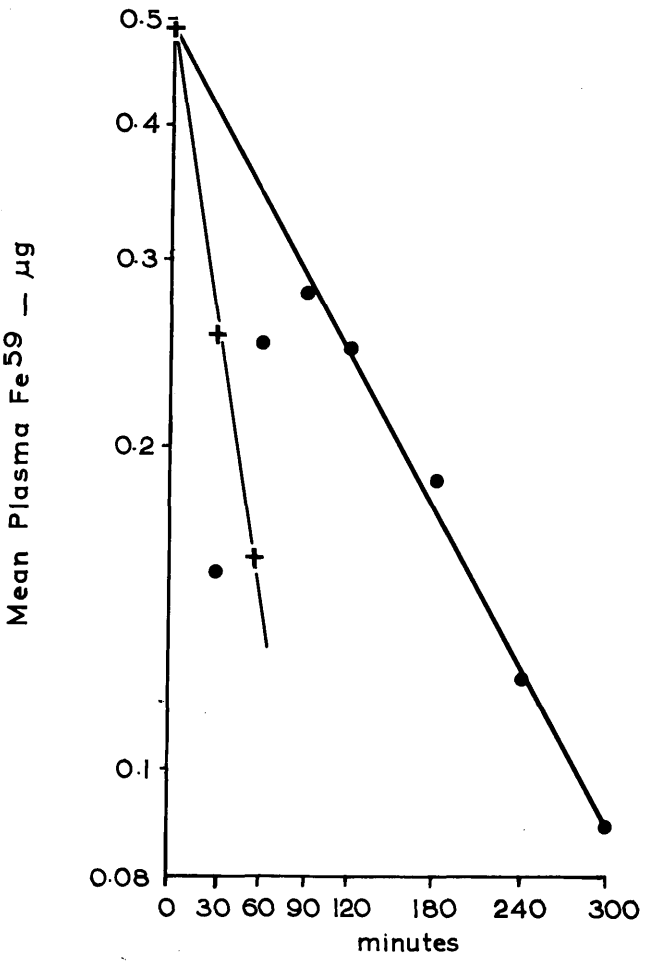


FIG. 35.

Semilogarithmic plot of the mean plasma levels of radioactive iron in the plasma during absorption of iron from isolated duodenal loops in 23 dogs, showing an exponential disappearance rate of iron from the plasma (Half time 118 min: rate 0.587% min.) and an exponential rate of appearance of iron in the plasma (Half time 38 min: rate 1.82% min.)

/the mean level of radioactive iron in the plasma was found when the line was extrapolated back to zero. The increasing values of plasma Fe 59 in the first 90 minutes were subtracted from this line and the resultant readings showed an exponential pattern (Fig. 35). This indicated that absorption into the blood was exponential. The rate was almost identical to that found from the direct scintillation counter placed over the loop. (1.82% per min., and 2.05% per min., respectively).

In 20 experiments the radioactivity of plasma simultaneously obtained from the portal vein and from the femoral artery was compared. The ratio of the portal to the femoral level was expressed as a percentage and was significantly greater than 100% at 30, 60 and 90 minutes (Table IX, Fig. 36). This indicated a higher level of radioactivity in the portal plasma at these times. The radioactive iron in the liver at the end of the 5 hour test was always less than the amount calculated from the arterio-venous difference and the assumed hepatic bloodflow. No appreciable radioactivity was in the lungs or myocardium.

The difference between portal, venous and systemic arterial radioactivity was diminished in the experiment in which the hepatic artery was ligated (Fig. 37). The amount /

Time after Iron given (min.)	Mean % Ratio Portal/Femoral Radioactivity	Difference from Equality (20 Tests) t	P
30	114.8 ± 3.51	4.21	< 0.001
60	103.4 ± 1.02	3.33	< 0.01
90	103.4 ± 1.01	3.37	< 0.01
120	100.7 ± 0.64	1.09	< 0.3
150	99.2 ± 0.70	1.14	< 0.3
180	100.7 ± 0.98	0.71	< 0.5
240	99.6 ± 1.02	0.39	< 0.8
300	100.0 ± 1.09	0	-

TABLE IX.

Comparison of the radioactivity in the portal and systemic arterial circulations during the absorption of iron from isolated intestinal loops in 20 dogs.

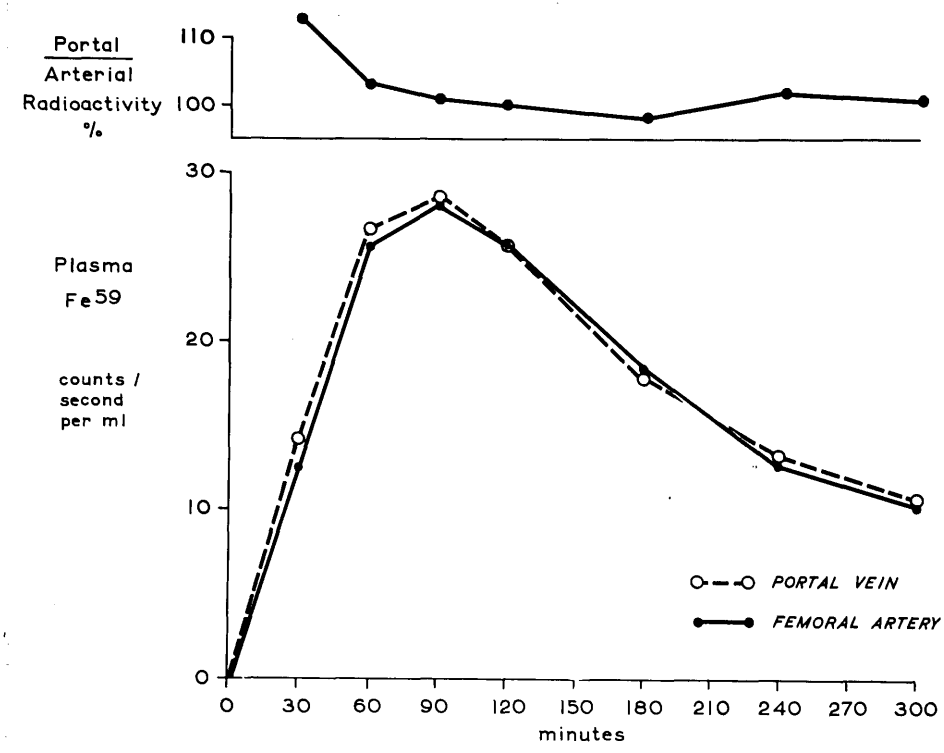


FIG. 36.

Radioactivity (Fe 59) in plasma from portal vein and right femoral artery during absorption of iron from isolated intestinal loops in dog S499. The ratio's of portal to femoral radioactivity are shown.

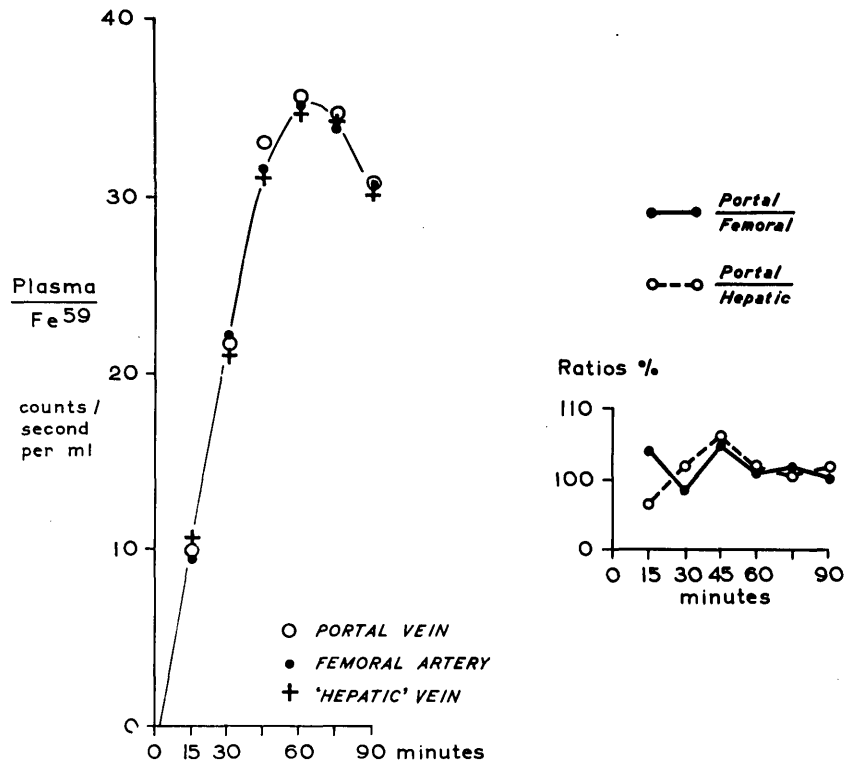


FIG. 37.

Radioactivity (Fe 59) in plasma from portal and hepatic veins and from femoral artery during absorption or iron from isolated duodenal loop after ligation of hepatic artery in dog G 1.

/amount of radioactive iron in the liver at the end of 90 minutes was less than the portal-"hepatic" vein difference multiplied by the estimated hepatic blood flow (17% of the dose as compared with 85%). Allowance was made for the ligation of the hepatic artery.

DISCUSSION.

The increase of the mean level of radioactive iron in the plasma to a maximum within $1\frac{1}{2}$ to 2 hours after placing the iron in the isolated loop is similar to that found both in man (Wasserman et al., 1952; Peterson and Ettinger, 1953; Bothwell et al., 1955) and in animals (Copp and Greenberg, 1946; Stewart and Gambino, 1954). This also conforms with the finding in the previous section that almost all the absorption of iron takes place in the first 2 hours.

The exponential disappearance rate of the radioactive iron from the plasma is similar to that previously described in dogs (Stewart and Gambino, 1954). The derivation from this exponential rate of an appearance rate of radioactive iron into the plasma which is the same as the rate of disappearance of radioactive iron from the isolated loop confirms the validity of the results. Despite these /

/these findings, a lack of uniformity is seen when individual tests are examined. Just over half of the curves of Fe 59 in the plasma have an exponential pattern. This may indicate limitations of the experimental technique. On the other hand, it is in keeping with the findings of Hallberg and Solvell (1960) in man. These workers found that the exponential disappearance rate from the blood of intravenously injected iron was altered during absorption of iron from the gastro-intestinal tract.

The well-recognised difficulty of using the alterations of the plasma level of a substance to estimate its absorption from the alimentary canal stems from the fact that more than one process affects the plasma levels; not only the intake from the gut but also the outflow in to the tissues. Much discussion has taken place in the past about the value of serum iron levels in estimating the absorption of iron (Laurell, 1952). More recently, Bothwell and co-workers (1955), using radioactive iron, concluded that the amount of iron in the blood was a reliable index of the amount absorbed in only half of the patients examined. The present study revealed a significant relationship between the maximal amount of radioactive iron in the plasma and the amount of /

/of iron absorbed in 5 hours. This correlation may be uncovered due to the avoidance of the variability of gastric emptying and of intestinal motility, due to the short period over which the absorption of iron is being measured and to the strictly controlled local condition in the isolated loops of intestine. However, the maximal level of radioactive iron in the plasma gave a reliable guide to the amount of iron absorbed only when more than 4% of the dose appeared in the plasma. Similar to the human studies, its value in individual cases is limited but in this series the limitation is clearly defined.

Comparisons of the radioactivity in the portal venous and systemic circulations have shown that the former was significantly greater for 90 minutes after the administration of iron. This agreed with the findings of Neander and Valquist (1949) who compared levels of serum iron in portal venous and inferior vena caval blood in rabbits after intragastric infusion of iron. They suggested that this difference indicated that the liver was taking up iron. This conclusion was supported by Schäfer and Breyer (1956) who estimated the levels of iron in the serum of portal and systemic blood in dogs. On the contrary, Jensen et al., (1956) did not demonstrate any difference between hepatic /

/blood.

CONCLUSIONS.

1. The maximal level of radioactive iron in the plasma during absorption of iron from isolated intestinal loops correlated with the amount of iron absorbed. It provided a measure of absorption of iron in over half the tests.

2. The greater radioactivity in the portal venous than in the systemic arterial circulation during the first 90 minutes after placing radioactive iron in the isolated intestinal loop was due to dilution of the portal blood by the hepatic arterial and vena caval blood and to removal of iron from the portal blood by the liver.

ABSORPTION OF IRON FROM THIRY-VELLA FISTULAE IN DOGS.

In the first section methods were established in

ABSORPTION OF IRON FROM THIRY-VELLA FISTULAE IN DOGS.

and of change of pH on this absorption have also been studied.

MATERIALS AND METHODS.

were done on four healthy mongrel
dogs. They were fed on a uniform
maintenance diet weighed

ABSORPTION OF IRON FROM THIRY-VELLA FISTULAE IN DOGS.

In the first section methods were established for estimating the absorption of iron from isolated loops of intestine in a series of acute experiments in dogs. These findings have now been applied to chronic Thiry-Vella fistulae. Previous work has been done with such fistulae in dogs in an attempt to estimate the amount of radioactive iron excreted into the bowel after oral administration (Salera et al., 1956). The possibility of obtaining repeatable estimations of the absorption of iron from chronic Thiry-Vella fistulae has been examined. The effect of varying doses of ascorbic acid and of change of pH on this absorption have also been studied.

MATERIALS AND METHODS.

Thirty tests were done on four healthy mongrel dogs weighing 8.5 to 13 Kg. They were fed on standard kennel diet and maintained their weight throughout the /

/the period of testing. In each, an accurately measured loop of duodenum was separated from the rest of the alimentary tract and both ends brought out through muscle splitting incisions to the right of the midline where permanent stomata were fashioned by suturing the mucosa to the skin. The openings in the muscle and fascia were made small to facilitate the closing of the stomata with balloons during tests of absorption of iron. The continuity of the gut was re-established by an end-to-end anastomosis. Full details of the operative procedure are on page 90. Ten to 12 weeks after this operation tests of absorption of iron were begun. Tests were at least 3 days apart.

The loops were kept healthy and free from cellular debris by twice weekly lavage and distension with physiological saline at 37°C. The loop capacity, without the dog showing signs of discomfort, was 40 to 50 ml. The irrigation or placing of test solutions into the Thiry-Vella fistulae was done through modified No. 16F gauge /

/gauge Foley catheters (Code et al., 1960) which had a 5 ml. balloon placed 16 cm. from the tip in lieu of the usual terminal balloon. The part of the catheter distal to the 5 ml. balloon had multiple perforations for ease in withdrawal of intestinal contents. After insertion of the catheter the balloon was inflated; then, by withdrawing the catheter, the balloon was pressed against the inside of the stoma so obtaining a watertight seal. The other stoma was blocked in a similar manner with a standard No. 16F gauge Foley catheter (Fig. 38a). In each test the fistula was irrigated with Tyrode's solution at 37°C until the returning fluid was clear; 30 minutes later, 25 ml. of test solution were pipetted into a syringe and put in through the indwelling catheter. The test solution contained 10 micrograms of ferric iron, including the radioactive label of 5 to 10 microcuries of Fe 59, in 25 ml. Tyrode's solution at 37°C and at pH 2 or 7. Dueterium oxide 0.2 ml. was used as a label for the water. In some cases varying amounts of ascorbic acid from 1 to 100 mg. were added, /

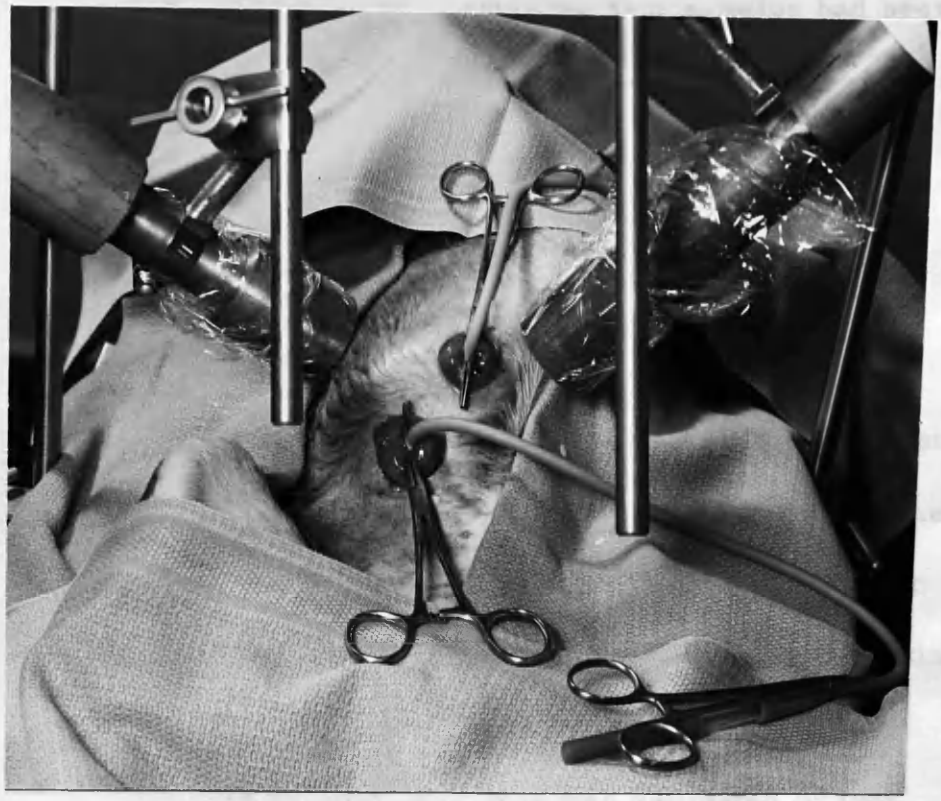


FIG. 38 (a).

Scintillation crystals mounted over Thiry Vella fistula of duodenum during test of absorption of iron showing 2 NaI crystals with lead sheathing protected by cellophane from accidental contamination. The stomata of the fistula are occluded with balloons which are held firmly in position by clamps applied to the catheters.

/added. In two tests juice secreted by innervated canine gastric corpus pouches in response to a meal of meat was used to make the test solution. The pH was adjusted to 2 with hydrochloric acid. After the test solution had been placed in the loop, specimens were taken in some tests at 15, 30, 60 and 90 minutes and pH measurements made. The tests lasted 120 minutes. In one test the loop was emptied of iron-containing fluid at 30 minutes and then irrigated with 100 ml. Tyrode's solution. Absorption was followed during the remaining 90 minutes of the test. The dog's temperature, measured on a rectal thermometer, was maintained at 37 to 39°C with the aid of an electric blanket, as a fall in body temperature can affect intestinal absorption (Lind, 1960).

The absorption of iron was estimated by two direct-writing scintillation counters fixed over the loop (Figs. 38a and b). This method, using only one counter, has been shown to give a good estimate of the amount of iron absorbed (page 39). The test period was two hours since it has been seen that the absorption of iron has almost completely stopped by this time in most cases (page 45). Two scintillation counters were used /

FIG. 38 (b).

Scintillation counters mounted over Thiry Vella fistula of duodenum during test of absorption of iron. Ratemeters and recorders from in vivo scintillation counters are shown.

/used to obviate any discrepancy in the recording due to movement of the loop relative to the crystal. For the same reason, light anaesthesia was maintained throughout the tests with intravenous sodium pentobarbitone.

The dueterium oxide content of the test solution and of the 15 min. sample of bowel fluid were estimated on the mass spectrometer (Solomons et al., 1950; Code et al., 1954) at intervals throughout the series of tests. The bidirectional rates of movement of water were calculated using the formula of Visscher and co-workers (1944). These estimations were done in order to provide a guide whether the function of the isolated loops of bowel changed from test to test.

Regular estimations were made of haemoglobin, packed cell volume and serum iron. At the completion of the series of tests the dogs were submitted to autopsy.

RESULTS.

The method was found to be reproducable in that repeated estimations in the same animal differed by 12% at the most with a S. E. M. of around 2% (Table X).

The amount of iron absorbed from these chronic Thiry Vella fistulae did not differ significantly from that /

Dog	No. of Readings	Absorption of Iron % Dose		
		Mean	Range	S. E. M.
P455	6	31.01	25.6 - 37.1	1.67
P777	4	16.78	14.0 - 19.2	1.25
P779	6	27.81	25.0 - 32.1	1.59
S192	4	14.12	11.2 - 18.9	2.06

TABLE X.

Percentage absorption of iron in repeated tests using 10 micrograms ferric iron plus 100 mg. ascorbic acid at pH of 2 in chronic Thiry Vella fistulae in 4 dogs.

/that in the acute isolated loops (Table XI). The addition of ascorbic acid gave a graded increase in the absorption of iron which levelled out at 10 to 20 mg. (Fig. 39, Table XII).

As with the acutely prepared loops, the addition of ascorbic acid at pH 7 gave the same absorption of iron as changing the pH to 2. Also, ascorbic acid at pH 2 resulted in doubling of absorption of iron (Fig. 40). Making the test solution in canine gastric juice gave no increment of absorption of iron above that from a similar solution in Tyrode's solution at the same pH, namely 2. When radioactive iron was removed at 30 minutes the absorption of iron proceeded up to 120 minutes from that left attached to the loop. The total amount absorbed was almost identical to that when the iron containing fluid was left undisturbed in the bowel during the whole two hours (2.58 micrograms as compared with 2.50 micrograms). In 3 of the 4 dogs the iron absorbed by the duodenal loop alone was from about one half to nearly twice the amount absorbed by the rest of the bowel which was still in continuity (Table XIII).

No significant change in the rates of water movement were found during the period of testing and haemoglobin, /

	Thiry Vella		Acute Loops	
	No. of tests	Mean % absorption	No. of tests	Mean % absorption
10 micrograms iron at pH 2 + Vit C	10	23.81% ± 2.50	4	18.07% ± 3.29*
Diff		5.74 ± 4.49**		
t		1.278		
P		< 0.3		

* S.E.M. ** S.E.M. Diff

TABLE XI.

Comparison of absorption of iron from a test solution at pH 2 with ascorbic acid placed in acutely isolated duodenal loops or in chronic duodenal Thiry Vella fistulae.

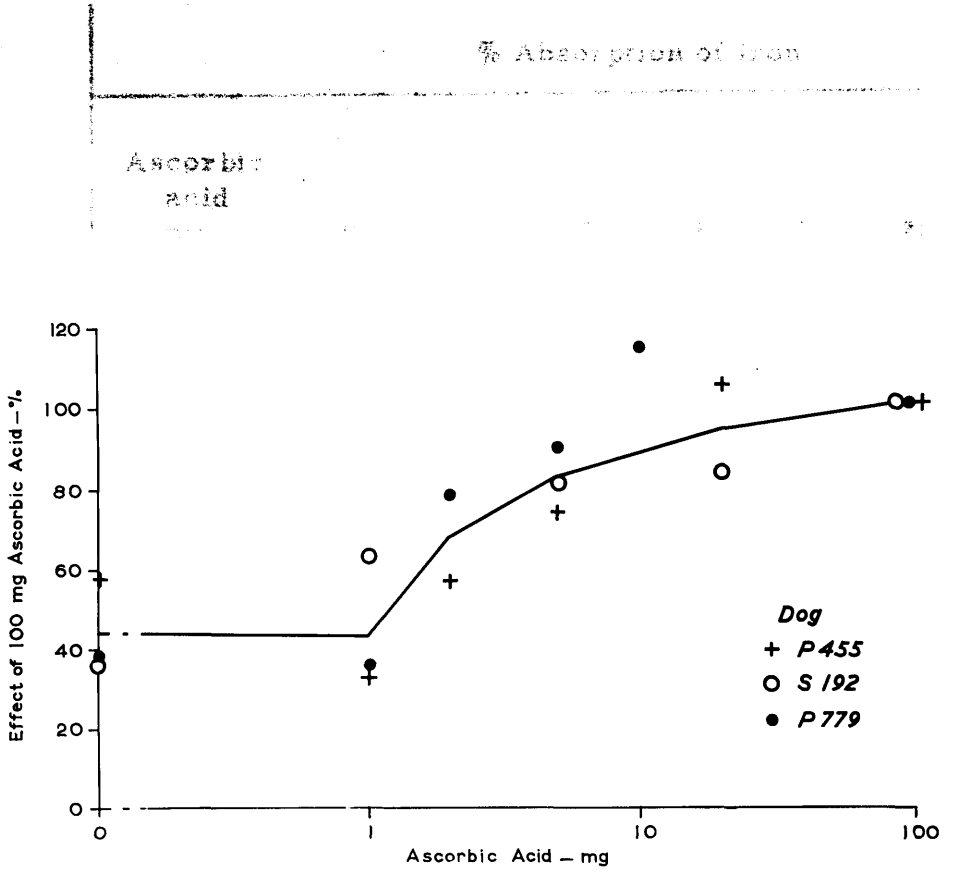


FIG. 39.

Effect of various doses of ascorbic acid on the absorption of 10 micrograms of ferric iron from Thiry Vella fistulae of the duodenum expressed as percentage of the absorption at a dose of 100 mg. ascorbic acid.

	% Absorption of Iron						
Ascorbic acid mg.	0	1	2	5	10	20	100
P455	18.8 (58)*	10.7 (33)	18.3 (57)	23.6 (74)	-	32.6 (105)	31.0 (100)
P779	10.8 (39)	10.1 (36)	21.8 (79)	25.0 (90)	32.0 (115)	-	27.8 (100)
S192	5.3 (38)	8.9 (63)	-	11.3 (80)	-	11.7 (83)	14.1 (100)

* Figures in brackets denote absorption of iron at varying dose of ascorbic acid expressed as a percentage of the absorption at a dose of 100 mg. ascorbic acid.

TABLE XII.

Effect of varying doses of ascorbic acid on percentage absorption of iron from chronic Thiry Vella fistulae in 3 dogs.

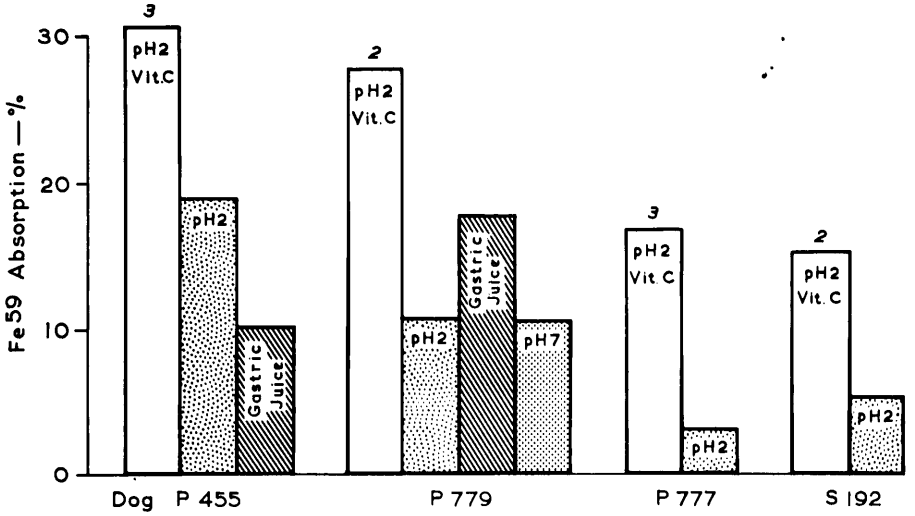


FIG. 40.

Percentage absorption of 10 micrograms ferric iron from isolated Thiry Vella fistulae of the duodenum in 4 dogs with various test solutions.

Dog	Test Conditions	A. From Isolated Duodenal Loops	B. From Remainder of Gut	$\frac{A}{B}$ %
P455	pH 2	18.8	10.0	180
	pH 2 Vit C	31.0	26.0	119
P777	pH 2	3.0	43.0	7
	pH 2 Vit C	16.8	59.5	28
P779	pH 2	10.8	24.0	46
	pH 2 Vit C	27.8	51.0	54
S192	pH 2 Vit C	14.1	32.0	44

TABLE XIII

Comparison of percentage of absorption of radioactive iron from isolated duodenal loops and from the rest of the alimentary tract in 4 dogs.

/haemoglobin, packed cell volume and serum iron showed small fluctuations within the normal range. At autopsy the muscular layers of the isolated loops were somewhat fibrosed. Histological examination showed no abnormality of the mucosal layer.

DISCUSSION.

The similarity between the results obtained from the acute isolated loops and the chronic Thiry Vella fistulae is in agreement with that found by Code and co-workers (1960) when using the same preparations to study absorption of water and electrolytes.

Amounts of ascorbic acid from 1 to 20 mg. showed an increasing effect in augmenting the absorption of iron from the Thiry Vella fistulae but there was only a small further increase up to 100 mg. If this promoting effect were due to formation of a ferric ascorbate alone, 0.2 mg. of ascorbic acid would be enough to combine with 10 micrograms of iron. However, no increase over control absorption occurred at a level of 1 mg. Thus, an excess of ascorbic acid is needed, either because a chelate can only be formed in such conditions or because the reducing power of ascorbic acid is the effective mechanism. The latter explanation seems likely, /

/likely, since the increase in absorption of iron continues to a dose of 10 to 20 mg. of ascorbic acid, an excess of 50 to 100 times the theoretical amount for chelation. However, it may be that both chelation and reducing power operate in conjunction. The absorption of iron by the Thiry Vella segment was from one half to nearly twice that of the same dose given via an intragastric tube in 3 or 4 dogs. In view of the small area of the separated loop it might appear that this isolated duodenal mucosa had a much greater absorptive capacity per unit area than the rest of the bowel. Factors increasing the absorptive power of the isolated bowel would be cleaning before testing which would remove any debris that might in the ordinary way become attached to the iron: also, the exclusion of the pancreatic secretion from the loop would increase absorption (Taylor et al., 1935; Gillman et al., 1947; Kinney et al., 1955; Kaufman et al., 1958). On the other hand, the scintillation counter measurement does not allow for the possibility of iron which became attached to the gut wall being absorbed after the two hour period. This would tend to counterbalance the above 2 factors. Another explanation for the apparent difference in absorptive capacity between these parts of the bowel is /

/is that the iron remains available for absorption only for a relatively short time as has been shown in the experiments in the acutely prepared isolated loops. This would mean that only a limited area of bowel would be reached by the iron in a state in which it could be absorbed. Therefore, the isolated loop was not, in fact, being compared with the remainder of the bowel in continuity but with a smaller area dependent on many factors including motility of the intestine and duration of availability of iron for absorption.

The question arises whether the mucosa of this isolated loop can be considered to be physiological especially in view of the tendency for the muscular coat to contract, although this was minimised by regular lavage and distension. In addition, recent work in the rat has suggested that the circulation of an isolated jejunal loop (Thiry Fistula) changes to divert blood away from the mucosa within 30 to 60 days after the loop being isolated (Nylander and Olerud, 1961). These possible shortcomings are unavoidable in a separated loop of bowel but from their nature would have combined to give a diminution in absorption of iron. Thus, our findings, if anything, may not reflect the full capacity of the duodenal mucosa, although they agree with the results in the acute isolated loops. /

/loops.

No extra factor was found in gastric juice to promote absorption of iron comparable to the action of intrinsic factor in the absorption of Vitamin B. 12.

CONCLUSIONS.

A reproducible method has been established for measuring absorption of iron from Thiry Vella fistulae in the dog. This absorption did not differ significantly from that in acutely prepared isolated loops.

The action of ascorbic acid in increasing absorption of iron is not solely due to formation of a chelate. Iron-containing solution in the bowel lumen ceases to have a material influence on absorption of iron after 30 minutes.

THE IMPORTANCE OF THE DUODENUM IN THE ABSORPTION OF IRON IN THE DOG.

THE IMPORTANCE OF THE DUODENUM IN THE ABSORPTION OF IRON IN THE DOG.

As noted in the introduction, the duodenum is thought by many to be the site of maximal absorption of iron and when it is by-passed after gastric operations its lack is thought to contribute to the occurrence of anaemia. Supporting evidence on this concept in the dog comes mainly from the Rochester, N. Y. group, using anaemic dogs with total gastric pouches or with jejunal fistulae (Hahn et al., 1943; Stewart et al., 1950). These observations have been extended using more recent techniques of assessing absorption of iron in non-anaemic dogs to study the effect of removal of the duodenum on the absorption of iron from the alimentary tract.

MATERIALS AND METHODS.

Four healthy mongrel dogs, weighing 8.5 to 13 Kg., were fed standard kennel diet and maintained their weight throughout the study. They had been dewormed and inoculated against distemper. Studies of the absorption of iron were made before and after separation of the duodenum. A test dose of 10 micrograms of ferric iron, including the radioactive label of 5 to 10 microcuries of /

/of Fe 59 was given by intragastric tube in 25 ml. Tyrode's solution followed by 200 ml. physiological saline to wash through the tube. Less than 1% of the dose adhered to the tube. Ten micrograms was chosen as a dose giving about 30% absorption so that any change in either direction, could be readily appreciated. In some tests 100 mg. ascorbic acid were added. The animals were fasted for 16 hours before and 2 hours after giving the iron at 8 to 9 a.m. The amount of radioactive iron absorbed was estimated by measuring how much was excreted in the faeces and subtracting this from the initial dose. The dogs were kept in metabolic cages and all stools were collected until less than 1% of the dose was excreted in 24 hours. Urine was also collected during this period which lasted for as long as 6 days. Utilisation of the isotope was determined by estimating the amount of radioactive iron in the peripheral blood 14 days after the iron was given. At least 3 weeks elapsed between tests.

The radioactivity of the blood was measured in a thallium-activated sodium iodide scintillation counter (Tracerlab.) with an automatic scaler. Duplicate specimens of 5 ml. external jugular venous blood haemolysed with saponin were used. In order to calculate accurately /

/accurately the radioactivity in the blood, Evans Blue was used to determine the plasma volume (Von Porat, 1951). The haematocrit, after correction for trapped plasma (Chaplin and Mollison, 1952) and for variation from the total body haematocrit (Chaplin et al., 1953) was used to calculate total blood volume. Haemoglobin (as cyanmethaemoglobin) packed cell volume (3,000 r.p.m. for 30 min.) and serum iron (Ramsay, 1957) were frequently estimated. Stools were examined for faecal occult blood and for intestinal parasites. Faeces were packed into an almost cylindrical plastic container (7 cm. x 5 to 6 cm. diam.) and the radioactivity measured by placing the container on top of the scintillation counter. Control experiments were run to assess the effect of the height of the faeces above the sodium crystal on the amount of radioactivity recorded. The mean of 6 such experiments was used as a correction curve (Fig. 41). In practice, the height in the plastic container of the specimen of faeces to be counted was measured and the readings corrected to a height of 6 cm. from the top of the plastic container.

An attempt was made to keep the body stores of iron constant despite the operation. The amount of blood lost at surgery was kept to a minimum and was measured by the loss of radioactivity from the blood. The /

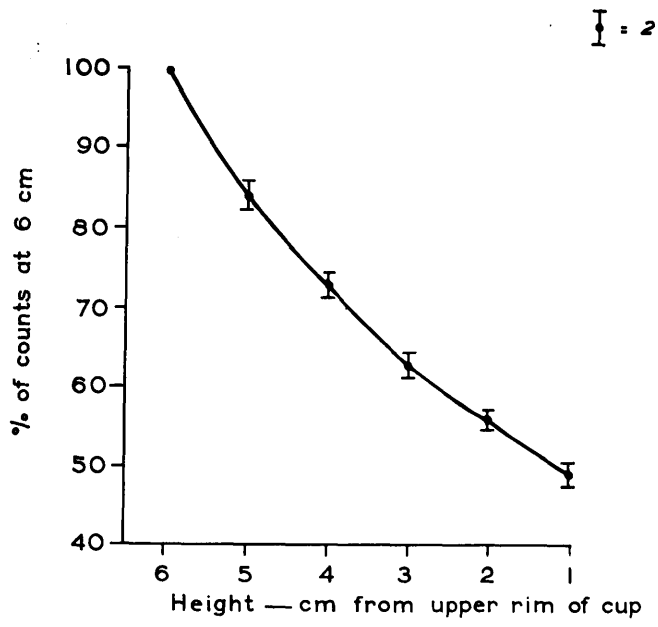


FIG. 41.

Effect of change of height of radioactive contents of plastic cup, measured in cm. from upper rim of cup, on the number of counts recorded by sodium iodide scintillation counter. Mean of 6 curves.

/The concentration of radioactive iron in the blood had stabilised before operation (Hahn et al., 1941) and a measurement of the total blood volume was made. These measurements were repeated a day or so after the operation and intramuscular iron was given to replace the iron lost.

Operative Procedure.

With the animal under pentobarbitone anaesthesia and using aseptic technique a midline epigastric incision was made. The duodenum was divided just distal to the site of entry of the major pancreatic duct. This level was chosen for two reasons:-

(1) The major pancreatic duct was preserved to avoid the augmentation of absorption of iron attendant on diminished or absent pancreatic secretion (Taylor et al., 1935; Gillman et al., 1947; Kinney et al., 1955; Kaufmann et al., 1958). Transplantation of the pancreatic duct could also have achieved this but with a greater operative risk and less certainty of having full pancreatic secretion.

(2) Any more proximal division of the duodenum to be worthwhile would have had to have been taken to the pylorus and reconstruction following this would have been liable to cause an increase in gastric acid secretion (Storer et /

/et al., 1952). Such a change might have invalidated the comparison of the tests before and after operation. The duodenum was divided a second time just distal to the duodeno-jejunal flexure. The ends of the separated segment of duodenum were brought out through incisions to the right of the midline and the mucosa sutured to the skin to make permanent stomata. Throughout the procedure care was taken not to damage the blood supply to the intestine. The exact measurements of the loop were recorded and the continuity of the gut was re-established by end-to-end anastomosis in two layers. The isolated segments were used for studies of the absorption of iron (see page 78). The postoperative recovery was uneventful in all cases and further tests of the absorption of iron were begun after 3 to 4 weeks.

Following one postoperative test, one dog (P455) was made anaemic by bleeding and another test of absorption of iron was made.

RESULTS.

The addition of ascorbic acid to the test solution doubled the absorption of iron both before and after operation (Fig. 42). After the removal of the segment of duodenum /

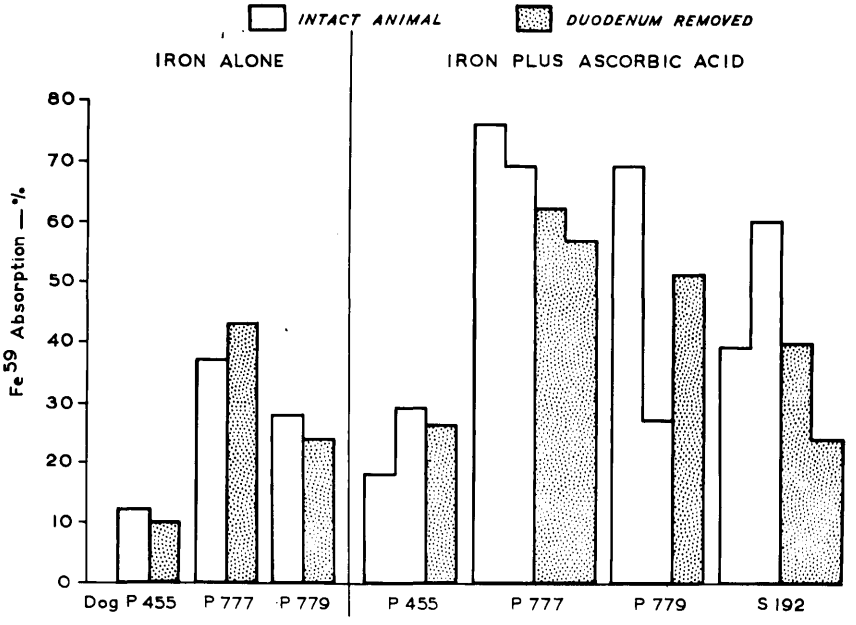


FIG. 42.

Percentage absorption of 10 micrograms of ferric iron with or without 100 mg. ascorbic acid given via an intragastric tube in dogs before and after removal of the duodenum from continuity with the rest of the alimentary tract.

/duodenum no constant change was found in absorption of iron: diminution in absorption occurred in 2 of 3 tests with ferric chloride alone and in 4 of 6 tests with ascorbic acid when compared with the pre-operative mean absorption (Fig. 42, Table XIV). However, only two of these postoperative results were well outside the range of findings in the intact animal. Utilisation of the iron absorbed was diminished in one dog (S192) and slightly diminished in another (P777) after separation of the duodenum (Fig. 43, Table XV).

The dog made anaemic after operation showed a marked increase in absorption of iron in response to the anaemia. With a dose of 10 micrograms of ferric iron with 100 mg. ascorbic acid, absorption rose from 26% after operation to 56% after the bleeding.

Haemoglobin, packed cell volume and serum iron results did not vary significantly at any of the tests of absorption with the exception of the one discussed immediately above.

DISCUSSION.

A valid comparison of the absorption of iron before and after the removal of the duodenum from continuity with the rest of the alimentary tract can only be made if there is no change in the general factors which control the /

	Absorption of Iron % of amount given					
	Before Operation			After Operation		
	No Vit C	Vit C		No Vit C	Vit C	
P455	12	18	29	10	26	56*
P777	37	76	69	43	62	57
P779	28	69	27	24	24	51
S192	-	39	60	-	40	24

* When dog was anaemic

TABLE XIV.

Percentage absorption of 10 micrograms ferric iron with or without 100 mg. ascorbic acid given via an intragastric tube in 4 dogs before and after operation to remove the duodenum from continuity with the rest of the alimentary tract.

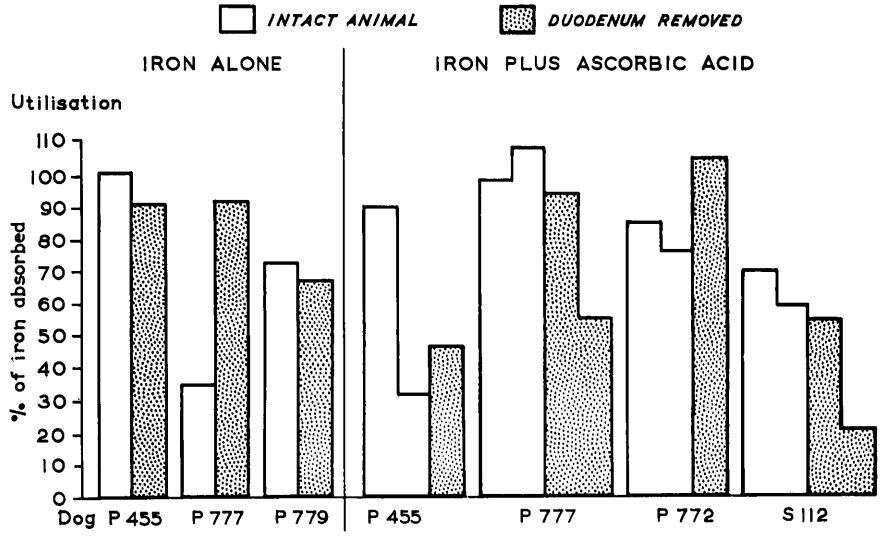


FIG. 43.

Utilisation of radioactive iron absorbed from 10 micrograms ferric iron with or without ascorbic acid given via an intragastric tube in 4 dogs before and after removal of the duodenum from continuity with the rest of the alimentary tract. Utilisation expressed as a percentage of the amount absorbed.

Utilisation of Fe 59 % of amount absorbed						
	Before Operation			After Operation		
	No Vit C	Vit C		No Vit C	Vit C	
P455	100	89	31	90	46	73*
P777	35	97	107	91	93	55
P779	72	84	75	67	104	-
S192	-	69	58	-	54	20

* When dog was anaemic

TABLE XV.

Utilisation of radioactive iron absorbed from 10 micrograms of ferric iron with or without 100 mg. ascorbic acid given via an intragastric rube in 4 dogs before and after operation to remove the duodenum from continuity with the rest of the alimentary tract.

/the absorption of iron. In particular, the body stores of iron must not change appreciably. It is well known that either an increase or a decrease in the body stores can influence the absorption of iron (Bothwell et al., 1958). It was thus important to replace accurately the iron lost at operation and to keep check on possible faecal loss from parasites.

One might expect a diminution in the absorption of iron when the duodenum was removed from contact with the iron given, as this part of the gut has been said to be the most important area in absorption of iron (Hahn et al., 1943; Endicott, 1949; Granick, 1949; Stewart et al., 1950). No support for this view came from the present study which is in agreement with previous work on absorption of iron in humans who had both a gastroduodenal and gastrojejunal anastomosis at different times after gastrectomy (Duthie, 1959). Possible objections to the above work in man might be that more was involved in the studies than the mere by-passing of the duodenum and that the admixture of the pancreatic juice with the iron would not have been so effective in the gastro-jejunal anastomosis (Polya) operation so that a diminution in the absorption of iron under these circumstances /

/circumstances might have been masked. Passage of food material into the by-passed duodenum has been reported by Kay (1957). Although barium meal studies did not demonstrate any reflux no absolute guarantee was given that some of the test solution might not have reached duodenal mucosa. The findings reported here avoid these objections by having complete separation of the duodenum from the rest of the gut and by preserving the pancreatic secretion.

The capability of patients after partial gastric resection to absorb iron is not significantly changed when they are not anaemic (Smith and Mallett 1957; Baird and Wilson, 1959), although Stevens and co-workers (1959) noted below average absorption of iron in 6 of 8 patients after gastrectomy. However, it is agreed that when anaemia supervenes there is a lack of the normal increase in absorption of iron which occurs in anaemic patients with intact stomachs (Baird and Wilson, 1959; Stevens et al., 1959). An increase in the absorption of iron was observed in the dog made anaemic after removal of the duodenum. This would suggest by-passing the duodenum is not the factor responsible for the failure of this response after gastrectomy.

The altered utilisation of iron in the later tests in /

/in two dogs might be within normal variation. On the other hand, it is known that inflammation can diminish utilisation of iron, at least temporarily, (Dubach et al., 1946; Wintrobe et al., 1947; Yuile et al., 1949). However, no sepsis was present and the general health of the dogs was good. A diminution in utilisation can take place when iron stores are increased. No evidence of such an increase was found in the present study.

The amount of iron given was small enough to be absorbed efficiently by the bowel without the duodenum and it is possible that a larger dose, putting greater stress on iron-absorbing capacity, might have revealed a change after removal of the duodenum.

CONCLUSION.

The removal of the duodenum from continuity with the rest of the gastrointestinal canal had no unequivocal effect on the absorption of iron at the dose levels used, although a slight tendency to diminution of absorption was noted in some tests after operation. This diminution was not definite enough to ascribe a predominant role to the duodenum in the absorption of iron. /

INTESTINAL ABSORPTION OF IRON

IN THE RAT.

MATERIALS AND METHODS.

Male Sprague Dawley rats weighing 170 to 200 gm were used. Except in Experiment IV below, they were

INTESTINAL ABSORPTION OF IRON IN THE RAT.

In the previous section it has been shown that removal of the duodenum has no definite effect on the gastrointestinal absorption of iron in the dog. A small dose of iron was used and it was felt that increasing the amount given might reveal the importance of the duodenum in the absorption of iron. In view of the large number of animals required the rat has been used for this study.

In addition to the above question of the importance of the duodenum, the study has investigated the gradient of absorption of iron along the alimentary tract and the effect upon any such gradient of bleeding and of an iron-deficient diet.

MATERIALS AND METHODS.

Male Sprague Dawley rats weighing 170 to 220 gm. were used. Except in Experiment IV below, they were given standard feed and had gained weight normally. The amount of iron given varied from 1 to 1,000 micrograms as ferric chloride with a radioactive label of 3 to 5 microcuries Fe 59 and with 5 mg. ascorbic acid in all cases. The iron /

/iron was given in 0.5 ml. 0.9% saline at a pH of 2.5 to 3. All animals fasted 12 to 16 hours prior to being given the iron in order to allow the small bowel to be empty so that food residues in the lower ileum would not affect the comparison with more proximal areas. Iron was administered from a calibrated tuberculin syringe via a fine intragastric polythene tube or via an intradermal needle into the gut at laparotomy. The maximum variation in giving repeated doses from the syringe was 3%. When iron was placed in the stomach no anaesthesia was given. Ether anaesthesia was used during laparotomy which was performed through a midline abdominal incision. Iron-containing solution was injected at one of the following sites:-

Duodenum	-	just distal to the pylorus.
Upper jejunum	-	5 cm. distal to the duodeno-jejunal flexure.
Mid jejunum	-	15 cm. distal to the duodeno-jejunal flexure.
Jejuno-ileum	-	30 cm. distal to the duodeno-jejunal flexure.
Ileum	-	10 cm. proximal to the ileo-caecal junction.

The total length of the jejunum and ileum in rats 170 - 220 gm. was about 60 cm.

The bowel was held firmly round the needle during /

/during injection and on withdrawal finger pressure was maintained for 30 seconds. To test whether retrograde spread of the iron had been avoided, 3 rats were injected in the upper jejunum, a fine silk ligature being used to mark the site of injection. The animals were sacrificed at 4, 8 and 12 hours and the small bowel excised. The jejunum proximal to the silk ligature was removed, the duodenum divided into two portions and the rest of the small bowel into six equal parts about 10 cm. in length, the proximal three being labelled jejunum and the remaining three ileum. The radioactivity of each of these nine portions was determined in a thallium activated sodium iodide scintillation well-type counter, corrected to the weight of the jejunal segment into which injection was made and compared with an equal weight of rectus abdominis muscle as a control. No significant retrograde spread of radioactivity was found. (Fig. 44). Precise examination of the segment of jejunum immediately proximal to the site of injection showed the maximal retrograde spread of radioactivity to be 1.5 cm. Iron reaches the colon of the rat within 3 hours after intragastric administration (Austoni and Greenberg, 1940; Mori et al., 1957). In keeping with this finding only a small proportion of the dose, less than 10%, was in the entire small bowel and its contents in our experiments. Almost all of this was in the segment, approximately 10 cm. long, at the site of /

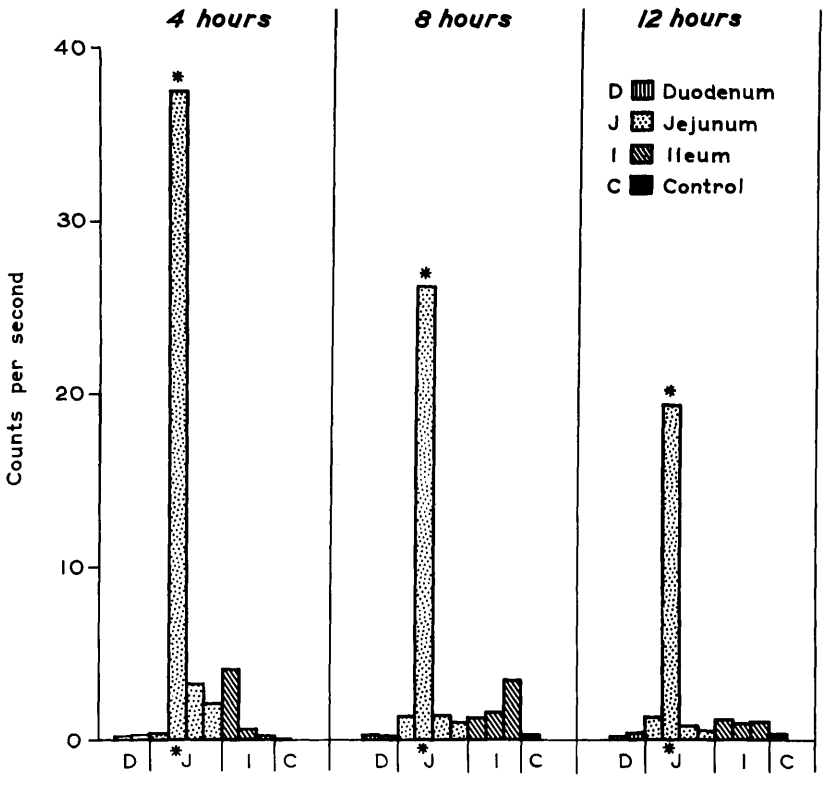


FIG. 44.

Distribution of radioactive iron in the small bowel of rats 4, 8, and 12 hours after injection into the upper jejunum. Values corrected to the weight of the jejunal segment (*) into which the injection was made and compared with an equal weight of rectus abdominis muscle.

/of injection.

The amount of iron absorbed was estimated by total body counting. The rat was placed on its side on the bottom of a glass beaker 9 cm. in diameter and held in place by another smaller beaker which was fixed inside the first leaving a space 7 cm. in height. (Fig. 45). In this space the rat moved round and so helped to compensate for any lack of homogenity in the distribution of the radioactivity. The beaker was placed 9 cm. above a thallium-activated sodium iodide scintillation crystal. This technique was found to give reproducible readings when the radioactive iron was distributed through the body several days after injection. Immediately after injection when the bolus of Fe 59 was still concentrated in the bowel a wide variation in duplicate measurements was found. However, when an 0.5 ml. control sample of the test solution was diluted in the beaker up to a height of 6 cm. the reading agreed well with the mean of repeated readings for a group of 13 rats immediately after injection. (Table XVI). A standard for each experiment was made in this manner and the absorption of iron was expressed as a percentage of the standard. Five microcuries of Fe 59 when diluted gave about 800 counts per second.

The time for measuring the retention of iron was chosen following daily total body counting of rats after /

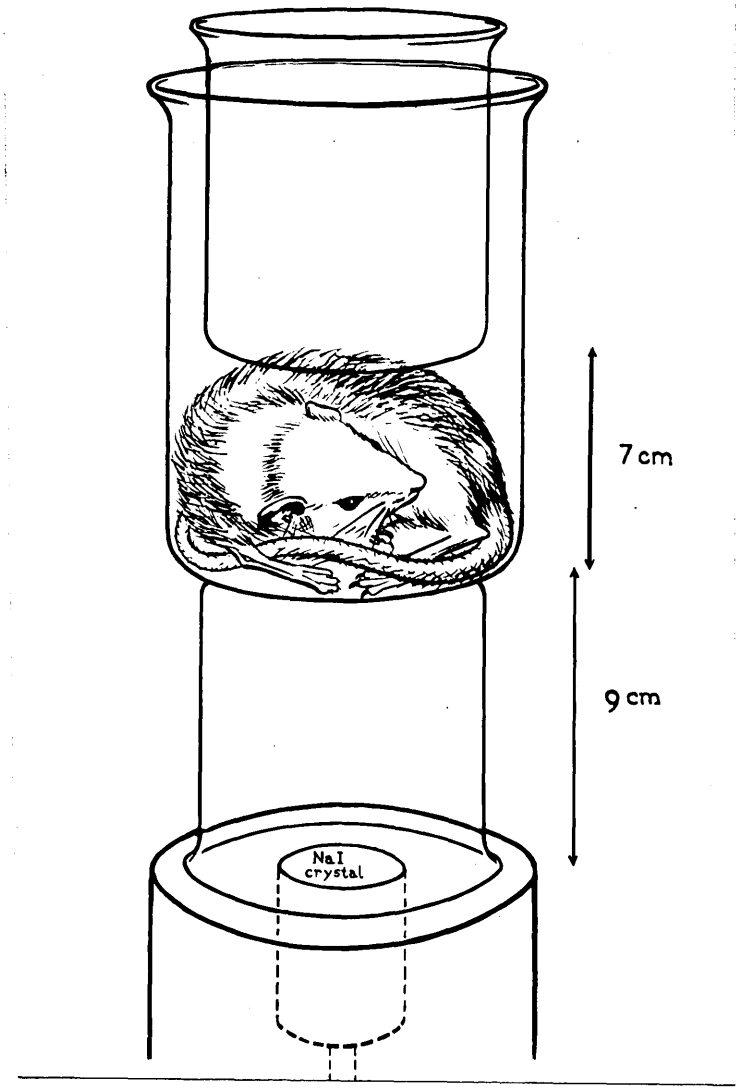


FIG. 45.

Diagram of method of total body counting of rats at a distance of 9 cms. from a sodium iodide crystal after giving radioactive iron into various areas of the bowel.

Rat No.	Net. Counts/sec.
1	858.83
2	799.50
3	773.67
4	858.56
5	834.87
6	822.51
7	829.80
8	773.30
9	666.17
10	696.25
11	1065.63
12	700.58
13	965.65
Mean	819.55
0.5 ml Fe59 in beaker	819.48

TABLE XVI.

Comparison of recording by sodium iodide crystals of counts from 5 microcuries Fe59 immediately after injection into rats and after dilution to an equivalent height in a beaker. (6 cm.)

/after injection of 1 microgram ferric iron into the duodenum, the jejunum or the ileum. Most iron was lost from the body in the first 48 hours and a stable level was maintained after 4 days (Fig. 46). Thus, total body counting was done in duplicate on 5th and 7th days after giving the iron.

The dependability of the total body counting method of estimating the absorption of iron is well established (Herndon et al., 1958; Beutler and Buttenweiser, 1960; Field et al., 1960; Greenberg et al., 1960). The present modification was checked against balance methods in eight rats. Intraduodenal or intra-jejunal injection of 10 micrograms of ferric iron with 5 mg. ascorbic acid was followed by putting the rats in individual metabolic cages. Faeces and urine were collected separately for 5 days. The radioactivity excreted was measured in a well-type scintillation counter. As other authors have found, negligible urinary excretion was noted and almost all faecal excretion occurred in 48 hours. The iron retained was calculated by subtracting the amount excreted from the dose given. It was compared with the estimate obtained by total body counting and gave closely similar results. (Table XVII).

Experiments were performed in four groups using a total of 165 rats. /

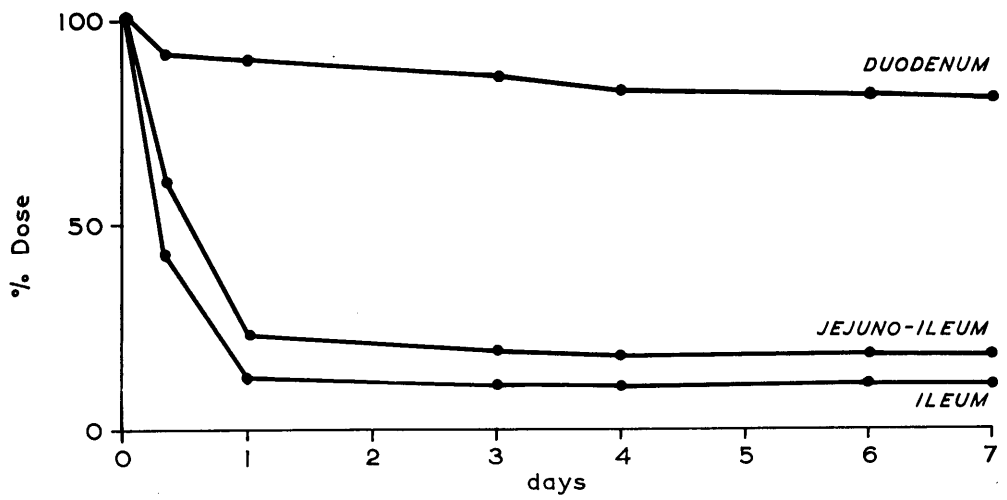


FIG. 46.

Total body counting of rats after injection of 1 microgram ferric iron with 5 mg. ascorbic acid into the duodenum, jejuno-ileum, and ileum showing no change in retention of iron expressed as a percentage of the dose, 4 to 7 days after injection.

Site of Injection	Absorption of Iron % Dose		
	Faecal Collection		Total body Counting
	Faecal Fe^{59}	∴ Retained	
Duodenum 1	17	83	85
2	21	79	74
3	21	79	72
4	71	29	35
Mean		67.5	66.5
Jejunum 1	57	43	42
2	58	42	35
3	29	71	69
4	66	34	30
Mean		47.5	44.0

TABLE XVII.

Comparison of faecal collection and total body counting of methods of estimating absorption of iron in the rat.

/rats.

I. Effect of by-passing duodenum.

Three dozen rats were used in groups of six. Two groups were studied at a time; one having the iron injected into the duodenum and the other into the upper jejunum. The doses given were 10, 100 and 1,000 micrograms.

II. Gradient of Absorption of Iron.

Six groups each of six rats were injected with 10 micrograms of iron. Two groups were studied at a time; one was given the iron into the duodenum; in the other the site was varied, being mid jejunum, jejuno-ileum or ileum respectively.

A group of 12 rats was given 10 micrograms of iron by intragastric tube, and compared with another six given iron by intraduodenal injection.

III. Effect of Bleeding.

Rats, lightly anaesthetised with ether, were bled 2.5 ml. per 100 mg. body weight by intra cardiac puncture on the 10th, 7th and 3rd days before the test of absorption of iron. Seventeen rats after bleeding were given 10 micrograms of iron; 16 were given 100 micrograms iron and 12 received 1000 micrograms.

IV. Effect of Iron Deficient Diet.

Thirty rats born at the same time were studied; /

/studied; 12 of these were put on an iron-free milk diet as weanlings (Copp and Greenberg, 1946) and 18 on standard feed (details in Appendix, p. 217). After 6 weeks on this diet when they were about 180 gm. they were given 100 micrograms iron by injection. Haemoglobin content of tail vein blood was measured as alkaline haematin.

RESULTS.

I. Effect of by-passing duodenum.

The absorption of iron after injection into the duodenum was significantly greater than after intrajejunal injection when 1000 micrograms of iron were given. It was also greater with 100 micrograms and probably statistically significant (Fig. 47). No significant difference was found at a dose of 10 micrograms (Table XVIII).

II. Gradient of Absorption of Iron.

Absorption of iron diminished as the injection was given more distally. The duodenum was used as a reference point. Injection at mid jejunum, jejuno-ileum and ileum, were all significantly different from the duodenum and from one another (Fig. 48, Table XIX). Taking the duodenal reading as 100% the absorption in the other areas was as low as 4% (Table XX). The absorption of iron given via an /

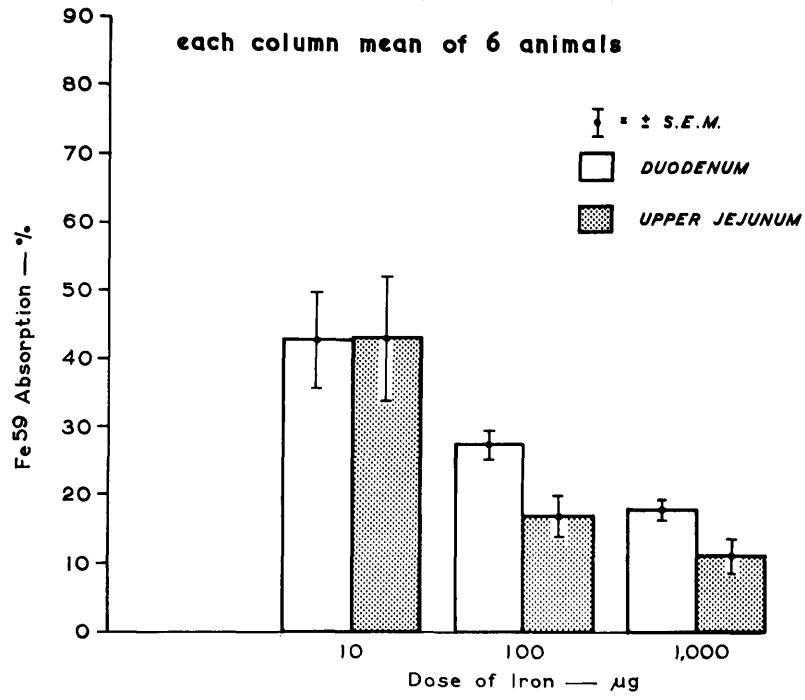


FIG. 47.

Percentage absorption of ferric iron injected into the duodenum and upper jejunum in groups of 6 rats.

Dose of Iron ug.	Mean absorption % dose in 6 rats		Mean Diff.	S.E.M. Diff.	t	P
10	Duod.	43.6 ± 6.95*	- 0.3	11.55	0.026	>0.9
	Upper Jej.	43.9 ± 9.21*				
100	Duod.	27.2 ± 2.29*	10.2	3.73	7.736	<0.05
	Upper Jej.	17.0 ± 2.94*				
1000	Duod.	18.1 ± 1.42*	7.2	1.58	4.55	<0.01
	Upper Jej.	10.9 ± 0.70				

* S.E.M.

TABLE XVIII.

Comparison of absorption of iron injected into the duodenum or upper jejunum in groups of 6 rats.

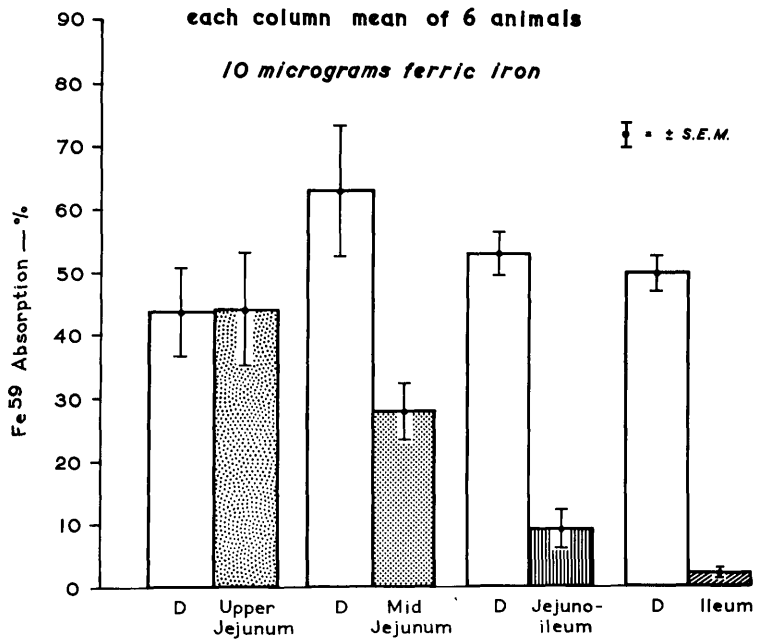


FIG. 48.

Percentage absorption of 10 micrograms ferric iron injected into the bowel of various sites in groups of 6 rats. In each experiment one group had intraduodenal injection and the other group mid-jejunal, jejuno-ileal or ileal site of infection.

Site of Injection	Mean absorption % dose in 6 rats.	Mean Diff.	S. E. M. Diff.	t	P
Duodenum Mid-jejunum	69.8 ± 6.05* 28.6 ± 4.08*	41.2	7.68	5.367	< 0.001
Duodenum Jejuno-ileum	53.1 ± 3.32* 9.2 ± 3.08*	43.9	4.53	9.696	< 0.001
Duodenum Ileum	50.4 ± 2.81* 2.1 ± 0.64*	48.3	1.80	26.792	< 0.001
Mid-jejunum Jejuno-ileum	28.6 ± 4.08* 9.2 ± 3.08*	19.4	5.12	3.788	< 0.01
Jejuno-ileum Ileum	9.2 ± 3.08* 2.1 ± 0.64*	7.1	3.14	2.258	< 0.05

* S. E. M.

TABLE XIX

Comparison of absorption of 10 micrograms of ferric iron injected at various sites in the small bowel in groups of 6 rats.

Site of Injection	Absorption of iron as % of absorption from duodenal site of injection					
	Normal			After Bleeding		
	10 ug	100 ug	1000 ug	10 ug	100 ug	1000 ug
Duodenum	100	100	100	100	100	100
Upper Jejunum	101	63	60	-	-	-
Mid Jejunum	41	-	-	46	57	104
Jejuno-Ileum	17	66	-	24	43	83
Ileum	4	9	-	15	37	52

TABLE XX.

Mean absorption of iron from various sites of injection into the bowel expressed as percentage of the absorption from intra-duodenal injection in normal rats, and rats after bleeding.

/an intragastric tube with no anaesthesia and no operation was not significantly different from that following intraduodenal injection of iron (Table XXI). When the logarithm of the amount of iron absorbed was plotted against the logarithm of the amount of iron injected, significant linear relationships were found for duodenal, jejunal and ileal sites of injection (Table XXII). The relationships were similar to those already found in the acutely prepared isolated intestinal loops in the dog (Table XXIII).

III. Effect of Bleeding.

Absorption of iron was increased in rats which had been bled when compared with normal rats. The increase was statistically significant in the duodenal site of injection at a dose of 10 micrograms and in the ileal site of injection at doses of 10 and 100 micrograms (Table XXIV). The augmentation of absorption of iron was relatively greater at the ileal site of injection (Table XXV, Fig. 49).

When the logarithm of the amount of iron absorbed was compared with the logarithm of the amount of iron injected, a significant linear relationship was found (Table XXVI). The slopes of these regression lines were compared with the lines obtained in the normal rats (Fig. 50). The /

	No. of rats	Mean absorption % dose	Diff. Means	S. E. M. Diff.	t	P
Intragastric tube	12	77.2 ± 1.44*	7.4	4.91	1.507	<0.2
Intraduodenal injection	6	69.8 ± 6.05*				

* S. E. M.

TABLE XXI.

Comparison of absorption of 10 micrograms ferric iron given by intragastric tube or by intraduodenal injection in the same experiment.

	No. of tests	Correlation of logarithm of amount of iron given and logarithm of amount of iron absorbed		
		Regression Coeff. b	Correlation Coeff. r	P
Duodenum	48	0.765	0.982	< 0.001
Jejuno-Ileum	18	0.751	0.706	< 0.01
Ileum	18	0.758	0.748	< 0.001

TABLE XXII.

Correlation of logarithm of amount of iron injected into the bowel of rats at various sites and the logarithm of the amount of iron absorbed.

Test Animal	No. of tests	Correlation of log of amount of iron given and log of amount absorbed.		
		Regression Coeff. b	Correlation Coeff. r	P
Duodenum				
Dog	17	0.967	0.901	< 0.001
Rat	48	0.765	0.982	< 0.001
Ileum				
Dog	10	1.149	0.872	< 0.001
Rat	18	0.758	0.748	< 0.001

TABLE XXIII.

Regression and correlation coefficient for the linear relationship between the logarithm of the amount of iron given and the logarithm of the amount of iron absorbed from isolated intestinal loops in the dog and after injection into the bowel in the rat.

Site of Injection	Dose of Iron ug	No. of rats	Mean absorption % Dose	Diff. Means	S. E. M. Diff.	t	P
Duodenum	10	Normal 24	54.2 ± 3.16*	19.8	2.19	9.029	<0.001
		Bled 5	74.0 ± 7.71*				
	100	Normal 12	28.0 ± 1.95*	7.7	3.72	2.069	<0.1
		Bled 4	35.7 ± 2.06*				
	1000	Normal 6	18.1 ± 1.42*	0.2	2.25	0.089	>0.9
		Bled 3	17.9 ± 8.71*				
Mid Jejunum	10	Normal 6	28.6 ± 4.08*	5.7	5.28	1.079	<0.4
		Bled 4	34.3 ± 3.05*				
	100	Bled 4	20.5 ± 1.26*				
	1000	Bled 3	18.6 ± 5.55*				

* S. E. M.

TABLE XXIV.

Comparison of absorption of ferric iron injected at various sites in the small intestine of normal rats and rats subject to repeated bleeding.

Site of Injection	Dose of Iron ug	No. of rats	Mean absorption % Dose	Diff. Means	S. E. M. Diff.	t	P
Jejuno-ileum	10	Normal 6	9.2 ± 3.08*	8.6	4.73	1.819	<0.2
		Bled 4	17.8 ± 0.60*				
	100	Normal 6	19.0 ± 6.49*	4.4	8.24	0.534	<0.7
		Bled 4	15.4 ± 1.80*				
	1000	Bled 3	14.7 ± 1.55*				
Ileum	10	Normal 6	2.1 ± 0.65*	9.0	2.05	4.398	<0.01
		Bled 4	11.1 ± 0.41*				
	100	Normal 6	2.6 ± 1.12*	10.5	1.57	6.662	<0.001
		Bled 4	13.1 ± 0.79*				
	1000	Bled 3	9.4 ± 1.19*				

* S. E. M.

TABLE XXIV.

Comparison of absorption of ferric iron injected at various sites in the small intestine of normal rats and rats subject to repeated bleeding.

Site of Injection	Absorption of Iron after Bleeding % of normal absorption		
	Dose of iron micrograms		
	10	100	1000
Duodenum	137	127	99
Jejuno-ileum	193	81	123 *
Ileum	552	505	520 *

* Assuming normal rats to have same relative absorption as at a dose of 100 micrograms.

TABLE XXV.

Mean absorption of iron injected at various sites into the bowel in rats after bleeding expressed as a percentage of the absorption of iron in normal rats.

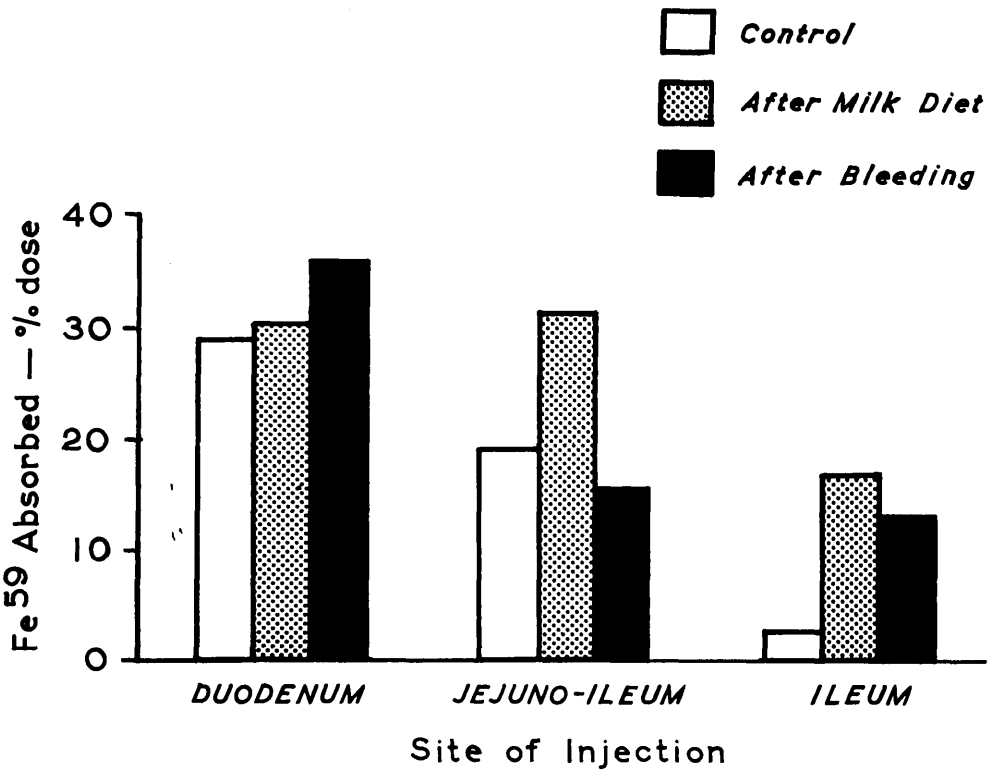


FIG. 49.

Mean percentage absorption of 100 micrograms ferric iron injected at various sites in the bowel of normal and control rats, rats after bleeding and rats fed on a milk diet, showing relatively large increase of absorption of iron at the ileal site of injection.

State of Rat	Site of Injection	No. of tests	Correlation of log of amount of iron given and log of amount absorbed		
			Regression Coeff. b	Correlation Coeff. r	P
Normal	Duodenum	48	0.765	0.982	< 0.001
After bleeding	"	12	0.695	0.991	< 0.001
Normal	Jejuno-ileum	18	0.751	0.706	< 0.01
After bleeding	" "	11	1.004	0.972	< 0.001
Normal	Ileum	18	0.758	0.748	< 0.001
After bleeding	"	11	1.032	0.963	< 0.001

TABLE XXVI.

Regression and correlation coefficient for linear relationships between logarithms of the amount of iron injected and the logarithms of the amount of iron absorbed after injection at various sites in the bowel of normal rats and rats after bleeding.

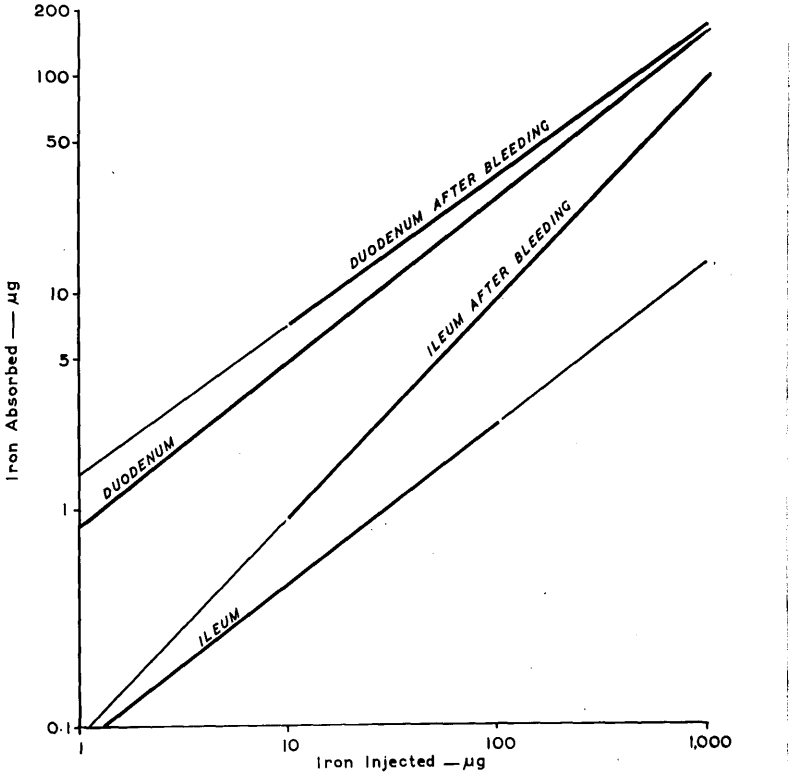


FIG. 50.

Regression lines of relationship between the logarithm of the amount of iron absorbed and the logarithm of the amount of iron injected into the duodenum or the ileum in normal control rats and rats after bleeding. The slope of the line for intraduodenal injection decreases slightly while the slope of the line for intraileal injection after bleeding increases.

(The thinner parts of the lines indicate extrapolation beyond limits of dats).

/The duodenal line after bleeding was slightly less steep. By contrast, the slope of the jejuno-ileal and ileal lines increased after bleeding (Table XXVI).

The gradient of absorption along the bowel showed the same trend in normal rats and those which had been bled. In the latter animals the superiority of the duodenal site of injection over the ileal site of injection was much less marked (Tables XX and XXVII).

IV. Effect of Iron-Deficient Diet.

The level of anaemia produced by feeding on the milk diet was less than found by the original authors (Copp and Greenberg, 1946), being 11.33G% as compared with 14.41 G% in controls (Table XXVIII). However, this agreed with the findings of Wack and Wyatt (1959) using a milk diet.

The absorption of iron showed the same pattern as in the rats which had been bled. The increase in absorption in the iron deficient animals was significant at the ileal site of injection (Table XXIX) and was also proportionately greater in ileum than in duodenal or jejuno-ileal sites of injection (Table XXVII).

DISCUSSION.

This method of comparing the absorption of various /

Site of Injection	Absorption of iron as % of absorption from duodenal site of injection		
	Normal	Bled	Milk Diet
Duodenum	100	100	100
Jejuno-ileum	66.2	42.0	104
Ileum	9.1	36.7	55.5

TABLE XXVII.

Ratio of mean absorption of 100 micrograms iron injected at various sites along the bowel to that from the duodenum in normal rats, rats after bleeding and rats having a milk diet deficient in iron.

	No.	Mean Haemoglobin G %	Diff.	t	P
Normal	18	14.41 ± 0.13*	3.08 ± 0.22**	14.318	0.001
Milk Diet	12	11.33 ± 0.18*			

* S. E. M. ** S. E. M. Diff.

TABLE XXVIII.

Comparison of mean levels of haemoglobin in the tail blood of normal rats and rats on a milk diet.

Site of Injection	No. of rats	Mean absorption % dose of ug.	Diff. of Means	S. E. M. Diff.	t	P
Duodenum	Normal 6 Deficient 4	28.7 ± 3.36* 30.1 ± 3.67*	2.4	4.57	0.525	< 0.7
Jejuno-ileum	Normal 6 Deficient 4	19.0 ± 6.49* 31.3 ± 7.13*	12.3	9.91	1.242	< 0.3
Ileum	Normal 6 Deficient 4	2.6 ± 1.12* 16.7 ± 2.33*	14.1	2.34	6.019	< 0.001

* S. E. M.

TABLE XXIX

Comparison of absorption of 100 micrograms ferric iron injected at various sites in the small bowel in normal rats and rats fed on a milk diet deficient in iron.

/various areas of bowel has the defect that the more proximal sites of injection obviously allow the iron to traverse more of the bowel and would thus bias the results in their favour from mere area of bowel even though the proximal bowel had no special importance in the absorption of iron. However, it has been shown already in the dog that the iron in the bowel lumen has little or no effect on absorption after $\frac{1}{2}$ to 1 hour. The iron by one hour has only passed through part of the small bowel from the duodenum (Mori et al., 1957). Thus, due to the relatively short time that iron remains available for absorption, little discrepancy should arise in the area of bowel it traverses in the absorbable state from any of the sites of injection. This is especially so in the experiments by-passing the duodenum which again demonstrate that the bowel can absorb the same amount of iron whether the duodenum takes part or not. It was only with the higher dosage (up to 5 mg. per Kg. body weight) that the duodenal site of injection gave significantly greater absorption. This would suggest that the duodenum has a greater reserve of iron-absorbing power and that when larger amounts are presented to the mucosa it is able to take up more than the more distal areas of the bowel. If it is permissible to transfer some of the results of these /

/these experiments to man, they would explain in part why the expected fall in iron absorption after gastrectomy has not been uniformly demonstrated (Smith and Mallett, 1957; Baird et al., 1957; Baird and Wilson, 1959). The distal bowel could take over some of the absorption of iron if it received the metal in a form suitable for absorption.

While several authors have discussed absorption of iron from the ileum and distal bowel (Hahn et al., 1943; Stewart et al., 1950; Finch and Finch, 1955), only a few direct measurements have been made. The findings here presented support for the concept that the distal small bowel is less important than the proximal small bowel, even when iron is presented to the different areas in the same solution which does not happen in the intact animal. Although the acidic solution is not usual for the distal small bowel it was felt that, as it gave maximal absorption in our experiments in the dog, it might give the best opportunity for absorption in the rat.

The findings in the second group of experiments with rats confirm the suggestion of a gradient of absorption along the alimentary tract (Hahn et al., 1943; Stewart et al., 1950). In the ileal injections, the iron still had to traverse the colon. The absorption of iron was only 2 to 3% so that /

/that the contribution of the large bowel was minimal at these dose levels. The relevance of these results to normal ingestion of iron is shown by the similarity of the absorption after intragastric instillation and after intraduodenal injection. This similarity excludes any depressing effect of the anaesthetic or of the operation on absorption. In addition, this last finding would suggest that not much absorption takes place in the stomach, although Hahn and co-workers (1943) found some absorption from a total gastric pouch in the dog.

The results of this section demonstrate that some absorption can take place in the more distal small bowel even in the non-anaemic animal. This is contrary to the opinion of the workers studying ferritin and using its presence as an index of the site of iron absorption (Granick, 1946 a, b, 1949). They believed that the duodenum was the main site of absorption. Wack and Wyatt (1959) using autoradiography also held that the duodenum was the site of absorption in the non-anaemic animal. However, the present tests differ fundamentally from the above in that iron was introduced at artificially distal parts of the gut and, while showing that absorption is possible there, do not give any evidence that this occurs after the oral ingestion of iron. It would seem unlikely that iron would remain available for absorption long enough to reach the /

/the distal small bowel.

The biological significance of the correlation between the logarithm of the amount of iron given and the logarithm of the amount of iron absorbed is not clear but, as in the dog, it allows any desired amount of absorption to be chosen by simple interpolation. As suggested previously, the similarity of the relationship in dog and rat may allow comparison of the data of the two species. The increased slope of the regression lines for the above relationship at the jejunal and ileal sites of injection in rats after bleeding shows an increase in the capacity to deal with larger doses of iron and may indicate some change in the process of absorption.

It has been held that anaemia in the rat increases the area of small bowel which absorbs iron as far as the caecum (Wack and Wyatt, 1959). This work was by autoradiography of the excised intestine after an intragastric dose of iron. Our results, with the rats after bleeding and with those on an iron deficiency regime, also suggest that the mucosa of the distal small bowel becomes more active. In anaemic rats iron takes longer than normal to pass along the bowel and this delay has been suggested as the cause of the increase in the absorption of iron in the anaemic state /

/state (Austoni and Greenberg, 1940; Mori et al., 1957). This slower movement of iron would explain to some extent the greater increase of absorption found from the intraileal injections in our experiments. In the normal rat, such injections do not stay long in the small bowel as they have only to travel 10 cm. before reaching the caecum. At this time much of the iron will still be available for absorption. In the anaemic animal, the delay in moving into the large bowel would allow this available iron to remain in contact with small bowel mucosa and so give an opportunity for more absorption.

Another possible explanation for the smaller increase in absorption from intraduodenal injection compared with that after intraileal injection is that the duodenal mucosa may be working at its optimum rate in the normal rat when iron and ascorbic acid are presented to it at a pH of 2. Thus, the duodenum responds less to stresses on iron metabolism than the ileum which is not so fully active until bleeding or iron deficiency supplies the stimulus

CONCLUSIONS.

The by-passing of the duodenum significantly diminished absorption of iron in the rat only when large dose /

/dose levels were reached.

Absorption of iron occurred from intrajejunal or intraileal injection in non-anaemic rats. A gradient of absorption of iron was found, greatest after intraduodenal injection of iron-containing solution, and diminishing along the small bowel to be least after intraileal injection.

Repeated bleeding or iron deficiency was followed by a greater increase in the amount of iron absorbed from intrajejunal or intraileal injection than from intraduodenal injection but did not reverse the gradient of absorption.

ABSORPTION OF IRON FROM ISOLATED SEGMENTS OF BOWEL IN MAN.

ABSORPTION OF IRON FROM ISOLATED SEGMENTS OF BOWEL IN MAN.

A modification of the technique used in the dog has been applied to man to study the relative absorption of iron from isolated segments of the upper jejunum, of the lower ileum and of the transverse colon.

MATERIALS AND METHODS.

Five patients were studied; two men had a gastro-jejunal anastomosis following partial gastrectomy for chronic duodenal ulcer; two men had an ileostomy following colectomy for chronic ulcerative colitis: one woman had a transverse colostomy as a temporary phase in the treatment of diverticulitis of the pelvic colon.

A segment of bowel was isolated by means of two balloons attached to a double lumen Miller-Abbott intestinal tube. One lumen was used to inflate the balloons which were 16 cm. apart. The other lumen was perforated at 2 cm. intervals in the length between the balloons to allow instillation and withdrawal of the test solution. Both lumina were occluded at the tip of the tube. Under radiological control the tube was passed into the upper jejunum of the two patients after gastrectomy. In the remaining /

/remaining three patients the tube was passed into the intestinal stoma to a length of 15 cm. beyond the second balloon.

After the balloons were inflated, the bowel was washed clean with 0.9% sodium chloride solution at 37°C. Thirty minutes later the iron-containing solution was introduced and left in the bowel for 60 minutes before being withdrawn. The inflation of the balloons caused a feeling of distension in the abdomen and, in two cases, colicky pain. These sensations diminished after 5 to 10 minutes but did not pass away completely and so the test period was limited to one hour. Blood samples were taken at 30 and 60 minutes.

The test solution was 25 ml. of 0.9% sodium chloride solution at 37°C and a pH of 2 containing 5 micrograms ferric iron labelled with 3 to 5 microcuries Fe 59 along with 100 mg. ascorbic acid. Polyethylene glycol (Molecular weight 4000) in a concentration of 0.1% was added as an indicator of any leak of fluid past the balloons.

A thallium-activated sodium iodide crystal was fixed 20 cm. above the anterior abdominal wall overlying the segment of bowel. The output of the crystal was /

/was recorded on a conventional scaler. Consecutive periods of 100 seconds were counted. The mean reading in the first 10 minutes of the test was compared with that in the period from 50 to 60 minutes to obtain an estimate of the radioactive iron which had left the loop during the test hour. After the test solution had been removed and the tube had been withdrawn at 60 minutes, further readings of the amount of radioactive iron remaining attached to the bowel were obtained.

A well-type thallium-activated sodium iodide scintillation counter was used to estimate the radioactivity in samples of intestinal fluid and blood. Aliquots were examined of the test solution, of the fluid withdrawn from the bowel at 60 minutes, of plasma obtained at 30 and 60 minutes and of blood obtained at 14 days. In order to calculate accurately the amount of radioactivity in the blood the blood volume was determined by the Evans Blue technique, packed cell volume being corrected for trapped plasma (Chaplin and Mollison, 1952) and for variation from the total body haematocrit (Chaplin et al., 1953). In addition the haemoglobin and serum iron were estimated.

The two patients with an ileostomy collected the /

/the ileostomy fluid for 48 hours after the test and aliquots of this were also counted in the scintillation counter.

Polyethylene glycol was estimated by a modification of the turbidometric method of Hydén (1955).

RESULTS.

The findings are summarised in Table XXX.

A variation of up to 5% was observed between successive readings lasting 100 seconds from the sodium iodide crystal fixed over the segment and no estimate of rate of absorption of iron could be made. In view of this variation the calculation of the amount of radioactive iron leaving the segment and its contents must also be only an approximation. It ranged from 0 in the ileum to 25% in the upper jejunum. A leak occurred past the balloons in the test on the transverse colon.

The amount of iron remaining attached to the segment of bowel at the end of the test hour was from 20 to 40% of the amount of iron given. Much of this radioactivity was apparently loosely attached to the bowel, since upwards of half of it was found within 48 hours in the ileostomy fluid of the two patients on whom ileal tests were done.

The amount of iron in the blood at 14 days /

Patient No.	Site of Segment	Radioactive Iron % Dose			
		% attached to loop in 1 hour	% excreted from bowel in 48 hours	% absorbed in vivo counting	% in blood at 14 days
1	Jejunum	31.3	-	24.1	30.5
2	"	26.7	-	25.2	21.3
3	Ileum	21.1	9.6	2.4	8.8
4	"	22.5	18.3	0	1.1
5	Colon	39.2	-	-	-

TABLE XXX.

Amount of iron attached to bowel in 1 hour, excreted in 48 hours, absorbed in 1 hour and found in blood at 14 days after placing 5 micrograms of radioactive iron at pH 2 and with 100 mg. ascorbic acid in isolated segments of bowel in 5 patients.

/days corresponded fairly well with the estimation from the in vivo scintillation counting of the amount absorbed from the isolated segments of bowel. It also showed that some absorption of iron can occur from the colon.

COMMENT.

These experiments have demonstrated absorption of iron from isolated segments of intestine in man contrary to the results of Groen and Taylor (1937). However, considering the variations in the in vivo counting of the radioactivity no stress can be laid on the quantitation of the absorption. In addition, utilisation of iron measured by the level of radioactivity in the blood at 14 days is known not to be a reliable index of the amount of iron absorbed in patients who are not anaemic (Dubach et al., 1948; Josephs, 1958).

The patients who had suffered from chronic peptic ulcer or chronic ulcerative colitis all had been iron deficient at the time of operation. They had all been given iron salts by mouth postoperatively and were not iron-deficient at the time of testing, as far as can be ascertained from serum iron and haemoglobin levels in the blood.

The inflation of the balloons within the intestine /

/intestine stimulated sensory nerves and gave rise to feelings of abdominal distension and colic. These segments of bowel cannot be considered to have been in a completely physiological state. This objection would apply equally to all areas of bowel examined and should not have materially affected the comparison between the upper and lower bowel. Mindful of these limitations, it is suggested that these results show that the upper jejunum can absorb ferric salts with ascorbic acid to a greater extent than the lower ileum in man.

GENERAL SUMMARY.

GENERAL SUMMARY.

Iron was one of the first specific remedies known to the medical profession and, although challenged severely during the last century, its therapeutic value is now universally accepted. Less generally held are the various opinions on the local conditions in the gut which affect absorption of the metal and the site in the alimentary canal at which this absorption takes place. This Thesis has presented work on these two topics.

LOCAL FACTORS INFLUENCING THE ABSORPTION OF IRON.

Much investigation has been done on the general factors affecting the absorption of iron from the gastro-intestinal tract and on its metabolism after absorption. Less is known about the effect of the environment within the bowel and full agreement exists only on the augmenting action of ascorbic acid. Attention has been drawn to the difficulties encountered previously in using isolated loops of intestine to try to measure the absorption of iron.

Methods have been established for the study of the absorption of radioactive iron (Fe^{59}) from acutely prepared /

/prepared isolated loops of small intestine in the dog. Sixty dogs were studied. The results obtained by simultaneous measurement of absorption in three ways were in close agreement. The amount of iron absorbed from these isolated loops of intestine had a direct relationship to the amount of iron absorbed when both were plotted logarithmically. This phenomenon has also been demonstrated in connection with another haematinic-vitamin B. 12 (Moertel et al., 1960). The relationship in our experiments was most significant when ascorbic acid was present in the test solution.

The action of ascorbic acid in promoting the absorption of iron was confirmed. Its effect was shown to take place in the lumen of the bowel rather than in the bowel wall. Its augmentation of the absorption of iron was thought more likely to be due to local reducing properties than to its effect on pH of the intestinal content or to formation of a stable chelate such as iron forms with versene. However, the fact that the linear relationship between the logarithm of the dose and the logarithm of the amount absorbed was most significant with ascorbic acid suggests that some chemical alteration may have taken place following the addition of ascorbic acid. While a low pH at the initial introduction of the iron into the /

/the loop promoted absorption in comparison with a neutral pH, it was observed that maintaining the pH of the contents of the loop at less than 3.5 for up to 5 hours did not further increase absorption of iron. The increase in absorption of iron produced by an initial pH of 2 was of the same order as that produced by the addition of ascorbic acid at neutral pH.

No real difference was noted in the absorption of ferrous or ferric iron. However, it may be that the proportionately large amount of ascorbic acid (100 mg.) and the high hydrogen ion concentration (pH2) maintained a ferrous state in most cases.

Absorption of iron from a single dose lasted for up to 2 hours in most cases and the amount absorbed varied with the duration of absorption. When the mean figures were taken for 31 dogs in which only a duodenal loop was isolated, an exponential rate of absorption was found in the first 2 hours (1 . 98%/min). Examination of individual tests revealed that only 9 of 31 closely approximated to an exponential rate and so consecutive 15 minute periods were studied to allow comparison of all tests in detail. The rate was assumed to be constant in these time in tervals, and was expressed as a percentage of dose still remaining in the loop per 100 sq. cm. serosal surface per minute. This /

/This rate was fastest in the period 15 to 30 minutes after placing the iron in the loop. The pattern of absorption of iron was not changed by aspirating the iron-containing fluid from the loop even as early as 15 minutes, although if removal was performed before 60 minutes the total amount absorbed was diminished. When absorption of iron had stopped, transfer of the iron-containing intestinal fluid to another freshly prepared loop in a different dog did not show any significant absorption. Thus, some change had occurred rendering the iron unavailable for absorption. This change could not be reversed by the addition of ascorbic acid or by re-acidification. Giving repeated doses of iron into the same loop revealed no evidence of "mucosal block" at these dose levels and showed that the cessation of absorption at 2 hours was not due to a change in the mucosa.

Using in vivo counting techniques, repeatable measurement[5] of the absorption of radioactive iron from chronic Thiry Vella fistulae of the duodenum were achieved in four dogs. Confirmation was obtained of the effect of acidity and of ascorbic acid noted above. Gastric juice gave no greater increase in absorption of iron than equivalent amounts of hydrochloric acid. Graded doses of ascorbic acid /

/acid revealed an increase in iron absorption beyond a threshold of 1 mg. and reaching a plateau about 20 mg. The threshold was too high for a direct non-ascorbate to be the only promoting change involved.

The applications of this work to the intact animal are complicated by the fact that the iron has been prevented from moving from the isolated loop. This is essential to evaluate the process of absorption by the one area of bowel and to be able to obtain a measurement of the rates of change in this region. As it has been demonstrated that the iron in the bowel lumen rapidly becomes unavailable for absorption, the artificial holding-up of the iron-containing solution may not give much distortion of what happens in the intact animal. With these reservations, the following hypothesis is advanced, on the basis of the findings reported, to explain the sequence of events after the introduction of iron into the small bowel of the dog (Fig. 51).

Iron enters the duodenum from the stomach in small amounts with the peristaltic action of the gastric antrum. The intraduodenal pH falls with each rush of gastric contents and the evidence of the action of a low pH would suggest that gastric acidity is important. During the first 15 minutes /

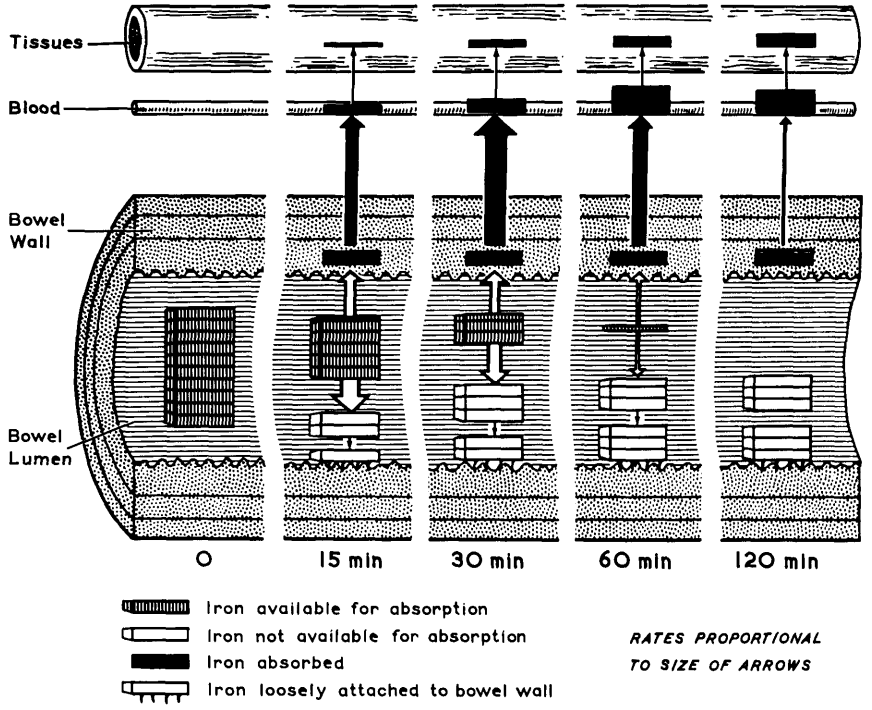


FIG. 51.

Diagram of suggested sequence of events after introduction of a dose of iron into the duodenum of a dog. (Two micrograms iron with 100 mg. ascorbic acid used as the example).

/minutes after entry into the duodenum removal of iron from the bowel lumen and bowel wall into the blood stream gains momentum and reaches a maximum in the second 15 minutes. Meanwhile, in the bowel lumen a rapid change is occurring in the iron which makes it unavailable for absorption, so that after 30 to 60 minutes it has no further effect on the absorption of iron. The change is delayed by the acidification of the iron-containing solution or by the addition of ascorbic acid. The latter has a different action from a true chelating agent. Part of the iron unavailable for absorption becomes loosely attached to the mucosa so that it cannot be aspirated but can be gently scraped off. Presumably this would happen with the passage of intestinal contents. The attachment is at an exponential rate. Absorption into the blood stream continues for up to 120 minutes, with a further slight absorption from 3 to 5 hours in some cases. Our observation period was five hours following the introduction of the iron into the loop and at the end of this time a small amount of iron, proportional to the amount absorbed, was still firmly attached to the bowel wall. This might have been able to be absorbed later or might be cast off with the changing mucosal cells in the next 2 to 4 days.

Also depicted on the diagram (Fig. 51) are the /

/the movements of absorbed iron into and out of the blood. In most experiments the rate of absorption from the loop and loop contents was greatest in the period 15 to 30 minutes. Using the data from 23 dogs in which only duodenal loops were isolated, it was found that the mean level of radioactive iron in the plasma showed an exponential disappearance into the tissues at a rate of 0.587% min. An exponential rate of appearance of radioactive iron in the plasma could also be obtained from these mean levels of Fe59. It was almost identical to the rate derived from the mean figures for absorption of iron from the isolated loop. However, individual experiments did not follow this exponential pattern so closely.

A significant correlation was found between the maximum amount of radioactive iron in the plasma and the amount of radioactive iron absorbed from isolated intestinal loops.

A comparison of the levels of radioactive iron in the plasma of the portal venous and femoral arterial blood in 20 dogs showed a significantly greater concentration in the portal blood during the first 90 minutes of absorption of iron from the isolated intestinal loops. This difference was /

/was due to removal of iron from the circulation by the liver and to dilution of the portal blood by hepatic arterial and systemic venous blood.

SITE OF ABSORPTION OF IRON IN THE INTESTINE.

In the review of the literature it was seen that the duodenum is thought to be the main site of absorption of iron but that few direct comparisons between it and other areas of bowel had been made and its importance relative to the rest of the alimentary tract had not been defined.

In the study on isolated loops in acute preparations in 20 dogs, the duodenum was found to absorb up to 14 times as much iron per 100 sq. cm. of serosal surface as the ileum. The difference was not significant unless the initial pH of the test solution was 2 and ascorbic acid was added. In other respects the two parts of small bowel exhibited a marked similarity. The response to increase in amount of iron given, to acidity of the test solution and to ascorbic acid were of the same order.

In four dogs the importance of the duodenum was assessed by measuring the absorption of radioactive iron by means of balance techniques before and after removal of the duodenum from continuity with the rest of gastrointestinal tract. Although there was a slight tendency /

/tendency for a diminution in absorption to occur after operation, no uniform trend was noted. The animal could absorb as much iron without the duodenum as when this part of the gut was in use. Thus, the duodenum is not essential to maintain absorption of small doses of inorganic iron. This supports evidence, in man, suggesting that by-passing the duodenum by a gastrojejunal anastomosis after gastric resection is not the main cause in producing iron-deficiency anaemia.

The above tests concerning the duodenum had all been performed with a small dose of iron (10 micrograms) and recourse was had to the rat to investigate the effect of varying dosage and to compare duodenum, jejunum and ileum while still in continuity. A total of 165 animals were studied. Ferric iron and ascorbic acid at a pH of 2 were injected into the chosen segment of bowel at laparotomy and the amount of radioactive iron retained was measured by a total body counting technique. Intraduodenal injection compared with injection 5 cm. distal to the Ligt of Treitz confirmed the findings in the dog. However, by increasing the amount of iron given to as much as 1 mg. a statistically significant difference was found, the duodenal site of injection resulting /

/resulting in greater absorption. The duodenum only makes an indispensable contribution to the absorption of iron when the stress of large dosage is applied. Little difference was found in comparison of intragastric and intraduodenal administration of iron, indicating that the stomach plays a small part in absorption of iron at these doses.

Intrajejunal and intraileal injection of iron gave smaller absorption than intraduodenal injection. A gradient of absorption of iron along the small bowel of the rat was confirmed. A direct relationship was found between the amount of iron given and the amount absorbed both plotted logarithmically. This held for duodenum and to a lesser extent for jejunum and ileum. The slope of the regression line for this relationship was similar for all three sites of injection, although that for the intrajejunal was set at a lower level than that for intraduodenal injection with a line for intraileal injection being lowest.

The increase of absorption of iron in response to repeated bleeding or to a milk diet, low in iron content, was proportionately smallest for the duodenal site of injection, greater for the jejunal site and greatest in the ileum. In addition, a correlation, on a log/log scale, still existed between the amount of radioiron absorbed and the amount /

/amount injected. In the case of the duodenum, the regression line had a slope almost the same as in the control animals but showing a slightly greater absorption at lower doses. Whereas both the jejunal and ileal sites of injection revealed an increase in absorption especially from the larger doses with an increase in the slope of the regression line.

Experiments on 5 patients have shown absorption of iron from segments of intestine isolated by means of balloons on an intestinal tube within the lumen. A greater amount of iron was absorbed from the upper jejunum than from the lower ileum.

Summarising these findings and mindful of the possible fallacies of arguing from one species to another, the following is suggested as the pattern of absorption of iron along the bowel. In the intact animal absorption in the stomach is not of great significance. The iron passes through the pylorus into the duodenum in an acid solution. Most absorption takes place in this segment: firstly, because it gets the first chance to absorb the iron and secondly, the iron is still in a state available for absorption. The upper jejunum has been shown to have almost as great a potentiality for absorption of iron as the duodenum and this section of the gut plays a part in the next 15 to 60 minutes while some of the iron remains available /

/available for absorption. Indeed, the duodenum is quite dispensable as far as the absorption of inorganic iron is concerned until larger doses are reached.

The duodenum seems to be absorbing iron almost at its maximum rate when the iron is presented in an acid solution with ascorbic acid directly into its lumen, for very little increase in absorption occurs in response to bleeding or to a diet low in iron, although both of these factors have been shown previously to augment considerably absorption of iron given by the intragastric route. By contrast, a notable increase in absorption of iron follows the injection of iron into the lower jejunum or into the ileum in either of the above circumstances. Thus the more distal small bowel is able to increase absorption to a proportionately greater extent than the proximal small bowel. No direct evidence has been presented regarding the role played by the colon.

These studies of the intestinal absorption of iron have given rise to more questions than they have answered. Some definition of the local factors influencing the absorption of iron has been achieved but, with the exception of ascorbic acid, no information about the possible mechanisms by which they act has been obtained. The generally held opinion of the primacy of the duodenum has been confirmed although some /

/some limitations on its fundamental importance have been formulated. No evidence of the processes involved in the altered response of the distal small bowel has emerged from these investigations. However, they may be of value in providing new methods of studying the absorption of iron from the small intestine and in clarifying understanding of some of the steps involved in this process.

REFERENCES.

REFERENCES.

AUSTONI, M.E., and GREENBERG, D.M. (1940) Studies in iron metabolism with the aid of its artificial radioactive isotope. The absorption excretion and distribution of iron in the rat on normal and iron-deficient diets. J. biol. Chem., 134, 27-41.

BADENOCH, J., EVANS, J.R., and RICHARDS, W.C.D. (1957) The stomach in hypochromic anaemia. Brit. J. Haemat., 3, 175-185.

BAIRD, I.M., PODMORE, D.A., and WILSON, G.M. (1957) Changes in iron metabolism following gastrectomy and other operations. Clin. Sci., 16, 463-473.

BAIRD, I.M., and WILSON, G.M. (1959) The pathogenesis of anaemia after partial gastrectomy. II. Iron absorption after partial gastrectomy. Quart. J. Med., 28, 35-41.

BARER, A.P., and FOWLER, W.M. (1937) Influence of gastric acidity and degree of anaemia on iron retention. Arch. intern. Med., 59, 785-792.

BESSIS, M., and BRETON-GORIOUS, J. (1958) Trois aspects du fer des coupes d'organes examinées au microscope électronique (ferritine et derivé dans les cellules intestinales, les erythroblastes et les cellules réticulaires). Sem. Hôp. Paris, 33, 4149.

BESSIS, M., BRETON, J., DREYFUS, J-C, and SCHAPIRA, G., (1958) Aspect au microscope électronique de l'apoferritine plus ou moins chargée en fer. Rev. franc. Ét. clin. biol., 3, 981-983.

BEUTLER, E., ROBSON, M.J., and BUTTENWEISER, E. (1958) A comparison of the plasma iron, iron-binding capacity sternal marrow iron and other methods in the clinical evaluation of iron stores. Ann. intern. Med., 48, 60-82.

BEUTLER, E., BUTTENWEISER, E. (1960) The regulation of iron absorption. I. A search for humoral factors. J. Lab. clin. Med., 55, 274-280.

BOHMANSSON, G. (1950) Prophylaxis and therapy in late postgastrectomy complications. Acta med. scand., Suppl., 246, 37-45.

BOLLMAN, J.L., KHATTAB, M., THORS, R., and GRINDLAY, J.H. (1953) Experimentally produced alterations of hepatic blood flow. A.M.A. Arch. Aurg., 66, 562-569.

BONNET, J.D. (1958) Studies on the metabolism of iron utilizing radioiron stressing the gastrointestinal absorption. Mayo Foundation Thesis M.S.

BONNET, J.D., HAGEDORN, S.B., and OWEN, C.A. Jr., (1960) A quantitative method for measuring the gastrointestinal absorption of iron. Blood, 15, 36-44.

BOTHWELL, T.H., Van DOORN-WITTKAMPF, H.W., Du PREEZ, M.L., and ALPER, T. (1953) The absorption of iron. Radioiron studies in idiopathic hemochromatosis, malnutrition cytosiderosis and transfusional hemosiderosis. J. Lab. clin. Med., 41, 863-847.

BOTHWELL, T.H., MALLETT, B., OLIVER, R., and SMITH, M.D. (1955) Inability to assess absorption of iron from plasma radioiron curves. Brit. J. Haemat., 1, 352-357.

BOTHWELL, T.H., and FINCH, C.A. (1957) The intestine in iron metabolism: its role in normal and abnormal states. Am. J. dig. Dis., 2, 145-158.

BOTHWELL, T.H., PIRZIO-BIROLI, G., FINCH, C.A. (1958) Iron absorption. I. Factors influencing absorption J. Lab. clin. Med., 51, 24-36.

BROCK, J.F., and HUNTER, D. (1937) The fate of large doses of iron administered by mouth. Quart. J. Med., n.s. 6, 5-16.

BROWN, E.B., and JUSTUS, B.W. (1954) In vitro absorption of radioiron by everted pouches of rat intestine. Am. J. Physiol., 194, 319-326.

BROWN, E.B., DUBACH, R., and MOORE, C.V. (1958) Studies in iron transportation and metabolism. XI. Critical analysis of mucosal block by large doses of inorganic iron in human subjects. J.Lab. clin.Med., 52, 335-355.

BRUMMER, P. (1950) The significance of achlorhydria for the etiology of iron-deficiency anaemia. Acta med. scand., 137, 43-47.

BRUUSGAARD, C. (1946) The operative treatment of gastric and duodenal ulcer. Acta chir.scand.Suppt., 117, 1-435.

CALLENDER, S.T. (1959) Iron absorption. Brit.med.Bull., 15, 5-8.

CALLENDER, S.T., MALLETT, B.J., and SMITH, M.D. (1957) Absorption of haemoglobin iron. Brit.J. Haemat., 3, 186-192.

CHAPLIN, H. (Jr.) and MOLLISON, P.L. (1952) Correction for plasma trapped in the red cell column of the haematocrit. Blood, 7, 1227-1238.

CHAPLIN, H. (Jr.), MOLLISON, P.L., and VETTER, J. (1953) The body/venous haematocrit ratio: its constancy over a wide haematocrit range. J.clin. Invest., 32, 1309-1316.

CHARLEY, P., and SALTMAN, P. (1960) The regulation of iron transport in the gut. Fed.Proc., 19, 248.

CHENEY, B., and FINCH, C.A. (1960) Effect of inosine on iron absorption in rats. Proc.Soc.exp.Biol., 103, 37-38.

CHODOS, R.D., ROSS, J.F., APT, L., POLLYCOVE, M., and HALKETT, J.A. (1957) The absorption of radioiron labelled foods and iron salts in normal and iron-deficient subjects and in idiopathic hemochromatosis. J.clin.Invest., 36, 314-326.

CHRISTIAN, H.A. (1903) A sketch of the history of the treatment of chlorosis with iron. Med.Lib. and Hist.J., 1, 176-180

CODE, C.F. (1960) The semantics of the process of absorption. Perspect. Biol. Med., 3, 560-562.

CODE, C.F., SCHOLER, J.F., HIGHTOWER, N.C. Jr., DIETZLER, F.K., and BALDES, E.J. (1954) Absorption of water from the upper part of the human gastro-intestinal tract. Proc. Mayo Clin., 29, 235.

CODE, C.F., and McINTIRE, F.C. (1956) Methods of biochemical analysis. Vol. 3, P. 66, Interscience, New York.

CODE, C.F., BASS, P., McCLARY, G.B. Jr., NEWNUM, R.L., and ORVIS, A.L. (1960) Absorption of water, sodium and potassium in small intestine of dogs. Amer. J. Physiol., 199, 281-288.

COPP, D.H., and GREENBERG, D.M. (1946) A tracer study of iron metabolism with radioactive iron: I. Methods: absorption and excretion of iron. J. biol. Chem., 164, 377-387.

DAVIDSON, W.M.B., and MARKSON, J.L. (1955) The gastric mucosa in iron deficiency. Lancet, 2, 639-643.

DEMULDER, R. (1958) Iron: metabolism, biochemistry and clinical pathological physiology: review of recent literature. A.M.A. Arch. intern. Med., 102, 254-301.

De VINALS, R.R., and De CAMPO, E.A. (1957) Démonstration histochimique de l'existence d'une barrière ferrique intestinale. Rev. Hémat., 12, 635-641.

DOWDLE, E.B., SCHACHTER, D., and SCHENKER, H. (1960) Active transport of Fe^{59} by everted segments of rat duodenum. Amer. J. Physiol., 198, 609-613.

DUBACH, R., MOORE, C.V., MINNICH, V. (1946) Studies in iron transportation and metabolism. V. Utilisation of intravenously injected radioactive iron for haemoglobin synthesis, and an evaluation of the radioactive iron method for studying iron absorption. J. Lab. clin. Med., 31, 1201-1222.

FISHER, R.A., and YATES, F. (1948) Statistical tables for biological, agricultural and medical research. 3rd edition. Oliver and Boyd, London.

FOY, H., FONDI, A., and AUSTIN, W.G. (1959) Effect of dietary phytate on faecal absorption of radioactive ferric chloride. Nature (Lond.), 183, 691-692.

FREEMAN, S., and BURRILL, M.W. (1945) Comparative effectiveness of various iron compounds in promoting iron retention and hemoglobin regeneration by anaemic rats. J.Nutr., 30, 293-300.

GILLMAN, J., GILLMAN, T., MANDELSTAM, J., and GILBERT, C. (1947) Cytosiderosis and the genesis of arteriosclerosis and other fibrous-tissue reactions. Nature (Lond.), 159, 875-876.

GILLMAN, T., and IVY, A.C. (1947) Histological study of participation of intestinal epithelium, reticulo-endothelial system and lymphatics in iron absorption and transport: preliminary report. Gastroenterology, 9, 162-169.

GOLDBERG, A., and LOCHHEAD, A. (1960) Personal communication.

GOLDWATER, L.J. (1935) A short history of iron therapy. Ann. med. Hist., 7, 261-267.

GORDON, A.S. (1959) Hemopoietine. Physiol.Rev., 39, 1-40.

GORDON, A.S. (1960) Humoral influences on blood cell formation and release. Haemopoiesis. Ciba Foundation, pp. 325-362. Ciba Foundation Symposium on Haemopoiesis, J. & A. Churchill Ltd., London.

GRACE, W.J., and DOIG, R.K. (1953) The relationship between gastric acidity and the absorption of iron from the upper intestinal tract. J.clin.Invest., 32, 571.

DUBACH, R., CALLENDER, S.T.E., and MOORE, C.V. (1948) Studies in iron transportation and metabolism. VI. Absorption of radioactive iron in patients with fever and with anaemia of varied etiology. Blood, 3, 526-540.

DUBACH, R., MOORE, C.V., and CALLENDER, S. (1949) Iron excretion in human subjects as measured by the isotope technique. Fed. Proc., 8, 353.

DUBACH, R., MOORE, C.V., and CALLENDER, S. (1955) Studies in iron transport and metabolism. IX. The excretion of iron as measured by the isotope technique. J. Lab. clin. Med., 45, 599-615.

DUTHIE, H.L. (1959) Absorption of inorganic iron after partial gastrectomy: comparison of the Polya and Billroth I operations. Scot. med. J., 4, 523-526.

ENDICOTT, K.M., GILMAN, T., BRECHER, G., NESS, A.T., CLARKE, F.A., and ADAMIK, E.R. (1949) A study of histochemical iron using tracer method. J. Lab. clin. Med., 34, 414-421.

FAIRMAN, E., and WHIPPLE, G.H. (1933) Bone marrow volume in adult dogs. Amer. J. Physiol., 104, 352-357.

FIELD, M., SEKI, M., MITCHELL, M.L., and CHALMERS, T.C. (1960) Studies in iron absorption. I. Determination in rats by measurement of total body radioactivity. J. Lab. clin. Med., 55, 929-935.

FINCH, S., HASKINS, D., and FINCH, C.A. (1950) Iron metabolism. Haematopoiesis following phlebotomy. Iron as a limiting factor. J. clin. Invest., 29, 1078-1086.

FINCH, S.C., and FINCH, C.A. (1955) Idiopathic hemochromatosis, an iron storage disease. Medicine (Balt.), 34, 381-430.

FINCH, C.A., and LODEN, B. (1959) Body iron exchange in man. J. clin. Invest., 38, 392-396.

FISHER, R.A. (1930) Statistical methods for research workers. Oliver and Boyd, London.

FISHER, R.A., and YATES, F. (1948) Statistical tables for biological, agricultural and medical research. 3rd edition. Oliver and Boyd, London.

FOY, H., FONDI, A., and AUSTIN, W.G. (1959) Effect of dietary phytate on faecal absorption of radioactive ferric chloride. Nature (Lond.), 183, 691-692.

FREEMAN, S., and BURRILL, M.W. (1945) Comparative effectiveness of various iron compounds in promoting iron retention and hemoglobin regeneration by anaemic rats. J.Nutr., 30, 293-300.

GILLMAN, J., GILLMAN, T., MANDELSTAM, J., and GILBERT, C. (1947) Cytosiderosis and the genesis of arteriosclerosis and other fibrous-tissue reactions. Nature (Lond.), 159, 875-876.

GILLMAN, T., and IVY, A.C. (1947) Histological study of participation of intestinal epithelium, reticulo-endothelial system and lymphatics in iron absorption and transport: preliminary report. Gastroenterology, 9, 162-169.

GOLDBERG, A., and LOCHHEAD, A. (1960) Personal communication.

GOLDWATER, L.J. (1935) A short history of iron therapy. Ann.med.Hist., 7, 261-267.

GORDON, A.S. (1959) Hemopoietine. Physiol.Rev., 39, 1-40.

GORDON, A.S. (1960) Humoral influences on blood cell formation and release. Haemopoiesis. Ciba Foundation, pp.325-362. Ciba Foundation Symposium on Haemopoiesis, J. & A. Churchill Ltd., London.

GRACE, W.J., and DOIG, R.K. (1953) The relationship between gastric acidity and the absorption of iron from the upper intestinal tract. J.clin.Invest., 32, 571.

GRANICK, S. (1946[a]) Ferritin. IX. Increase of the protein apoferritin in the gastrointestinal mucosa as a direct response to iron feeding. The function of ferritin in the regulation of iron absorption. J. biol. Chem., 164, 737-746.

GRANICK, S. (1946[b]) Ferritin: its properties and significance for iron metabolism. Chem. Rev., 38, 379-403.

GRANICK, S. J. (1949) Iron metabolism and haemachromatosis. Bull N. Y. Acad. Med., 25, 403-428.

GREENBERG, M. S., WONG, H., MILLER, S. A., SCARLATA, R. W., and CHALMERS, T. C. (1960) Iron absorption and turnover in anoxia. Abstract - Am. Soc. Clin. Invest., 52nd Ann. Meeting Program, p. 28.

GRINDLAY, J. H., HERRICK, J. F., and MANN, F. C. (1941) Measurement of the blood flow of the liver. Amer. J. Physiol., 132, 489-496.

GRINDLAY, J. H., MANN, F. C., BOLLMAN, J. L. (1951) Effect of occlusion of the arterial blood supply to the normal liver. An experimental study. A. M. A. Arch. Surg., 62, 806-810.

GROEN, J., and TAYLOR, F. H. L. (1937) Absorption of iron compound from the upper part of the small intestine. Proc. Soc. exp. Biol. (N. Y.), 36, 694-695.

GROEN, J., Van Der BROEK, W. A., and VELDMAN, H. (1947) Absorption of iron compounds from the small intestine in the rat. Biochem. biophys. Acta, 1, 315-326.

GUBLER, C. J. (1956) Absorption and metabolism of iron. Science, 123, 87-90.

GURNEY, C. W., and FILMANOWICZ, E. (1960) Kinetics of erythropoiesis. Am. Soc. Clin. Res. Abstract, p. 30.

HAHN, P. F., ROSS, J. F., BALE, W. F., and WHIPPLE, G. H. (1940) The utilization of iron and the rapidity of haemoglobin formation in anaemia due to blood loss. J. exper. Med., 71, 731-736.

HAHN, P.F., BALE, W.F., and BALFOUR, W.M. (1941) Radioactive iron used to study red blood cells over long periods. The constancy of the total blood volume in the dog. Amer.J.Physiol., 135, 600-605.

HAHN, P.F., BALE, W.F., ROSS, J.F., BALFOUR, W.M., and WHIPPLE, G.H. (1943) Radioactive iron absorption by gastrointestinal tract. Influence of anaemia, anoxia and antecedent feeding. Distribution in growing dogs. J.exp.Med., 78, 169-188.

HAHN, P.F., JONES, E., LOWE, R.C., MENEELY, G.R., and PEACOCK, W. (1945) The relative absorption and utilisation of ferrous and ferric iron in anaemia as determined with the radioactive isotope. Amer.J. Physiol., 143, 191-197.

HAHN, P.F., BALE, W.F., and WHIPPLE, G.H. (1946) Effects of inflammation (Turpentine Abscess) on iron absorption. Proc.Soc.exp.Biol.(N.Y.), 61, 405-406.

HALLBERG, L., SÖLVELL, L., and BRISE, H. (1959) Studies över järnresorptionens patofysiologi. Nord.Med., 61, 532-538.

HALLBERG, L., and SÖLVELL, L. (1960) Iron absorption studies. Acta med.scand. Suppl., 538, 168, 1-108.

HEATH, C.W., MINOT, G.R., POHLE, F.J., and ALSTED, G. (1938) The influence of mucin on the absorption of iron in hypochromic anaemia. Amer.J.med.Sci., 195, 281-286.

HEILMEYER, L., and PLÖTNER, H. (1936) Eisenmangelzustände und ihre Behandlung. Klin. Wschr., 15, 1669-1673.

HEILMEYER, L., KEIDERLING, W., and WÖHLER, F. (1957) Existiert bei des Eisenresorption im Dünndarm ein Mucosablock? Klin. Wschr., 35, 690.

HENLEY, F.A. (1952) Gastrectomy with replacement: a preliminary report. Brit.J.Surg., 40, 118-128.

HERNDON, J.F., RICE, E.G., TUCKER, R.G., VAN LOON, E.J., and GREENBERG, S.M. (1958) Iron absorption and metabolism. III. The enhancement of iron absorption in rats by D-sorbitol. J.Nutr., 64, 615-623.

HURTADO, A., MARINO, C., and DELGADO, E. (1945) Influence of anoxaemia on the hemopoietic activity. Arch. intern. Med., 75, 284-323.

HYDE, A.S. (1957) Absorption of radioiron perfused through the duodenum of the rat. Amer. J. Physiol., 191, 265-270.

HYDÉN, S. (1955) A turbidometric method for determination of higher polyethylene glycols in biological materials. K. Lantbr. Hogskolans Annaler., 22, 139-145.

INGALLS, R.L., and JOHNSTON, F.A. (1954) Iron from gastro-intestinal sources excreted in the feces of human subjects. J.Nutr., 53, 351-363.

JENSEN, W.N., BUSH, J.A., ASHENBRUCKER, H., CARTWRIGHT, G.E., and WINTROBE, M.M. (1956) The kinetics of iron metabolism in normal growing swine. J. exp. Med., 103, 145-159.

JOSEPHS, H.W. (1958) Absorption of iron as a problem in human physiology: a critical review. Blood, 13, 1-54.

KAUFMAN, N., KLAVINS, J.V., and KINNEY, T.D. (1958) Excessive iron absorption in rats fed low protein, high fat diets. Lab. Invest., 7, 369-376.

KAY, A.W. (1958) The pyloric antrum and peptic ulceration. Gastroenterologia, 89, 282-286.

KELLOG, F., and METTIER, S.R. (1936) Effect of alkaline therapy for peptic ulcer on utilisation of dietary iron in the regeneration of haemoglobin. Arch. intern. Med., 58, 278-284.

KINNEY, T.D., HEGSTED, D.M., and FINCH, C.A. (1949) The influence of diet on iron absorption. I. The pathology of iron excess. J. exp. Med., 90, 137-156.

KINNEY, T.D., KAUFMAN, N., and KLAVINS, J. (1955) Effect of ethionine-induced pancreatic damage on iron absorption. J. exp. Med., 102, 151-156.

KRANTZ, S., GOLDWASSER, E., and JACOBSON, L.O. (1959) Studies on erythropoiesis. XIV. The relationship of humoral stimulation to iron absorption. Blood, 14, 654-661.

LAURELL, C.B. (1952) Plasma iron and the transport of iron in the organism. Pharmacol. Rev., 4, 371-395.

LEES, F., and ROSENTHAL, F.D. (1958) Gastric mucosal lesions before and after treatment in iron deficiency anaemia. Quart. J. Med., 27, 19-26.

LIND, J.F. (1960) Personal communication.

LINMAN, J.W., and BETHELL, F.H. (1960) Factors in the control of haemopoiesis. Ciba Foundation Symposium on Haemopoiesis, pp. 369-392. J. & A. Churchill Ltd., London.

LINTZEL, W. (1933) Zum Nachweis der Resorption des Nahrungeisens als Ferroion. Biochem. Z., 263, 173-186.

LUCAS, G.H.W., and SUMMERFELDT, P. (1939) The absorption and utilisation of iron salts. Canad. med. Ass. J., 40, 588-591.

McCALLUM, A.B. (1894) On the absorption of iron in the animal body. J. Physiol., 16, 268-297.

McCANCE, R.A., and WIDDOWSON, E.M. (1937) Absorption and excretion of iron. Lancet, 2, 680-684.

McCANCE, R.A., and WIDDOWSON, E.M. (1938) Absorption and excretion of iron following oral and intravenous administration. J. Physiol., 94, 148-154.

McCANCE, R.A., EDGECOMBE, C.N., and WIDDOWSON, E.M. (1943) Phytic acid and iron absorption. Lancet, 2, 126-128.

MEHNERT, B., MEHNERT, H., and STUHLFAUTH, K. (1958) Untersuchungen zur Frage der vitaminsparenden Eigenschaft des Sorbits. Klin. Wschr., 36, 1029-1030.

METTIER, S.R., and MINOT, G.R. (1931) The effect of iron on blood formation as influenced by changing the acidity of the gastro-duodenal contents in certain cases of anaemia. Amer.J.med.Sci., 181, 25-36.

METTIER, S.R., KELLOG, F., and RINEHART, J.F. (1933) Etiologic relationship of achlorhydria to the anaemia, with special reference to the effect of large doses of iron, organic (dietary) iron and of predigested food upon formation of erythrocytes. Amer.J.med.Sci., 186, 694-704.

MILLER, T.G., and ABOTT, W.O. (1936) Intubation studies of the human small intestine. III. A technic for the collection of pure intestinal secretion and for the study of intestinal absorption. J.Amer.med.Ass., 106, 16-18.

MINOT, G.R., and HEATH, C.W. (1932) The response of the reticulocytes to iron. Amer.J.med.Sci., 183, 110-121.

MOERTEL, C.G., SCUDAMORE, H.H., OWEN, C.A. Jr., and BOLLMAN, J.L. (1959) Intestinal absorption of cobalt^{60}-labeled vitamin B_{12} in the normal male albino rat. Amer.J.Physiol., 197, 347-350.

MOORE, C.V. (1955) The importance of nutritional factors in the pathogenesis of iron-deficiency anaemia. Amer.J. clin.Nutrition, 3, 3-10.

MOORE, C.V., ARROWSMITH, W.R., WELCH, J., and MINNICH, V. (1939) Studies in iron transportation and metabolism. IV. Observations on the absorption of iron from the gastrointestinal tract. J.clin.Invest., 18, 553-580.

MOORE, C.V., DUBACH, R., MINNICH, V., and ROBERTS, H.K. (1944) Absorption of ferrous and ferric radioactive iron by human subjects and by dogs. J.clin.Invest., 23, 755-767.

MOORE, C.V., and DUBACH, R. (1951) Observations on the absorption of iron from foods tagged with radio-iron. Trans.Ass.Amer.Phycns., 64, 245-256.

MORGAN, T.B., and YUDKIN, J. (1957) The vitamin sparing action of Sorbitol. Nature, 180, 543-545.

MORI, H.D., BARKER, P.A., JURAS, D.S., and WISSLER, R.W. (1957) Study of the mechanism of increased iron absorption in hamsters fed tween 20. Lab. Invest., 6, 421-446.

MORONEY, M.J. (1953) Facts from figures, 2nd Ed., Penguin Books, London.

MOURIQUAND, C., and ÉDEL, C. (1958) Recherches histologiques sur l'absorption duodénale du fer chez le cobaye (guinea pig) normal et chez le cobaye scorbutique. C. rend. Acad. Sc., 246, 1908-1911.

MOURIQUAND, C., and MILLER, M. (1958) Comportement du fer dans la muqueuse duodénale du cobaye (guinea pig) au cours de l'anémie expérimentale par hémorragie. C. rend. Soc. biol., 152, 781-783.

NAKAMURA, F.I., and MITCHELL, H.H. (1943) The utilization for haemoglobin regeneration of the iron in salts used in the enrichment of flour and bread. J. Nutr., 25, 39-48.

NEANDER, G., and VALQUIST, B. (1949) Studies on the absorption of iron. II. Experimental studies on the serum iron levels in the portal vein during iron absorption tests. Acta physiol. scand., 17, 97-109.

NYLANDER, G., and OLERUD, S. (1961) The vascular pattern of an isolated jejunal loop. A microangiographic study in the rat. Acta chir. scand., 121, 39-46.

PALAY, S.L., and KARLIN, L.J. (1959) An electron microscopic study of the intestinal villus. I. The fasting animal. J. Biophys. Biochem. Cytol., 5, 363-371.

PETERSON, R.E., and ETTINGER, R.H. (1953) Radioactive iron absorption in siderosis (Hemochromatosis) of the liver. Amer. J. Med., 15, 518-524.

PIRZIO-BIROLI, G., BOTHWELL, T.H., and FINCH, C.A. (1958) Iron absorption. II. The absorption of radioiron administered with a standard meal in man. J.Lab. clin.Med., 51, 37-48.

PIRZIO-BIROLI, G., and FINCH, C.A. (1960) Iron absorption. III. The influence of iron stores on iron absorption in the normal subject. J.Lab.clin.Med., 55, 216-220.

RAMSAY, W.N. (1957) The determination of iron in blood plasma or serum. Clin.chem.Acta, 2, 214-220.

REISMANN, K.R., COLEMAN, T.J., BUDAI, B.S., and MORIARTY, L.R. (1955) Acute intestinal iron intoxication. I. Iron absorption, serum iron and autopsy findings. Blood, 10, 35-45.

REYNAFARJE, C., LOZANO, R., VALDIVIESO, J. (1959) The polycythaemia of high altitudes: iron metabolism and related aspects. Blood, 14, 433-455.

RICHTER, G.W. (1958) Electron microscopy of hemosiderin: presence of ferritin and occurrence of crystalline lattices in hemosiderin deposits. J.Biophys. Biochem. Cytol., 4, 55-58.

ROBSCHIET-ROBBINS, F.S. (1929) The regeneration of hemoglobin and erythrocytes. Physiol.Rev., 9, 666-709.

SALERA, U., TAMBURINO, G., and MATRONOLA, F. (1956) Ricerche sull'eliminazione del ferro attraverso la mucosa intestinale dell'ansa isolata alla Vella. Rass. fisiopat., 28, 954-957.

SCHÄFER, K.H., and BREYER, A. (1956) Experimentelle Untersuchungen zur Frage der Eisenresorption im Infekt. Klin.Wschr., 34, 54-55.

SEBRELL, W.H. Jr., and HARRIS, R.S. (1954) The vitamins. Chemistry, physiology, pathology. Academic Press Inc., New York.

SHARPE, L.M., PEACOCK, W.C., COOKE, R., and HARRIS, R.S. (1950) The effect of phytate and other food factors on iron absorption. J.Nutr., 41, 433-446.

SHIELDS, R., and CODE, C.F. (1961) The effect of increased portal pressure on the sorption of water and sodium from the ileum of dogs. Amer.J.Physiol. In press.

SMITH, D.L., BIERWAGEN, M.E., and EARLY, T.K. (1957) Effect of environmental oxygen tension on intestinal iron absorption. Proc.Soc.exp.Biol., N.Y., 94, 710-711.

SMITH, M.D., and MALLETT, B. (1957) Iron absorption before and after partial gastrectomy. Clin.Sci., 16, 23-33.

SMITH, M.D., and PANNACCIULLI, I.M. (1958) Absorption of inorganic iron from graded doses: its significance in relation to iron absorption tests and the "mucosal block" theory. Brit.J.Haematol., 4, 428-434.

SMITH, M.D. (1959) Iron metabolism: a review in relation to clinical problems. Scot.med.J., 4, 467-472.

SOLOMON, A.K., EDELMAN, I.S., and SOLOWAY, S. (1950) The use of the mass spectrometer to measure dueterium in body fluids. J.clin.Invest., 29, 1311-9.

STEINKAMP, R., DUBACH, R., and MOORE, C.V. (1955) Studies in iron transportation and metabolism. VIII. Absorption of radio-iron from iron-encircled bread. Arch.intern.Med., 95, 181-193.

STEVENS, A.R., PIRZIO-BIROLI, G., HARKINS, H.N., NYHUS, L.M., and FINCH, C.A. (1959) Iron metabolism in patients after partial gastrectomy. Ann.Surg., 149, 534-538.

STEWART, W.B., YUILE, C.L., CLAIBORNE, H.A., SNOWMAN, R.T., and WHIPPLE, G.H. (1950) Radioiron absorption in anaemic dogs. Fluctuations in the mucosal block and evidence for a gradient of absorption in the gastro-intestinal tract. J.exp.Med., 92, 375-382.

STEWART, W.B., and GAMBINO, S.R. (1954) Dynamics of iron absorption. Fed.Proc., 13, 444.

STORER, E.H., OBERHELMAN, H.A.Jr., WOODWARD, E.R., SMITH, C.A., and DRAGSTEDT, L.R. (1952) Effect of the Exalto-Mann-Williamson procedure on gastric secretion. A.M.A.Arch.Surg., 64, 192-199.

STREET, H.R. (1943) A study of the availability of the iron in enriched bread. J.Nutr., 26, 187-195.

SYDENHAM, T. (1681) The works of Thomas Sydenham M.D., translated from the Latin edition of Dr. Greenhill. R.G.Latham, M.D., Sydenham Society, London, 1850. Vol. 2, p.97-98.

TAYLOR, J., STIVEN, D., and REID, E.W. (1935) Experimental and idiopathic siderosis in cats. J.Path.Bact., 41, 397-405.

TINSLEY, J.C.Jr., MOORE, C.V., DUBACH, R., MINNICH, V., GRINDSTEIN, M. (1949) The role of oxygen in the regulation of erythropoiesis. Depression of the rate of delivery of new red cells to the blood by high concentrations of inspired oxygen. J.clin.Invest., 28, 1544-1564.

TOMPSETT, S.L. (1940) Factors influencing the absorption of iron and copper from the alimentary tract. Biochem. J.,34, 961-969.

UNDERWOOD, E.J. (1938) A comparison of ferrous and ferric iron in the nutrition of the rat. J.Nutr., 16, 299-308.

VAN DYKE, D.C. (1960) Sources and properties of human urinary erythropoietin. Ciba Foundation Symposium on Haemopoiesis. J. & A. Churchill Ltd., London.

VISSCHER, M.B., FLETCHER, E.E.Jr., CARR, C.W., GREGOR, M.R., BUSHY, M.S., and BAKER, D.E. (1944) Isotopic tracer studies on the movement of water and ions between intestinal lumen and blood. Amer.J. Physiol., 142, 550-575.

Von PORAT, B.T.D. (1951) Blood volume determinations with the Evans Blue Dye method. Acta med.scand.Suppl. 256, 1-108.

WACK, J.P., and WYATT, J.P. (1959) Studies of ferrodynamics. I. Gastrointestinal absorption of Fe59 in the rat under differing dietary states. A.M.A. Arch. Path., 67, 237-242.

WALLENSTEN, S. (1954) Results of the surgical treatment of peptic ulcer by partial gastrectomy according to Billroth I and II methods. A clinical study based on 1256 operated cases. Acta chir. scand., Suppt., 191, 1-161.

WALLENSTEN, S. (1958) Iron absorption after Billroth I and II partial gastrectomy. Acta chir. scand., 115, 270-275.

WARREN, R. (1939) Serosal and mucosal dimensions at different levels of the dog's small intestine. Anat. Rec., 75, 427-437.

WASSERMAN, L.R., RASHKOFF, I.A., LEAVITT, D., MAYER, J., and PORT, S. (1952) The rate of removal of radioactive iron from the plasma - an index of erythropoiesis. J. clin. Invest., 31, 32-39.

WATSON, A.B. (1947) Partial gastrectomy for simple ulcer. A review of the end results of 132 cases, with a criticism of the Polya operation. Brit. J. Surg., 34, 353-366.

WHIPPLE, G.H., and ROBSCHEIT-ROBBINS, F.S. (1930) Blood regeneration in severe anaemia. XVI. Optimum iron therapy and salt effect. Amer. J. Physiol., 92, 362.

WHIPPLE, G.H., and ROBSCHEIT-ROBBINS, F.S. (1936) Iron and its utilisation in experimental anaemia. Amer. J. med. Sci., 191, 11-24.

WILL, J.J., and VILTER, R.W. (1954) A study of the absorption and utilization of an iron chelate in iron deficient patients. J. Lab. clin. Med., 44, 499-505.

WILLIAMS, J. (1959) The effect of ascorbic acid on iron absorption in postgastrectomy anaemia and achlorhydria. Clin. Sci., 18, 521-526.

WINTROBE, M.M. (1956) Clinical hematology. 4th Ed. Henry Kimpton, London.

WINTROBE, M.M., GREENBERG, G.R., HUMPHREY, S.R., ASHENBRUCKER, H., WORTH, W., and KRAMER, R. (1947) The anaemia of infection. III. The uptake of radio-active iron in iron-deficient and in pyridoxine-deficient pigs before and after acute inflammation. J. clin. Invest., 26, 103-113.

WISSLER, R.W., BETHARD, W.F., BARKER, B., and MORI, H.D., (1954) Effects of polyoxyethylene Sorbitan Monolaurate (Tween 20) upon gastrointestinal iron absorption in Hamsters. Proc. Soc. exp. Biol., 86, 170-177.

WITTS, L.J. (1936) The therapeutic action of iron. Lancet, 1, 1-5.

WOHLER, F., HEILMEYER, L., EMRICH, D., and KANG, S.H., (1957) Zur Funktion des Ferritins bei der Eisenresorption. Arch. exper. Path. u Pharmakol. 230, 107-124.

WOOTTON, A.C. (1910) Chronicles of pharmacy. McMillan & Co., Ltd., London.

YUILE, C.L., BLY, C.G., STEWART, W.B., IZZO, A.J., WELLS, J.C., and WHIPPLE, G.H. (1949) Plasma and red cell radioiron following intravenous injection Turpentine abscesses in normal and anaemic dogs. J. exp. Med., 90, 273-282.

YUILE, C.L., HAYDEN, J.W., BUSH, J.A., TECLUK, H., and STEWART, W.B. (1950) Plasma iron and saturation of plasma iron-binding protein in dogs as related to gastrointestinal absorption of iron. J. exp. Med., 92, 367-373.

APPENDIX.

RESULTS OF STUDIES OF ABSORPTION OF IRON FROM ACUTELY PREPARED INTESTINAL LOOPS IN DOGS.

5462	15.4	[illegible]	[illegible]	[illegible]
5407	16.0	15.9	47.5	[illegible]
5499	18.3	14.2	45	[illegible]
5554	14.6	[illegible]	43.5	[illegible]
5553	17.3	16.2	45	12
5571	16.4	14.5	44.5	

Dog	Weight Kg	Haemoglobin Gm %	P. C. V. %	Serum Iron ug %
P661	15.0	15.5	55	-
P710	14.8	13.4	48	-
P808	19.2	16.1	52	-
P815	18.0	15.9	48.5	-
P843	15.0	13.1	42	135
P907	16.3	15.1	48	175
P926	15.0	16.6	51	154
P941	19.0	14.8	45	131
P976	18.8	17.9	53	144
S 22	22.0	16.4	49	165
S 38	15.7	14.9	45.5	155
S 52	20.2	17.7	55.5	154
S 73	16.4	16.6	50.0	167
S 93	20.6	13.8	42	182
S151	14.0	17.2	55	233
S194	20.4	16.2	50.5	-
S222	18.0	15.9	48.5	147
S242	16.2	15.9	50	124
S344	19.0	15.7	49.5	-
S365	20.2	14.7	46	152
S390	18.1	14.8	45.5	161
S415	16.1	14.8	46	180
S424	17.4	14.0	44.5	166
S445	19.7	16.5	49.5	179
S462	25.4	12.7	40	129
S487	16.0	15.0	47.5	147
S499	18.3	14.2	46	123
S554	14.8	14.0	43.5	141
S555	17.3	15.6	48	128
S571	16.4	14.5	44.5	-

Weight haemoglobin and serum iron values for 30 dogs used in experiments on absorption of iron from acutely prepared isolated loops of intestine.

Dog	Weight Kg	Haemoglobin Gm %	P. C. V. %	Serum Fe ug %
S589	18.2	13.8	43.5	169
S608	18.5	15.3	50	189
S609	15.8	14.3	47	150
S619	17.9	15.8	48	172
S620	17.0	14.7	46	199
S805	20.0	13.4	43	127
S806	19.2	16.8	53	144
S821	12.0	14.0	44	140
S822	11.4	14.6	47	150
S849	18.0	16.3	50	-
S850	18.1	14.0	43	126
S862	20.0	13.7	43	143
S863	18.8	15.3	47	163
S874	20.0	14.8	47.5	182
S886	16.0	14.3	47	132
S894	16.0	10.8	33	61
S933	16.4	13.1	40.5	130
S942	15.0	13.0	40.5	135
S943	16.4	14.3	47.5	141
S967	19.0	17.2	53	169
S982	18.8	15.0	48	159
S983	19.1	14.0	43.5	140
S999	18.2	15.0	48	177
S1000	16.5	14.3	44	136
T 15	19.3	16.9	52	-
T 16	17.3	14.6	47	137
T 36	18.0	15.6	49	-
T 37	16.4	13.7	42	126
T 52	16.3	14.6	47.5	131
T 53	19.5	15.6	49	157
G 1	17.0	14.0	43	-

Weight haemoglobin and serum iron values for 31 dogs used in experiments on absorption of iron from acutely prepared isolated loops of intestine.

Dog	Absorption of Iron %		
	Method A	Method B	Mean
Duodenum			
P907	15.0	15.5	15.25
P926	14.3	9.4	11.85
P941	2.6	1.5	2.05
S 22	18.1	15.4	18.75
S 38	23.7	20.6	22.15
S 73	10.5	14.2	12.35
S 93	23.2	17.4	20.30
S151	26.0	20.6	23.30
S194	30.4	32.8	31.60
S222	40.3	23.3	36.80
S242	12.6	13.2	12.90
S344	18.7	11.0	14.85
S415	38.8	54.2	46.50
S424	14.0	15.0	14.50
S445	20.7	29.1	24.90
S487	16.0	13.4	14.70
S499	33.3	41.6	37.45
S541	16.6	21.1	18.85
S571	1.9	0.1	1.00
S589	9.6	8.9	9.35
S805	23.1	33.9	28.50
S806	17.9	20.1	19.00

Comparison of methods A and B of measuring the absorption of iron from isolated loops in the dog in 5 hour tests.

Dog	Absorption of Iron %		
	Method A	Method B	Mean
Duodenum			
S821	34.7	28.2	31.45
S822	34.7	28.8	31.75
S849	30.3	27.8	29.05
S850	15.2	13.9	14.55
S862	20.4	11.4	15.90
S933	12.2	7.3	9.75
S943	22.0	13.5	17.75
S983	28.9	23.9	26.40
S999	17.6	13.8	15.70
S1000	24.4	21.1	22.75
T15	21.0	22.8	21.90
T16	2.6	0.5	1.55
T52	17.7	20.9	19.30
T53	24.3	21.2	22.75
Ileum			
S344	1.0	1.0	1.00
S424	14.5	7.3	10.90
S445	14.8	7.9	11.35
S499	6.6	3.9	5.25
S541	7.0	7.7	7.35
S571	2.0	2.0	2.00
S589	4.0	7.9	5.95

Mean A	18.19 ± 1.57 S.E.M.
Mean B	16.61 ± 1.76 S.E.M.
Reliability coefficient r	0.8579
d.f. = 41	$P = < 0.001$
Linear regression equation	$A = 5.52 + 0.7628\ B$

Comparison of methods A and B of measuring the absorption of iron from isolated loops in the dog in 5 hour tests.

ONE LOOP

Dog	Absorption of Iron %	
	I Mean of methods A & B	II Method C
S805	28.5	21.1
S806	19.0	12.7
S821	31.4	33.0
S822	31.7	27.4
S849	29.0	28.6
S850	14.5	4.4
S862	15.9	12.9
S863	11.1	4.5
S933	9.7	1.0
S943	17.7	15.3
S983	26.4	26.8
S999	15.7	5.1
S1000	22.7	15.1
T15	21.9	12.4
T16	1.5	0.4
T36	1.9	2.4
T52	19.3	9.0
T53	22.7	25.4

Mean I	18.22 ± 2.21*
Mean II	15.12 ± 2.48*
Reliability coefficient	0.8283
d.f. 15	$P < 0.001$

Linear Regression Equation

$$II = -1.37 + 0.905\ I$$

*S. E. M.

Comparison of method C for measuring absorption of iron with methods A and B in experiments with one isolated loop in 5 hour tests.

TWO LOOPS

Dog	Absorption of Iron %	
	I Methods A & B	II Method C
P808	1.2	0.2
P815	14.0	8.2
P843	10.0	2.0
P907	17.4	14.0
P926	10.1	4.3
P941	1.7	2.0
S 22	12.5	10.2
S 38	10.2	18.5
S 73	12.1	13.7
S 93	13.4	13.9
S194	24.4	16.4
S222	24.2	21.3
S242	10.2	7.3
S344	6.1	5.6
S415	37.1	47.5
S424	10.6	4.3
S445	21.0	17.9
S487	7.8	9.1
S499	23.7	16.2
S571	1.1	0.4
S589	10.1	4.0

Mean I	13.28 ± 1.93*
Mean II	11.29 ± 2.29
Reliability coefficient	0.8879
d.f. 19	$P < 0.001$

Linear Regression Equation

$II = -2.71 + 1.054\ I$

*S. E. M.

Comparison of method C for measuring absorption of iron with methods A & B in experiments with two isolated loops in 5 hour tests.

Dog	Experimental Conditions	Dose of Ferric Iron ug	Absorption of Iron % Duodenum	Ileum	Ratio Duod./Ileum	Difference from unity of ratio: statistics using logs.*
S242	pH2 Vit C	0.5	12.9	7.3	1.77	
S344		0.5	14.8	1.0	14.80	
S222		1.0	36.8	24.9	1.48	
S415		2.0	46.5	19.6	2.37	t = 3.39
S499		2.0	37.4	3.9	9.59	d.f. 8
S194		3.0	31.6	16.2	1.95	P = <0.01
S424		5.0	14.5	10.9	1.33	
S 73		10.0	12.3	9.8	1.26	
S 93		10.0	20.3	7.1	2.86	
S487	pH2 No Vit C	1.0	14.7	7.0	2.10	
P907		1.5	15.2	17.5	0.87	t = 1.26
S445		2.0	24.9	11.3	2.20	d.f. 5
P926		4.0	11.8	9.8	1.20	P = >0.2
P941		5.0	2.0	2.5	0.80	
S462		5.0	1.0	1.0	1.00	
P843	pH7 No Vit C	1.5	9.7	9.6	1.01	t = 0.96
P815		2.0	11.8	19.6	0.63	d.f. 5
P808		3.0	0.1	0.1	1.00	P = >0.05

* Logarithms were used because of the skew distribution of the ratios.

Comparison of absorption of iron from duodenal and ileal loops prepared in the same dog in 5 hour tests.

DUODENAL LOOPS								
Dog	S821	S822	S893	S999	S1000	S943	T 15	T 53
Ferrous Iron ug	5.0	1.0	10.0	10.0	2.0	2.0	3.0	4.0
pH	2	2	2	2	2	7	7	7
Vit C	+	+	+	+	+	+	+	+
Time	Radioactive iron recorded by Method (A) % dose per 100 sq. cm. serosal surface							
15	97.3	95.3	96.2	93.6	96.3	97.3	98.4	96.3
30	93.2	90.6	93.4	91.4	93.4	91.9	95.3	91.9
45	86.2	84.7	88.5	89.9	89.0	89.2	91.4	87.4
60	80.0	80.0	84.3	88.4	86.2	87.2	88.3	84.7
75	73.6	75.3	81.5	87.7	84.3	85.2	86.7	82.9
90	68.6	70.6	77.7		82.4	83.2	85.9	82.0
105	67.1	68.8	74.9		80.5	81.9	85.0	81.1
120	66.6	67.6	73.5		78.6	80.6	84.1	80.2
135	66.2		73.2	87.3	77.6			79.3
150	65.8		72.9		76.6			78.4
165	65.3		72.6		76.1	79.9		
180			72.3		75.6	79.2		
195			72.0			78.6	82.5	
210			71.7			78.0	81.7	
225			71.4				80.9	
240		67.0	71.1				79.7	
255		66.4					79.0	
270		65.8						
295		65.3						
300	65.3	65.3	71.1	87.3	75.6	78.0	79.0	78.4
Disappearance Non-Exponential			✓	✓				
Exponential								
$T\frac{1}{2}$						37		34.5
r						1.87		2.01
Two components								
(1) $T\frac{1}{2}$	97.5	90			97.5		82	
r	0.71	0.77			0.71		0.85	
(2) $T\frac{1}{2}$	25	37			45		39	
r	2.77	1.87			1.54		1.78	

Readings from direct writing scintillation counter expressed as percent of dose of radioactive iron given per 100 sq. cm. serosal surface of duodenal loops. The form of the disappearance curves of iron from the loop are noted below and where appropriate the half time ($T\frac{1}{2}$) and rate (% min.) are given.

DUODENAL LOOPS							
Dog	S849	S850	S862	T 52	S859	S805	S806
Ferrous Iron ug	2.0	3.0	1.0	4.0	2.0	2.0	3.0
pH	2	2	2	2	2	2	2
Vit C	-	-	-	-	+	+	+
Time	Radioactive iron recorded by Method (A) % dose per 100 sq. cm. serosal surface						
15	97.1	97.0	93.0	97.8	97.0	97.2	94.7
30	88.6	94.0	82.6	94.8	93.3	93.7	89.4
45	85.0	91.0	79.1	92.2	92.6	88.8	88.1
60	82.3	88.0	76.5	90.0	92.3	85.3	86.8
75	80.5	86.2	75.6	87.8		83.2	
90	79.1	84.8		85.6		81.8	
105	77.4			84.1		80.4	85.5
120	76.0			83.2		79.0	84.7
135	74.3			82.3			83.4
150	73.9						82.1
165	72.5						
180	71.4				91.6		
195	69.7				91.3	78.1	
210					91.0	77.6	
225					90.7	77.2	
240					90.4	76.9	
255							
270							
285							
300	69.7	84.8	75.6	82.3	90.4	76.9	82.1
Disappearance Non-exponential	✓				✓	✓	✓
Exponential							
$T\frac{1}{2}$			31	51			
r			2.24	1.36			
Two components							
(1) $T\frac{1}{2}$		56					
r		1.24					
(2) $T\frac{1}{2}$		15					
r		4.62					

Readings from direct writing scintillation counter expressed as percent of dose of radioactive iron given per 100 sq. cm. serosal surface of duodenal loops. The form of the disappearance curves of iron from the loop are noted below and where appropriate the half time ($T\frac{1}{2}$) and rate (% min.) are given.

DUODENAL LOOPS								
Dog	P907	P926	S22	S38	S445	S487	S571	S73
Ferric Iron ug.	1.5	4.0	1.5	1.5	10.0	1.0	2.0	10.0
pH	2	2	2	2	2	2	7	2
Vit C	-	-	-	-	-	-	-	+
Time	Radioactive iron % dose							
15	98.9	96.0	96.1	92.3	96.7	96.5	100	97.3
30	92.1	91.6	91.2	91.1	92.5	93.5	99.4	93.9
45	87.6	88.7	88.3	83.6	89.5	92.3	98.9	91.2
60	85.7	86.2	86.3	83.1	86.5	91.1	98.4	89.5
75	85.0	85.7	85.2	80.6	85.5	90.5	98.1	
90			84.1	78.1	84.5	89.9		
105			84.1	77.3		89.5		
120			84.1			89.0		
135			83.0			88.3		
150			81.9			87.6		
165						87.1		
180						86.5		
195					83.6	86.1		
210					82.7	85.8		
225					81.8	85.5		
240					80.9	85.2		
255					80.2	84.9		
270					79.8	84.6		
285						84.3		
300	85.0	85.7	81.9	77.3	79.8	84.0	98.1	89.5
Nonexponential					✓	✓	✓	
Exponential		✓	✓					✓
$T\frac{1}{2}$		20	45					28
r % min.		3.46	1.54					2.47
2 Rates	✓			✓				
(1) $T\frac{1}{2}$	78			43				
r % min.	0.89			1.61				
(2) $T\frac{1}{2}$	21			20				
r % min.	3.30			3.46				

Readings from direct writing scintillation counter expressed as percent of dose of radioactive iron given per 100 sq. cm. serosal surface of duodenal loops. The form of the disappearance curves of iron from the loop are noted below and where appropriate the half time ($T\frac{1}{2}$) and rate (% min.) are given.

DUODENAL LOOPS								
Dog	S93	S194	S242	S344	S415	S424	S499	S933
Ferric Iron ug.	10.0	3.0	0.5	0.5	2.0	5.0	2.0	2.0
pH	2	2	2	2	2	2	2	7
Vit C	+	+	+	+	+	+	+	+
Time	Radioactive iron % dose							
15	97.5	91.3	96.3	94.8	92.5	94.2	94.4	98.8
30	92.5	87.0	94.2	87.1	84.3	91.3	88.5	92.7
45	89.5	82.7	93.1	85.7	78.7	89.8	82.2	91.5
60	88.3	80.1	92.0	84.4	74.3	88.3	76.6	89.9
75	85.9	78.4	90.7	83.0	70.5	86.8	74.6	89.3
90	84.1	77.5	89.4	82.7	68.0	86.1	72.1	88.7
105	82.3			81.3	65.5		70.8	88.4
120	81.1				63.3		69.5	88.1
135					62.1		68.0	87.8
150					61.8		67.3	
165	79.9				61.5		67.0	
180	79.0				61.2		66.7	
195	78.4	75.0						
210	77.8	73.5						
225	77.2	71.6	88.9					
240	76.7	70.6	88.4					
255		69.6	88.0					
270			87.6					
285								
300	76.7	69.6	87.6	81.3	61.2	86.1	66.7	87.8
Nonexponential		✓	✓	✓		✓		✓
Exponential	✓				✓		✓	
$T\frac{1}{2}$	37.5				35		37	
r % min.	1.85				1.98		1.87	
2 Rates (1) $T\frac{1}{2}$ r % min. (2) $T\frac{1}{2}$ r % min.								

Readings from direct writing scintillation counter expressed as per cent of dose of radioactive iron given per 100 sq. cm. serosal surface of duodenal loops. The form of the disappearance curves of iron from the loop are noted below and where appropriate the half time ($T\frac{1}{2}$) and rate (% min.) are given.

Time min.	Radioactive Iron Mean % Remaining 31 Duod. Loops	S. E. M.
15	96.05	0.36
30	91.61	0.59
45	88.25	0.75
60	85.83	0.91
75	84.16	1.07
90	82.83	1.23
105	82.00	1.33
120	81.42	1.40
135	81.04	1.43
150	80.81	1.45
165	80.63	1.48
180	80.45	1.48
195	80.14	1.50
210	79.95	1.50
225	79.75	1.51
240	79.57	1.51
255	79.45	1.52
270	79.39	1.52
285	79.37	1.53
300	79.37	1.53

Mean amount of radioactive iron remaining in 31 isolated duodenal loops during test of absorption.

Form of Disappearance Curves of Fe^{59} from Loop	No.	Mean Time to Half Concentration	Mean rate % min.
Non Exponential	14		
Exponential	10	35.6 min.	1.9466
Two components	7	(1) 77 min.	(1) 0.90
		(2) 27.17 min.	(2) 2.5506

Form of disappearance curve of radioactive iron from 31 isolated duodenal loops.

DUODENAL LOOPS								
Dog	P907	P926	S 22	S 38	S445	S487	S571	S 73
Ferric Iron Micro-gram	1.5	4.0	1.5	1.5	10.0	1.0	2.0	10.0
pH	2	2	2	2	2	2	7	2
Vit C	-	-	-	-	-	-	-	+
Time	Rate of disappearance of Fe^{59} from loop % dose remaining/100 sq. cm. serosal area/min.							
0- 15	0.075	0.264	0.262	0.583	0.270	0.240	0.000	0.181
16- 30	0.455	0.297	0.326	0.092	0.340	0.205	0.040	0.226
31- 45	0.332	0.219	0.223	0.562	0.226	0.085	0.033	0.192
46- 60	0.176	0.189	0.156	0.037	0.205	0.086	0.033	0.193
61- 75	0.090	0.039	0.081	0.189	0.082	0.044	0.025	
76- 90			0.082	0.193	0.050	0.044		
91-105				0.098		0.045		
106-120			0.080			0.018		
121-135			0.082			0.054		
136-150						0.036		
151-165						0.038		
166-180						0.046		
181-195					0.110	0.037		
196-210					0.094	0.028		
211-225					0.095	0.019		
226-240					0.098			
241-255					0.079			
256-270					0.060			
271-285								
286-300								

Rates of disappearance of radioactive iron in consecutive 15 min. periods from isolated duodenal loops prepared in dogs expressed as percent of dose remaining in loop per 100 sq. cm. of serosal surface per min.

DUODENAL LOOPS								
Dog	S 93	S194	S242	S344	S415	S424	S499	S933
Ferric Iron Micrograms	10.0	3.0	0.5	0.5	2.0	5.0	2.0	2.0
pH	2	2	2	2	2	2	2	7
Vit C	+	+	+	+	+	+	+	+
Time	Rate of disappearance of Fe^{59} from loop % dose remaining/100 sq. cm. serosal area/min.							
0- 15	0.167	0.580	0.246	0.347	0.501	0.390	0.376	0.081
16- 30	0.324	0.318	0.147	0.511	0.545	0.207	0.396	0.406
31- 45	0.221	0.300	0.076	0.094	0.403	0.107	0.421	0.085
46- 60	0.092	0.210	0.038	0.095	0.381	0.109	0.376	0.047
61- 75	0.187	0.144	0.097	0.096	0.352	0.110	0.200	0.054
76- 90	0.145	0.074	0.078	0.097	0.250	0.056	0.165	0.056
91-105	0.146			0.098	0.251		0.084	0.025
106-120	0.099				0.175		0.085	0.027
121-135					0.069		0.065	0.029
136-150					0.035		0.044	
151-165	0.100				0.035		0.022	
166-180	0.087				0.035		0.022	
181-195	0.052	0.224						
196-210	0.053	0.193						
211-225	0.053	0.159	0.040					
226-240	0.027	0.082	0.041					
241-255		0.082	0.042					
256-270			0.020					
271-285								
286-300								

Rates of disappearance of radioactive iron in consecutive 15 min. periods from isolated duodenal loops prepared in dogs expressed as percent of dose remaining in loop per 100 sq. cm. of serosal surface per min.

DUODENAL LOOPS							
Dog	P849	S850	S862	T 52	S589	S805	S806
Ferrous Iron Micrograms	2.0	3.0	1.0	4.0	2.0	2.0	3.0
pH	2	2	2	2	2	2	2
Vit C	-	-	-	-	+	+	+
Time	Disappearance Rate						
0- 15	0.191	0.197	0.463	0.148	0.210	0.186	0.355
16- 30	0.571	0.202	0.694	0.198	0.252	0.232	0.381
31- 45	0.278	0.205	0.273	0.155	0.060	0.326	0.102
46- 60	0.218	0.207	0.213	0.158	0.030	0.276	0.104
61- 75	0.151	0.168	0.073	0.162		0.187	
76- 90	0.116	0.170		0.164		0.122	
91-105	0.157			0.105		0.125	0.106
106-120	0.121			0.057		0.127	0.054
121-135	0.159			0.035			0.107
136-150	0.121						0.108
151-165	0.159						
166-180	0.122						
181-195	0.123					0.065	
196-210	0.124					0.033	
211-225	0.044					0.033	
226-240						0.033	
241-255							
256-270							
271-285							
286-300							

Rates of disappearance of radioactive iron in consecutive 15 min. periods from isolated duodenal loops prepared in dogs expressed as percent of the dose remaining in the loop per 100 sq. cm. serosal surface per minute.

DUODENAL LOOPS								
Dog	S821	S822	S983	S999	S1000	S943	T 15	T 53
Ferrous Iron Micro-grams	5.0	1.0	10.0	10.0	2.0	2.0	3.0	4.0
pH	2	2	2	2	2	7	7	7
Vit C	+	+	+	+	+	+	+	+
Time	Disappearance Rate							
0- 15	0.183	0.314	0.190	0.424	0.248	0.178	0.105	0.180
16- 30	0.275	0.321	0.194	0.149	0.206	0.357	0.214	0.363
31- 45	0.458	0.400	0.338	0.102	0.292	0.196	0.262	0.328
46- 60	0.417	0.364	0.320	0.104	0.271	0.165	0.236	0.166
61- 75	0.428	0.387	0.198	0.052	0.156	0.170	0.112	0.114
76- 90	0.359	0.412	0.201		0.242	0.136	0.063	0.088
91-105	0.101	0.165	0.204		0.246	0.095	0.064	0.059
106-120	0.024	0.089	0.113		0.177	0.097	0.065	0.060
121-135	0.025		0.071	0.053	0.092			0.061
136-150	0.026		0.072		0.093			0.062
151-165	0.027		0.073		0.046	0.061		
166-180			0.045		0.024	0.062		
181-195			0.045			0.063	0.127	
196-210			0.038			0.064	0.068	
211-225			0.038				0.070	
226-240		0.057	0.031				0.106	
241-255		0.057					0.072	
256-270		0.058						
271-285		0.058						
286-300								

Rates of disappearance of radioactive iron in consecutive 15 min. periods from isolated duodenal loops prepared in dogs expressed as percent of the dose remaining in the loop per 100 sq. cm. serosal surface per minute.

Time min.	Mean rate % dose remaining per 100 sq. cm. per min.	S. E. M.
0- 15	0.287	0.0159
16- 30	0.316	0.0247
31- 45	0.232	0.0193
46- 60	0.170	0.0172
61- 75	0.121	0.0174
76- 90	0.100	0.0164
91-105	0.060	0.0114
106-120	0.041	0.0078
121-135	0.025	0.0060
136-150	0.016	0.0050
151-165	0.012	0.0041
166-180	0.010	0.0033
181-195	0.023	0.0073
196-210	0.017	0.0059
211-225	0.014	0.0054
226-240	0.012	0.0045
241-255	0.009	0.0040
256-270	0.008	0.0037
271-285	0.004	0.0023
286-300	0.003	0.0016

Mean rate of disappearance of radioactive iron from 31 isolated duodenal loops, calculated for successive 15 min. perids and expressed as percent of dose remaining in loop per min. per 100 sq. cm. serosal surface.

Dog	S344	S499	S589	S487	S445
Dose of Iron ug.	0.5	2.0	2.0	1.0	2.0
pH	2	2	2	2	2
Vit C	+	+	+	-	-
Time min.	Absorption rate % per min. per 100 sq. cm. serosal area				
0 - 15		0.348	0.082	0.204	0.503
16 - 30			0.335	0.421	0.089
31 - 45			0.089	0.224	0.090
46 - 60				0.232	0.055
61 - 75					
76 - 90					
91 - 105					
106 - 120					
121 - 135	0.094				
136 - 150	0.057				
151 - 165	0.059				
166 - 180	0.050				

Rate of absorption of radioactive iron from isolated ileal loops in 5 dogs expressed as a percent. of the dose unabsorbed per 100 sq. cm. serosal surface per min.

Dog	Dose of Ferrous Iron Micro-grams	Absorption of Iron Method A In Vivo Counting			
		Max. rate % min.	Time of max. rate min.	*Duration of absorption (min.)	Amount absorbed micro-grams
S849	2.0	0.571	16 - 30	195	0.61
S850	3.0	0.199	46 - 60	90	0.30
S862	1.0	0.694	16 - 30	75	0.24
T 52	4.0	0.198	16 - 30	135	0.97
S589	2.0	0.252	16 - 30	60	0.19
S805	2.0	0.326	31 - 45	120	0.46
S806	3.0	0.356	16 - 30	60	0.54
S821	5.0	0.459	46 - 60	165	1.73
S822	1.0	0.314	31 - 45	120	0.35
S983	10.0	0.328	31 - 45	240	2.89
S999	10.0	0.424	0 - 15	75	1.26
S1000	2.0	0.292	31 - 45	180	0.49
S943	2.0	0.357	16 - 30	120	0.44
T 15	3.0	0.262	31 - 45	120	0.63
T 53	4.0	0.360	16 - 30	150	0.71

* First phase of absorption only: any later phase i.e. around 3 hours is not included.

Maximal rate of absorption of iron measured by in vivo scintillation counter, time of maximal rate and duration of absorption from isolated duodenal loops in dogs.

Dog	Ferric Iron Dose Micro-grams	Absorption of Iron Method A In Vivo Counting			
		Max. rate % min.	Time of max. rate (min.)	Duration of* absorption (min.)	Amount absorbed ug
P907	1.5	0.455	16 - 30	75	0.23
P926	4.0	0.297	16 - 30	75	0.47
S 22	1.5	0.326	16 - 30	105	0.41
S 38	1.5	0.583	0 - 15	105	0.57
S445	10.0	0.340	16 - 30	90	0.40
S487	1.0	0.240	0 - 15	225	0.16
S571	2.0	0.190	16 - 30	60	0.04
S 73	10.0	0.226	16 - 30	60	1.31
S 93	10.0	0.324	16 - 30	120	2.32
S194	3.0	0.580	0 - 15	90	0.91
S242	0.5	0.490	0 - 15	90	0.06
S344	0.5	0.511	16 - 30	105	0.09
S415	2.0	0.503	16 - 30	180	0.77
S424	5.0	0.390	0 - 15	90	0.70
S499	2.0	0.378	31 - 45	180	0.74
S933	2.0	0.406	16 - 30	135	0.22

* First phase of absorption only: any later phase at around 3 hours is not included.

Maximal rate of absorption of iron measured by in vivo scintillation counter, time of maximal rate and duration of absorption from isolated duodenal loops in dogs.

Dog	Test Solution Conditions	Ferrous Iron Dose ug	Iron not able to be aspirated* from Duodenal Loop (A) % Dose								Form of curve of (A)		
			15 min.	30	45	60	75	90	105	120	Non Expon^{tl}	Exponential $T_{\frac{1}{2}}$ min.	Exponential Rate % min.
S849	pH2	2.0	39.1	62.5	71.9		-					10.5	6.60
S850	"	3.0	22.2	31.7	-	37.1	-	44.4			+		
S862	"	1.0	25.4	47.6	-	51.6	-	53.2				10.5	6.60
T 52	"	4.0	31.9	46.0	-	68.9	-	75.3				19.0	3.65
S589	pH2 Vit C	2.0	25.9	46.3	-	63.0	-	66.7	-	70.4		19.5	3.55
S805	"	2.0	29.8	57.7	-	80.8						17.5	3.96
S806	"	3.0	9.1	39.4	69.7						+		
S821	"	5.0	19.1	35.5	47.1							16.5	4.20
S822	"	1.0	34.1	51.1	67.1							15.0	4.62
S983	"	10.0	18.5	35.2	-	50.0	-	56.6				19.0	3.65
S999	"	10.0	14.7	21.6	-	28.0	-	32.7	-	37.4		16.5	4.20
S1000	"	2.0	19.5	33.4	-	46.0	-	52.9				21.0	3.30
S943	pH7 Vit C	2.0	35.8	56.7	-	64.2	-	67.1				12.0	5.78
T 15	"	3.0	40.0	56.0	-	60.0	-	62.0				8.0	8.67
T 53	"	4.0	23.8	42.1	-	55.8	-	59.1				14.0	4.95

* Blank spaces at later times indicate equilibrium at last value given

Amounts of iron which could not be aspirated from the isolated duodenal loops during tests of absorption of iron in dogs.

Dog	Test Solution Conditions	Dose of Ferric Iron ug	Iron not able to be aspirated from loop* % dose A								Form of Curve		
			15	30	45	60	75	90	105	120	Non Exponential	Exponential $T\frac{1}{2}$ min.	Exponential Rate % min.
P907	pH2	1.5	46.2	57.2	58.7	60.0						7.5.	9.24
P926	"	4.0	30.0	43.3	52.2	56.7	-	58.9				13.5	5.13
S 22	"	1.5	20.2	29.7	35.8	37.8	34.5	42.9	43.6			15.0	4.62
S 38	"	1.5	40.0	47.5	55.0	62.5						13.5	5.13
S445	"	10.0	43.9	61.0	-	73.2	-	75.6				12.0	5.78
S487	"	1.0	59.5	71.2	72.6							8.0	8.67
S571	pH7	2.0	25.0	28.6	-	32.4	-	35.3	-	38.3	+		
S 73	pH2 Vit C	10.0	25.0	39.5	52.0	59.1	-	71.1	-	77.0		31.0	2.24
S 93	"	10.0	31.0	41.2	52.8	54.9	-	64.8	-	67.7		20.0	3.47
S194	"	3.0	8.9	11.5	-	14.6						14.0	4.95
S242	"	0.5	41.8	56.5	-	73.6	-	78.9	-	84.2		19.5	3.55
S344	"	0.5	31.4	40.0	-	54.3						14.5	4.78
S415	"	2.0	30.3	39.4	-	45.5						10.5	6.60
S424	"	5.0	31.0	47.9	-	53.5	-	63.1	-	71.9	+		
S499	"	2.0	58.7	66.0	-	67.0						6.0	11.55
S933	pH7 Vit C	2.0	13.7	21.2	-	35.0	-	-	-	37.5	+		

* Blank spaces at later times indicate equilibrium at last value given.

Amount of iron which could not be aspirated from the isolated duodenal loops during tests of absorption of iron in dogs.

Test Solution Conditions	No. of dogs	Rate at which iron became unavailable for aspiration from duodenal loops $T\frac{1}{2}$ min.	Diff.	t	P
pH2	7	12.17 ± 1.20 *	5.01 ± 1.92**	2.6120	< 0.02
pH2 Vit. C	14	17.18 ± 1.33 *			
All Conditions	26	14.77 ± 0.68			

* S.E.M.
** S.E.M. Diff.

Mean half times for change in radioactive iron so that it could not be aspirated from isolated duodenal loops during tests of absorption of iron in dogs.

DUODENAL LOOPS

Dog	Test Solution Conditions	(D) Dose of Iron micrograms	Iron absorption		Iron in Bowel Wall	
			% Dose	(E) Micro-grams	% Dose	(F) Micro-grams
S487	pH2	ferric 1.0	14.7	0.15	4.6	0.05
P907	"	" 1.5	15.2	0.23	6.4	0.10
S 22	"	" 1.5	18.7	0.28	3.8	0.05
S 38	"	" 1.5	22.2	0.35	12.7	0.19
S445	"	" 2.0	24.9	0.50	-	-
P926	"	" 4.0	11.8	0.47	5.2	0.21
P941	"	" 5.0	2.1	0.11	1.9	0.10
S862	pH2	ferrous 1.0	15.9	0.16	4.1	0.04
S849	"	" 2.0	29.0	0.58	4.7	0.09
S850	"	" 3.0	14.5	0.44	1.9	0.09
T 53	"	" 4.0	22.7	0.91	7.8	0.31
S863	"	" 5.0	11.1	0.56	8.9	0.45
T 36	"	" 10.0	1.9	0.19	0.9	0.09

Correlation of log. dose given (D) and log. amount absorbed (E) at pH2

Regression coefficient = Slope of regression line = 0.2846
Correlation coefficient $r = 0.6629$ d.f. 11 $P < 0.01$

Amount of iron absorbed and amount in bowel wall at the end of a 5 hour test of absorption of iron from isolated duodenal loops prepared in dogs. The test solution was given at pH2.

DUODENAL LOOPS

Dog	Test Solution Conditions	(D) Dose of Iron micrograms	Iron absorption		Iron in Bowel Wall	
			% Dose	(E) Micro-grams	% Dose	(F) Micro-grams
S242	pH2 Vit C	ferric 0.5	12.9	0.06	6.4	0.03
S344	"	" 0.5	14.8	0.07	2.4	0.01
S222	"	" 1.0	36.8	0.37	5.0	0.05
S415	"	" 2.0	46.5	0.93	9.1	0.18
S499	"	" 2.0	37.5	0.75	8.2	0.16
S194	"	" 3.0	31.6	0.95	5.0	0.15
S424	"	" 5.0	14.5	0.72	10.1	0.50
S 73	"	" 10.0	12.3	1.23	8.2	0.82
S 93	"	" 10.0	20.3	2.03	7.1	0.71
S822	"	ferrous 1.0	31.7	0.32	6.7	0.07
S589	"	" 2.0	9.3	0.18	3.3	0.07
S805	"	" 2.0	28.5	0.57	6.2	0.12
S1000	"	" 2.0	22.7	0.45	3.3	0.07
S806	"	" 3.0	19.0	0.57	8.1	0.24
S821	"	" 5.0	31.4	1.57	3.1	0.15
S983	"	" 10.0	26.4	2.64	2.8	0.28
S999	"	" 10.0	17.6	1.76	1.6	0.16

Correlation of log. dose of iron given (D) with log amount of iron absorbed (E).

Regression Coefficient b 0.9670

Correlation Coefficient r 0.9007 d.f. 15 $P < 0.001$

Amount of iron absorbed and amount in bowel wall at the end of a 5 hour test of absorption of iron from isolated duodenal loops prepared in dogs. The test solution was given at pH2 with ascorbic acid 100 mg.

DUODENAL LOOPS						
Dog	Test Solution Conditions	(D) Iron Dose Micrograms	Iron Absorbed % Dose	Iron Absorbed (E) Micrograms	Iron in Bowel Wall % Dose	Iron in Bowel Wall (F) Micrograms
P843	pH 7 Vit C	Ferric 1.5	9.7	0.15	3.9	0.06
P815	" "	" 2.0	8.3	0.16	6.0	0.12
S933	" "	" 2.0	12.2	0.24	1.8	0.04
P808	" "	" 3.0	0.1	0.03	0.5	0.15
S943	pH 7 Vit C	Ferrous 2.0	17.7	0.35	5.8	0.12
T 15	" "	" 3.0	21.9	0.66	3.4	0.10
T 53	" "	" 4.0	19.3	0.77	5.0	0.20
S571	pH 7	Ferric 2.0	1.0	0.02	1.5	0.03
T 16	"	" 2.0	1.5	0.03	0.4	0.01

Correlation between log dose of iron (D) given and log amount absorbed (E).

Regression Coeff. b. 1.6013
Correlation Coeff. r. 0.3982 df. 7 $P > 0.1$

Amount of iron absorbed and amount in bowel wall at the end of a 5 hour test of absorption from isolated duodenal loops prepared in dogs. The test solution was given at pH 7 with or without ascorbic acid 100 mg.

DUODENAL LOOPS

Correlation of Duodenal loops	No. of pairs	Regression Coefficient b	r	d.f.	P
Log Dose of Iron (D) / Log Amount absorbed (E)	39	$\frac{\log E}{\log D} = 0.8067$	0.5080	37	< 0.01
Amount absorbed (E) / Amount in bowel wall (F)	38	$\frac{(F)}{(E)} = 0.1675$	0.5843	36	< 0.001

Correlations from dose of iron given, amount absorbed and amount in bowel wall in 5 hour tests in isolated duodenal loops in dogs.

ILEAL LOOPS						
Dog	Test solution Conditions	(D) Iron Dose Micrograms		Iron absorbed % Dose	Iron absorbed (E) Micrograms	Iron in Bowel Wall % Dose	Iron in Bowel Wall (F) Micrograms
S589	pH2 Vit C	ferrous	2.0	6.0	0.12	6.2	0.12
S242		ferric	0.5	7.3	0.04	3.4	0.02
S344		"	0.5	1.0	0.01	1.2	0.01
S222		"	1.0	24.9	0.25	15.3	0.15
S415		"	2.0	19.6	0.39	8.4	0.17
S499		"	2.0	5.2	0.10	18.0	0.36
S194		"	3.0	16.2	0.49	8.0	0.24
S424		"	5.0	10.9	0.54	6.5	0.32
S 73		"	10.0	9.8	0.98	8.4	0.84
S 93		"	10.0	7.1	0.71	12.1	1.21
S487	pH2	ferric	1.0	7.0	0.07	0.9	0.01
P907	"	"	1.5	17.5	0.26	3.2	0.05
S 22	"	"	1.5	0.1	0.002	3.4	0.05
S 38	"	"	1.5	2.1	0.03	4.9	0.07
P926	"	"	4.0	9.8	0.39	1.8	0.07
P941	"	"	5.0	2.8	0.14	3.5	0.17
P843	pH7 Vit C	ferric	1.5	9.6	0.14	2.2	0.03
P815	"	"	2.0	19.6	0.39	0.8	0.02
P808	"	"	3.0	2.3	0.07	0.2	0.006
S571	pH7	ferric	2.0	2.0	0.04	0.9	0.02

Amount of iron absorbed and amount in the bowel wall at the end of a 5 hour test of absorption of iron from isolated ileal loops prepared in dogs.

Ileal Loops Correlation	No. of pairs	Regression Coefficient b	r	d.f.	P
Log Dose of Iron (D) / Log Amount absorbed (E)		log E/log D			
1. All loops	20	0.8476	0.5444	18	<0.02
2. At pH2 with Vit C	10	1.1495	0.8722	8	<0.001
3. At pH2 without Vit C	5	0.4908	0.5068	3	>0.1
Amount absorbed (E) / Amount in bowel wall (F)	20	F/E 0.2085	0.3019	18	>0.1

Correlations from dose of iron given, amount absorbed and amount in gut wall in 5 hour tests in isolated ileal loops in dogs.

EFFECT OF REMOVAL OF RADIOACTIVE IRON FROM LOOP AT VARIOUS INTERVALS ON ABSORPTION OF IRON FROM ISOLATED DUODENAL LOOPS IN 5 DOGS.

Dog	Initial Dose Iron ug	pH	Vit C mg.	Absorption % by 15 min.	30 min.	60 min.	120 min.	ug by time loop emptied	Loop emptied at mm.	Further Absorption % by 30 min.	60 min.	90 min.	120 min.	300 min.	ug by 300 min.
S619	2	2.0	-	10.3	10.3	12.8	18.0	0.31	120	-	-	-	-	-	-
S620	2	2.0	-	11.1	14.8	14.8	-	0.26	60	-	-	0	2.8	2.8	0.05
S874	2	2.0	-	5.3	8.8	-	-	0.17	30	-	2.3	2.9	2.9	3.1	0.06
S886	2	2.0	-	3.8	-	-	-	0.08	15	0.4	1.5	1.7	2.0	2.0	0.04
S894	10	2.0	100	9.3	18.5	-	-	2.4	30	-	3.3	3.9	4.8	4.8	0.48

Percentage distribution in the tissues of radioactive iron absorbed from the intestine with ascorbic acid.

Dog	Marrow	Plasma	Liver	Kidney	Spleen
P808	58.48	28.77	10.63	1.59	0.53
P815	46.44	41.15	10.62	1.30	0.40
P843	42.87	40.72	14.92	1.21	0.28
S933	43.15	29.45	24.66	1.37	1.37
S943	58.94	28.19	11.27	1.26	0.34
T 15	56.55	22.36	19.71	0.85	0.53
T 53	80.61	7.48	8.87	0.63	2.41
S 73	47.95	33.48	17.14	0.69	0.74
S 93	27.71	53.71	17.07	1.07	0.44
S194	79.16	10.69	8.94	0.72	0.49
S222	89.90	5.74	3.44	0.45	0.47
S242	81.73	9.73	7.96	0.46	0.12
S344	82.78	7.94	4.70	1.33	3.25
S415	87.19	3.93	6.82	0.62	1.44
S424	43.36	38.44	16.77	1.19	0.24
S499	68.47	28.04	1.69	1.04	0.76
S589	27.89	51.32	19.04	1.26	0.49
S805	81.42	6.36	9.22	0.38	2.62
S806	74.10	15.06	9.73	0.39	0.72
S821	76.50	9.83	12.69	0.90	0.08
S822	85.37	3.98	9.67	0.39	0.59
S983	60.70	18.55	14.80	1.12	4.83
S999	82.64	10.33	5.31	0.92	0.80
S1000	52.65	30.02	16.27	0.64	0.42
Mean S. E. M.	64.02 ± 3.93	22.30 ± 6.87	11.75 ± 1.16	1.02 ± 0.07	0.91 ± 0.24
Geom. Mean	60.77	16.83	10.15	0.97	0.64

Percentage distribution in the tissues of radioactive iron absorbed from the intestine with or without versenate.

Dog	Marrow	Plasma	Liver	Kidney	Spleen
Iron alone					
P907	88.22	5.37	5.61	0.40	0.40
P926	85.86	7.12	6.17	0.62	0.23
P941	55.85	23.36	13.34	2.75	1.70
S 22	30.20	47.24	19.70	2.17	0.69
S 38	84.87	5.09	7.12	1.33	1.60
S445	90.36	1.98	6.10	0.43	1.13
S487	80.33	6.24	11.56	1.21	0.66
S571	46.92	32.66	17.68	2.20	0.54
S849	84.09	4.99	9.41	0.54	0.97
S850	72.00	15.71	11.35	0.68	0.26
S862	69.14	2.81	27.27	0.38	0.40
S863	62.55	19.83	15.64	1.31	0.67
T 16	60.99	17.91	17.74	2.44	0.92
T 35	87.73	5.71	4.46	0.87	1.23
T 52	23.07	42.58	32.67	1.40	0.28
Mean S.E.M.	68.35 ± 5.18	16.11 ± 3.83	12.72 ± 2.13	1.25 ± 0.21	0.78 ± 0.12
Geom. Mean	63.77	12.14	9.85	1.02	0.65
With Versene					
S 52	55.33	33.03	9.68	1.16	0.80
S365	18.52	62.41	15.06	3.22	0.78
S390	46.31	32.94	12.75	4.48	0.52
Mean S.E.M.	40.05 ± 11.08	42.79 ± 9.81	12.46 ± 1.56	2.95 ± 0.96	0.70 ± 0.09
Geom. Mean	36.21	40.80	12.30	2.56	0.69

Dog	Dose of Iron ug	Vit C	Time of Test hrs	Fe 59 remaining within range of in vivo counter % dose min. 15	30	45	60	75	90	105	120
S554	2	-	0	89.5	77.8	74.1	72.2	70.3	68.0	68.0	68.0
	2	-	2½	86.8	78.9	76.8	75.2	74.2	72.3	72.3	72.3
	2	-	5	89.8	84.7	84.7	84.7	82.4	79.6	79.6	79.6
S942	2	-	0	89.5	79.0	76.9	72.7	71.6	70.5	70.5	70.5
	2	-	2½	90.4	81.6	76.0	74.8	74.8	73.7	73.7	73.7
	2	-	5	95.5	84.3	75.3	74.2	73.0	73.0	74.2	74.2
S608	10	+	0	94.0	92.5	90.3	87.5	84.3	82.2	81.4	81.4
	10	+	2½	94.9	92.8	89.9	87.9	85.6	83.1	83.1	83.1
	10	+	5	94.3	92.0	90.8	87.7	84.9	82.9	82.9	82.9

*pH maintained $\angle$3.5 throughout test.

Readings from in vivo scintillation counter over isolated duodenal loops after repeated tests with radioactive iron expressed as percentage of dose given.

Dog	Dose of Iron ug	Vit C	Time of Test hrs	Fe 59 remaining within range of in vivo counter %dose min. 15	30	45	60	75	90	105	120
S967	2	-	0	91.8	90.7	89.8	90.0	89.8	89.6	89.6	89.6
	2	+	2½	96.1	90.2	85.6	82.5	79.3	78.2	76.5	76.5
	2	-	5	95.3	92.3	92.3	90.0	83.1	77.7	77.7	77.7
T37	2	-	0	95.1	87.8	78.0	78.0	77.2	77.2	76.4	76.4
	2*	-	2½	95.0	83.3	80.0	76.7	75.9	75.0	74.1	76.7
	2	-	5	95.0	89.6	86.2	82.7	79.3	77.5	75.9	74.1
S982	2	-	0	94.3	91.4	91.4	91.4	91.4	90.0	87.8	87.8
	2	I.V.	2½	95.3	94.2	93.0	91.9	91.9	87.1	87.1	87.1
	2	+	5	90.6	87.1	86.2	85.3	82.8	81.9	80.0	78.5

*pH maintained $\angle$3.5 throughout test.

Readings from in vivo scintillation counter over isolated duodenal loops after repeated tests with radioactive iron expressed as percentage of dose given.

pH readings after placing solution of radioactive iron at pH2 with ascorbic acid in isolated duodenal loops.

Dog	Time: min. 0	15	30	60	90	120	180	240	300
S 73	2	3.29	4.6	5.59	6.43	6.60	6.75	6.97	6.95
S 93	2	2.54	3.66	4.69	5.92	6.29	6.74	6.76	6.84
S194	2	3.78	4.35	5.55	6.10	6.21	6.31	6.40	6.51
S222	2	3.62	4.86	6.85	6.95	7.03	7.00	6.93	6.96
S242	2	2.95	3.88	5.42	6.00	6.17	6.26	6.22	6.30
S344	2	3.76	4.19	6.06	6.63	6.72	6.72	6.75	6.81
S415	2	3.76	4.04	6.02	6.43	6.68	6.68	6.59	6.71
S424	2	2.86	4.46	5.17	5.82	6.28	6.42	6.58	6.60
S499	2	2.82	3.79	4.86	5.77	6.21	6.29	6.32	6.33
P863	2	3.91	5.40	6.77	-	6.95	-	-	-
S589	2	4.85	5.50	6.32	6.43	6.48	6.40	6.50	6.47
S805	2	4.25	5.50	6.36	6.40	6.51	6.53	6.60	6.58
S806	2	3.52	4.90	6.32	6.89	6.81	6.71	6.66	6.72
S821	2	3.63	3.95	4.78	6.04	6.23	6.32	6.52	6.55
S822	2	3.54	4.28	5.86	6.53	6.75	6.68	-	-
S983	2	3.54	4.22	5.42	6.20	-	-	-	-
S999	2	2.85	3.81	4.68	5.72	6.22	6.59	-	-
S1000	2	2.74	3.65	5.52	5.95	6.20	-	-	-
Mean	2	3.46	4.37	5.68	6.25	6.49	6.56	6.60	6.64
S.E.M.		± 0.14	± 0.14	± 0.16	± 0.07	± 0.07	± 0.06	± 0.06	± 0.06

pH readings after placing solution of radioactive iron at pH2 without ascorbic acid in isolated duodenal loops.

Dog	Time: min. 0	15	30	60	90	120	180	240	300
P907	2	6.03	6.90	7.02	-	6.95	6.91	-	-
P926	2	3.40	5.82	6.74	6.66	6.72	6.82	-	-
P941	2	3.54	6.09	6.77	6.93	6.81	6.82	-	-
S445	2	2.26	4.09	6.31	6.80	6.85	6.79	6.83	6.84
S487	2	3.22	5.88	6.36	6.47	6.50	6.50	6.68	6.70
S849	2	4.58	6.22	6.53	6.58	-	-	-	-
S850	2	5.01	6.55	6.91	7.21	7.18	7.32	7.28	7.07
S862	2	3.42	5.60	6.54	6.72	6.69	-	-	-
S863	2	5.30	6.18	6.48	6.47	-	-	-	-
T 36	2	6.50	7.01	6.82	6.70	-	-	-	-
T 52	2	3.48	5.82	6.47	6.49	-	-	-	-
Mean	2	4.25	6.01	6.63	6.70	6.81	6.86	6.91	6.87
S.E.M.		± 0.40	± 0.23	± 0.07	± 0.07	± 0.08	± 0.11	± 0.21	± 0.11

Isolated Tests Loop Conditions	No. of tests	Mean pH at min. 15	30	60	90	120	180	240	300
Duod. Vit C	18	3.46 ± 0.14*	4.37 ± 0.15*	5.68 ± 0.16*	6.25 ± 0.07*	6.49 ± 0.07*	6.56 ± 0.06*	6.60 ± 0.06*	6.64 ± 0.06*
No Vit C	11	4.25 ± 0.40*	6.01 ± 0.24*	6.63 ± 0.07*	6.70 ± 0.07*	6.81 ± 0.08*	6.86 ± 0.11*	6.91 ± 0.21*	6.87 ± 0.11
Diff.		0.89 ± 0.36**	1.64 ± 0.26**	0.95 ± 0.22**	0.45 ± 0.10**	0.32 ± 0.12**	0.30 ± 0.11**	0.31 ± 0.16**	0.23 ± 0.13**
t		2.495	6.284	4.404	4.334	2.629	2.680	1.985	1.707
P		< 0.02	< 0.001	< 0.001	< 0.001	< 0.02	< 0.02	< 0.1	< 0.2

* S. E. M. ** S. E. M. Diff.

Comparison of pH of fluid in 29 isolated duodenal loops after giving iron in solution at pH 2 with or without ascorbic acid.

pH readings after placing solution of radioactive iron at pH2 in isolated ileal loops

Dog	Ascorbic Acid	0	15	30	60	90	120	180	240	300
S 73	+	2	3.58	5.40	7.09	7.58	7.62	7.88	7.69	7.82
S 93	+	2	3.50	5.18	7.32	7.57	7.67	7.88	7.89	7.84
S194	+	2	4.02	6.04	7.00	7.45	7.67	7.62	7.72	7.78
S222	+	2	3.72	4.87	6.75	7.22	7.42	7.50	7.69	7.72
S242	+	2	3.43	5.68	7.02	7.40	7.48	7.68	7.62	7.71
S344	+	2	4.07	6.08	7.28	7.63	7.74	7.82	7.84	7.79
S415	+	2	3.90	4.80	6.21	6.85	7.08	7.21	7.42	7.53
S424	+	2	2.91	4.66	6.71	7.20	7.51	7.60	7.64	7.61
S499	+	2	3.42	5.55	7.02	7.33	7.48	7.48	7.54	7.56
S589	+	2	3.48	4.40	6.22	7.01	7.22	7.32	7.70	7.72
Mean		2	3.60	5.27	6.86	7.32	7.50	7.60	7.68	7.71
S.E.M.			± 0.11	± 0.18	± 0.12	± 0.08	± 0.07	± 0.08	± 0.04	± 0.03
P907	-	2	5.93	6.95	7.67	-	7.80	-	-	-
P926	-	2	5.68	6.90	7.72	7.91	8.10	-	-	-
P941	-	2	5.99	6.98	7.61	7.88	-	-	-	-
S445	-	2	5.31	6.70	7.16	7.35	7.42	7.51	7.63	7.65
S487	-	2	5.17	6.62	7.23	7.41	7.53	7.60	7.71	7.74
Mean		2	5.62	6.83	7.48	7.64	7.71	7.55	7.67	7.69
S.E.M.		-	± 0.16	± 0.06	± 0.12	± 0.15	± 0.15	± 0.05	± 0.04	± 0.05

Test Conditions	No. of tests	Mean pH at min. 15	30	60	90	120	180	240	300
Vit C	10	3.60 ± 0.11	5.27 ± 0.18	6.86 ± 0.12	7.32 ± 0.08	7.50 ± 0.07	7.60 ± 0.08	7.68 ± 0.04	7.71 ± 0.03
No Vit C	5	5.62 ± 0.16	6.83 ± 0.07	7.48 ± 0.12	7.64 ± 0.15	7.71 ± 0.15	7.55 ± 0.05	7.67 ± 0.04	7.69 + 0.05
Diff.		2.02 ± 0.19**	1.55 ± 0.27**	0.62 ± 0.20**	0.32 ± 0.16**	0.21 ± 0.14**	-0.05 ± 0.19**	-0.01 ± 0.10**	-0.02 ± 0.08**
t		10.515	5.764	3.447	2.020	1.497	0.027	0.010	0.003
P		<0.001	<0.001	<0.01	<0.1	<0.2	>0.9	>0.9	>0.9

* S. E. M. ** S. E. M. Diff.

Comparison of pH of fluid in 15 isolated ileal loops after giving iron in solution at pH2 and with or without ascorbic acid.

Dog	Dose of Iron micrograms	Vit C	Time of Start of Test hrs.	pH of intestinal fluid at mins. 15	30	60	120
S554	2	-	0	2.7	4.6	6.5	6.6
	2	-	2½	2.6	4.0	6.2	6.5
	2	-	5	2.6	3.3	4.9	6.2
S942	2	-	0	6.4	6.8	6.7	6.8
	2	-	2½	6.0	6.4	6.6	6.8
	2	-	5	5.9	6.8	6.8	6.8
S608	10	+	0	3.9	4.5	5.5	6.5
	10	+	2½	3.5	5.0	5.3	6.5
	10	+	5	3.7	4.1	5.4	6.8
S967	2	-	0	5.3	6.4	6.5	6.6
	2	+	2½	3.1	3.9	4.7	6.1
	2	-	5	3.4	6.0	6.5	6.9
S982	2	-	0	4.7	6.0	6.3	6.4
	2	I.V.	2½	3.6	5.6	6.4	6.5
	2	+	5	3.8	4.2	5.0	6.0
T37	2	-	0	3.0	5.4	6.0	6.2
	2*	-	2½	3.0	2.9	2.9	2.8
	2	-	5	2.9	3.6	4.3	5.4

* pH maintained < 3.5 throughout test

pH of intestinal fluid from isolated duodenal loops following repeated doses of iron-containing solution at pH 2.

From the work of Visscher et al., (1944) the rate of movement of water out of the gut into the blood, i.e. insorption is given by

$$\frac{V_o R_o - V_t R_t}{\frac{R_o + R_t}{2}}$$

where V_o = volume of water at time zero.

V_t = " " " " time t.

R_o = specific activity of dueterium oxide at time zero.

R_t = " " " " " " time t.

Exsorption, i.e., movement from blood to gut, is obtained by the difference of insorption from the net movement.

Movement of water in isolated duodenal loops in 4 dogs after giving radioactive iron in 50 ml. Tyrode's solution at a pH of 7.

Dog	Movement of water Ml/15 min.		
	Insorption	Exsorption	Net*
P960	23.5	19.4	+ 4.1
S 52	28.9	31.9	- 3.0
S390	29.7	27.7	+ 2.0
S571	40.5	41.9	- 1.4
Mean S.E.M.	30.65 ± 3.56	41.9 ± 4.68	0.42 ± 1.41

* + = Absorption - = Enterosorption

Movement of water in isolated duodenal loops after giving radioactive iron in 50 ml. Tyrode's solution at a pH of 2.

Dog	Movement of Water Ml/15 min.		
	Insorption	Exsorption	Net*
P907	32.8	38.8	- 6.0
P926	25.4	23.7	+ 1.7
P941	30.5	33.5	- 3.0
P976	34.6	37.0	- 2.4
S 22	37.6	33.3	+ 4.3
S 38	41.5	36.0	+ 4.5
S110	18.8	25.0	- 6.2
S151	24.3	30.1	- 6.8
S172	28.5	22.7	+ 5.8
S194	28.8	35.8	- 7.0
S202	44.7	36.9	+ 7.8
S222	31.5	35.7	- 4.2
S445	25.7	21.6	+ 4.1
S462	23.7	29.9	- 6.2
S487	30.6	34.9	- 4.3
S619	29.4	29.8	- 0.4
S620	30.6	29.7	+ 0.9
S849	34.3	33.5	+ 0.8
S850	21.7	37.8	-16.1
S862	30.3	29.7	+ 0.6
S863	25.3	29.3	- 4.0
S874	25.3	34.1	- 8.8
S886	24.4	31.3	- 6.9
Mean	29.14	31.74	- 2.60
S.E.M.	± 1.41	± 1.03	± 1.16

* + = Absorption - = Enterosorption

Movement of water in isolated duodenal loops after giving radioactive iron in 50 ml. Tyrode's solution with ascorbic acid at pH2 or pH7.

Dog	pH	Movement of Water Ml/15 min.		
		Insorption	Exsorption	Net*
P815	7	20.3	21.3	- 1.0
P843	7	32.6	38.6	- 6.0
S933	7	13.7	30.8	-17.1
S943	7	31.0	25.0	+ 6.0
Mean S.E.M.		24.43 ± 4.02	28.93 ± 2.94	- 4.5 ± 3.79
P863	2	34.6	37.6	- 3.0
S 73	2	27.4	28.7	- 1.3
S 93	2	19.5	26.0	- 7.5
S134	2	28.1	26.3	+ 1.8
S242	2	33.0	36.2	- 3.2
S344	2	25.1	38.7	-13.6
S357	2	26.9	35.1	- 8.2
S415	2	27.6	27.1	+ 0.5
S499	2	34.5	27.8	+ 6.7
S541	2	27.3	31.3	- 4.0
S589	2	23.9	30.8	- 6.8
S805	2	23.6	32.9	- 9.3
S806	2	25.4	27.4	- 2.0
S821	2	29.1	37.7	- 8.6
S822	2	30.0	25.7	+ 4.3
S894	2	37.7	32.7	+ 5.0
S983	2	19.6	19.6	-
Mean S.E.M.		27.84 ± 1.72	30.68 ± 1.36	- 2.84 ± 1.42

* + = Absorption _ = Enterosorption

	No. of tests	Movement of Water ML/15 min.		
		Insorption	Exsorption	Net. *
(1) pH 7: no ascorbic acid	4	30.65 ± 3.56	30.23 ± 4.68	+ 0.42 ± 1.41
(2) pH 2: no ascorbic acid	23	29.14 ± 1.41	31.74 ± 1.03	- 2.60 ± 1.16
(3) pH2 : with ascorbic acid	17	27.84 ± 1.72	30.68 ± 1.36	- 2.84 ± 1.42
(4) pH 2 : with or without ascorbic acid	40	28.34 ± 3.47	31.29 ± 2.58	- 2.85 ± 2.82
(5) All experiments	48	28.20 ± 1.01	31.01 ± 0.79	- 2.81 ± 0.83
Comparison	Diff.	S. E. M. Diff.	t	P
(1) - (2) Insorption	1.51	±3.7576	0.4015	>0.6
(1) - (3)	2.81	±3.8433	0.7311	>0.4
(1) - (4)	2.31	±2.0446	1.1298	>0.2
(1) - (2) Exsorption	1.51	± 3.0588	0.4937	>0.7
(1) - (3)	0.45	± 3.5056	0.1284	>0.8
(1) - (4)	1.06	± 2.2120	0.4792	>0.6
(1) - (2) Net	3.02	± 2.8962	1.0427	>0.2
(1) - (3)	3.26	± 2.9722	1.0968	>0.2
(1) - (4)	3.27	± 2.8408	1.1511	>0.2

* + = absorption - = enterosorption

Mean movements of water (ml per 15 minutes per 100 sq. cm. serosal surface) from isolated duodenal loops.

Movement of water in isolated ileal loops in 4 dogs after giving radioactive iron in 50 ml. Tyrode's solution at pH 7.

Dog	Movements of water Ml/15 min.		
	Insorption	Exsorption	Net*
P960	25.6	13.8	+11.8
S 52	26.0	16.5	+ 9.5
S390	32.0	16.9	+15.1
S571	32.1	30.5	+ 1.6
Mean S.E.M.	28.93 ± 1.81	19.43 ± 3.70	+ 9.50 ± 3.37

* + = Absorption - = Enterosorption

Movement of water in isolated ileal loops after giving radioactive iron in 50 ml. Tyrode's solution.

Dog	pH	Ascorbic Acid	Movement of Water Ml/15 min.		
			Insorption	Exsorption	Net*
P815	pH7	+	20.3	21.3	- 1.0
P843	"	+	10.1	12.1	- 2.0
Mean			15.2	16.7	- 1.5
S. E. M.					
P863	pH2	+	37.9	36.9	+ 1.0
S 73	"	+	28.9	21.2	+ 7.7
S 93	"	+	44.1	35.1	+ 9.0
S134	"	+	32.6	23.6	+ 8.0
S242	"	+	37.4	29.6	+ 7.8
S344	"	+	35.6	28.4	+ 7.2
S357	"	+	41.6	38.8	+ 2.8
S415	"	+	14.7	14.6	+ 0.1
S499	"	+	35.9	27.2	+ 8.7
S541	"	+	32.1	30.5	+ 1.6
S589	"	+	27.6	22.1	+ 5.5
Mean			33.48	28.09	+ 5.4
S. E. M.			± 2.59	± 2.35	± 1.12
P907	pH2	-	35.9	25.9	+ 6.0
P926	"	-	32.2	22.0	+10.2
P941	"	-	38.8	27.8	+11.0
P976	"	-	30.4	18.2	+12.2
S 22	"	-	65.2	47.9	+17.3
S 38	"	-	32.1	23.6	+ 8.5
S110	"	-	15.9	13.3	+ 2.6
S151	"	-	35.5	28.3	+ 7.2
S172	"	-	28.8	19.5	+ 9.3
S194	"	-	35.0	27.0	+ 8.0
S202	"	-	41.9	26.1	+15.8
S222	"	-	38.4	29.6	+ 8.8
S455	"	-	39.8	29.3	+10.5
S462	"	-	31.7	27.3	+ 4.4
S487	"	-	34.5	27.8	+ 6.7
Mean			35.74	26.24	+ 9.5
S. E. M.			± 2.63	± 1.96	± 0.86

* + = Absorption - = Enterosorption

	No. of tests	Movement of Water ML/15 min.		
		Insorption	Exsorption	Net. *
(1) pH 7: no ascorbic acid	4	28.93 ± 1.81	19.43 ± 3.70	+9.50 ± 3.37
(2) pH 2: no ascorbic acid	15	35.74 ± 2.63	26.24 ± 1.96	+9.50 ± 0.86
(3) pH 2: with ascorbic acid	11	33.48 ± 2.59	28.08 ± 2.35	+5.4 ± 1.12
(4) pH 2: with or without ascorbic acid	26	34.93 ± 1.56	26.83 ± 1.48	+8.10 ± 1.56
(5) All experiments	32	32.95 ± 1.42	25.28 ± 2.17	+7.63 ± 0.69
Comparison	Diff.	S.E.M. Diff.	t	P
Insorption (1) - (2)	6.81	±5.2726	1.2916	>0.2
(1) - (3)	4.55	±4.3278	1.0513	>0.3
(1) - (4)	6.00	±4.0172	1.4936	>0.1
Exsorption (1) - (2)	6.81	±4.2707	1.5945	>0.1
(1) - (3)	8.65	± 4.4056	1.9634	>0.05
(1) - (4)	7.40	±4.0025	1.8488	>0.05
Net (1) - (2)	-	-	-	-
(1) - (3)	4.1	±2.6994	1.5188	>0.1
(1) - (4)	1.4	±2.2387	0.6254	>0.5

* + = absorption - = enterosorption

Mean movements of water (ml per 15 minutes per 100 sq. cm serosal surface) from isolated ileal loops.

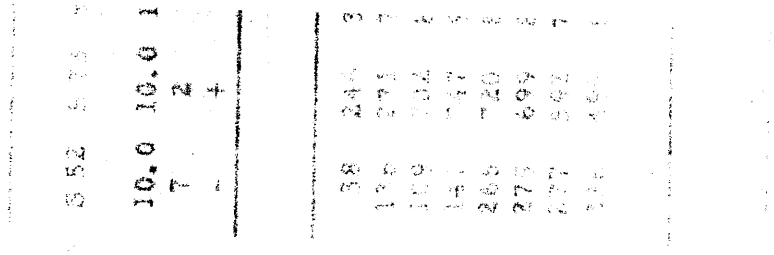
Dog	Time	Movement of Water ML/15 min. Insorption	Exsorption	Net *
S554	0	20.7	20.4	+ 0.3
	2½	21.7	21.3	+ 0.4
		(105)	(104)	
	5	24.1	27.6	- 3.5
		(116)	(135)	
S608	0	22.9	27.5	- 4.6
	2½	15.2	18.1	- 2.9
		(66)	(66)	
	5	19.4	20.7	- 1.3
		(85)	(75)	
S942	0	33.3	39.6	- 6.3
	2½	31.4	33.6	- 2.4
		(94)	(85)	
	5	28.3	31.1	- 2.8
		(85)	(79)	
S967	0	31.6	38.6	- 7.0
	2½	39.0	41.3	- 2.3
		(124)	(107)	
	5	36.6	43.6	- 7.0
		(116)	(113)	
S982	0	32.9	39.3	- 6.4
	2½	34.8	35.8	- 1.0
		(106)	(91)	
	5	38.8	40.4	- 1.6
		(118)	(103)	
T37	0	28.9	29.7	- 0.8
	2½	23.6	31.0	- 7.4
		(82)	(104)	
	5	23.7	30.8	- 7.1
		(82)	(104)	

* + = absorption - = enterosorption

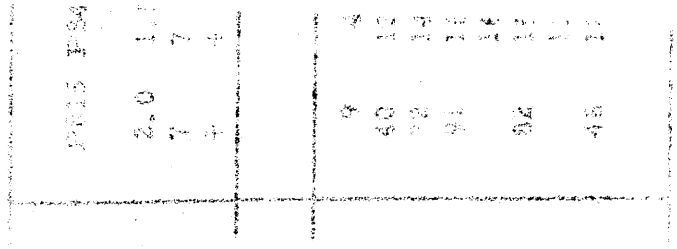
Figures in brackets are % of control reading at zero time.

TABLE

Movement of water in repeated tests on isolated duodenal loops in 5 dogs.



RESULTS OF STUDIES OF LEVELS OF RADIOACTIVE IRON IN THE PLASMA OF DOGS.



Dog	P815	P843	P863	P907	P926	P941	P976	S 22	S 38	S 52	S 73	S 93
Dose of Iron Micrograms	2.0	1.5	10.0	1.5	4.0	1.5	3.0	1.5	1.5	10.0	10.0	10.0
pH	7	7	2	2	2	2	2	2	2	7	2	2
Vit C	+	+	+	-	-	-	-	-	-	-	+	+
Time	Millimicrograms Fe^{59} per ml. plasma											
30	9	4	29	99	44	42	380	36	54	38	246	308
60	40	12	54	93	89	54	500	76	110	136	271	704
90	72	14	65	81	78	49	480	94	118	109	702	866
120	91	16	90	60	65	49	370	110	100	147	747	913
150		16	130		48	41	240	110	74	266	720	877
180	82	15	141	33	36	39	180	114	52	279	699	869
240		13	173	14	21	36	75	120	31	277	592	737
300	48	12	188	8.4	11	31	43	106	17	236	495	627

Levels of radioactive iron in the plasma in 12 dogs during absorption of iron from isolated duodenal and ileal loops in the same animal.

Dog	S194	S222	S242	S344	S390	S415	S445	S462	S487	S499	S571	S589
Dose of Iron Micrograms	3.0	1.0	0.5	0.5	2.0	2.0	10.0	2.0	1.0	2.0	2.0	2.0
pH	2	2	2	2	7	2	2	2	2	2	7	2
Vit C	+	+	+	+	-	+	-	-	-	+	-	+
Time	Millimicrograms Fe^{59} per ml. plasma											
30	145	25	5	5	36	251	282	12	45	131	2.0	18
60	255	41	11	11	82	499	280	26	60	258	4.6	37
90	279	37	12	11	104	447	189	19	58	277	5.2	42
120	237	35	13	9.2	118	364	133	16	48	248	5.1	43
180	165	24	9.7	6.2	117	189	52	6.5	29	178	5.1	38
240	105	15	5.6	3.7	97	92	18	3.5	15	129	4.6	36
300	61	11	3.5	2.2	81	48	8	3.5	6	104	3.0	32

Levels of radioactive iron in the plasma in 12 dogs during absorption of iron from isolated duodenal and ileal loops in the same animal.

Dog	S619	S620	S874	S886	S805	S806	S821	S822	S849	S850	S862
Dose of Iron Micrograms	2.0	2.0	2.0	2.0	2.0	3.0	5.0	1.0	2.0	2.0	1.0
pH	2	2	2	2	2	2	2	2	2	2	2
Vit C	-	-	-	-	+	+	+	+	-	-	-
Time	Millimicrograms Fe^{59} per ml. plasma										
30	18	47	10	36	139	104	405	114	416	82	60
60	19	90	15	20	301	188	851	199	475	103	82
90	23	107	15	14	304	238	891	218	395	90	64
120	17	95	17	10	242	234	585	211	315	75	46
150	-	-	15	4.4	-	-	-	-	-	-	32
180	15	72	13	-	131	167	238	180	149	46	21
210	-	-	-	-	-	-	-	-	-	-	14
240	12	45	11	2.8	79	108	80	128	54	28	9.8
270	-	-	-	-	-	-	-	-	-	-	6.3
300	9.2	23	9.6	-	32	66	80	39	33	24	4.5
Exponential Disappearance Rate	+	-	-	-	+	+	-	-	+	-	+

Levels of radioactive iron in the plasma in 11 dogs during absorption of iron from isolated duodenal loops.

Dog	S863	S894	S933	S943	S983	S999	S1000	T 15	T 16	T 36	T 52	T 53
Dose of Iron Micrograms	5.0	10.0	2.0	2.0	10.0	10.0	2.0	3.0	2.0	10.0	4.0	4.0
pH	2	2	7	7	2	2	2	7	7	2	2	7
Vit C	-	+	+	+	+	+	+	+	-	-	-	+
Time	Millimicrograms Fe^{59} per ml. plasma											
30	45	1068	23	63	642	38	13	160	2.2	227	117	436
60	111	729	49	161	1868	164	235	352	3.4	201	238	704
90	138	381	33	210	2500	260	275	398	2.2	175	297	764
120	140	198	-	228	2540	263	280	382	2.2	144	282	692
150	-	108	33	216	2189	218	262	346	2.2	109	274	568
180	115	63	25	209	1874	172	231	305	2.2	83	260	441
210	100	36	25	189	1430	134	202	256	2.2	52	228	322
240	81	27	18	169	1237	99	173	200	2.2	39	200	232
270	69	18	10	145	829	69	143	160	2.2	22	181	147
300	56	12	10	114	630	61	120	123	2.2	18	157	115
Exponential Disappearance Rate	+	+	-	-	+	-	+	+	-	+	+	+

Levels of radioactive iron in the plasma in 12 dogs during absorption of iron from isolated duodenal loops.

Time Min.	Mean Fe 59 in Plasma Millimicrograms	S. E. M.
30	154.7	34.13
60	251.1	46.98
90	281.7	61.77
120	249.3	57.60
180	188.6	43.38
240	122.0	31.19
300	83.6	22.51

Mean radioactive iron levels in plasma in 47 dogs during absorption of iron from isolated loops of small intestine.

Dog	Maximal Fe 59 in Plasma		Absorption of Fe 59	
	% Dose	Micrograms	% Dose	Micrograms
S619	1.2	0.024	15.6	0.31
S620	5.3	0.107	13.2	0.26
S874	0.9	0.017	11.9	0.23
S886	1.8	0.036	5.9	0.11
S805	15.2	0.303	32.8	0.65
S806	7.9	0.237	19.0	0.57
S821	21.8	0.890	31.7	1.57
S822	17.8	0.218	31.4	0.32
S849	23.8	0.475	29.1	0.58
S850	3.4	0.103	3.9	0.12
S862	8.2	0.082	15.9	0.16
S863	2.8	0.140	11.1	0.56
S894	10.7	1.068	19.7	1.97
S933	2.4	0.049	9.3	0.19
S943	11.4	0.228	17.7	0.35
S983	25.4	2.539	26.4	2.64
S999	2.6	0.263	15.7	1.57
S1000	14.0	0.280	22.7	0.45
T 15	13.3	0.397	21.9	0.66
T 16	0.2	0.003	0.2	0.004
T 36	2.3	0.227	2.0	0.20
T 52	19.1	0.764	22.8	0.91
T 53	7.4	0.296	19.3	0.79

	% Dose	Micrograms
Mean Fe 59 in plasma	9.52	0.38
Mean Fe 59 absorbed	17.35	0.66
Regression coeff. b.	1.019 ± 0.133*	0.726 ± 0.0834*
Correlation coeff. r.	0.8588	0.8854
P	< 0.001	< 0.001

* S. E.

Maximal level of radioactive iron in the plasma and amount of radioactive iron absorbed in 23 dogs with isolated duodenal loops.

Dog	Test Conditions	Ratios $\frac{\text{Portal}}{\text{Femoral}}$ Plasma Fe 59 % at time intervals after giving Fe 59 - min. 30	60	90	120	150	180	240	300
P907	pH 2	119	103	105	101	-	110	103	100
P926	"	114	101	104	101	96	94	102	108
P941	"	114	97	106	97	97	100	106	99
S445	"	103	101	97	96	-	101	101	96
S487	"	102	101	100	99	-	100	99	101
S 2	"	107	100	100	98	101	99	103	113
S 22	"	140	104	112	100	-	105	102	100
S 38	"	116	110	103	100	99	100	102	96
S 73	pH 2 Vit C	116	107	102	101	103	100	99	100
S 93	"	115	101	97	99	97	101	98	96
S194	"	111	100	99	98	101	98	100	101
S222	"	118	99	110	107	-	101	91	106
S344	"	126	99	106	107	-	98	94	95
S415	"	112	106	-	101	-	97	96	99
S424	"	112	101	102	99	-	99	98	96
S499	"	113	103	101	100	-	98	102	101
S151	"	146	113	106	101	98	103	105	103
S172	"	112	101	105	100	100	96	101	100
P815	pH 7 Vit C	107	111	110	102	-	102	-	99
P843	"	102	110	100	102	100	104	90	91
Mean		114.8	103.4	103.4	100.7	99.2	100.7	99.6	100.0
S.E.M.		3.51	1.02	1.01	0.64	0.70	0.98	1.02	1.09

Ratios of levels of radioactive iron in portal venous and removal arterial plasma obtained simultaneously during absorption of iron from isolated loops of small intestine expressed as a percentage.

RESULTS OF CHRONIC EXPERIMENTS ON ABSORPTION OF IRON IN DOGS.

Height	Radioactivity as measured by NaI crystal %						
Cm. from upper rim of cup	Tests						Mean
	1	2	3	4	5	6	
6	100	100	100	100	100	100	100
5	86.1	85.1	83.4	84.0	83.2	-	84.36 ± 0.610*
4	72.4	72.4	76.5	74.0	71.3	71.3	72.97 ± 0.889*
3	62.4	61.4	63.6	62.6	62.9	62.6	62.59 ± 0.319*
2	56.2	56.4	56.8	57.5	56.2	53.6	56.11 ± 0.593*
1	48.0	48.6	49.2	50.6	48.5	49.3	49.03 ± 0.405*

* S. E. M.

Radioactivity of solution diluted to various heights in plastic cup placed over a sodium iodide scintillation crystal, expressed as a percentage of the reading at a level of 6 cm. from the upper rim of the cup.

Dog	Absorption of Iron % Dose				Utilisation of Iron % Dose			
	No Vit C		With Vit C		No Vit C		With Vit C	
	Preop.	Postop.	Preop.	Postop.	Preop.	Postop.	Preop.	Postop.
P455	12	10	18	26	12	9	16	12
			29	56*			9	41*
P777	37	43	76	62	13	39	74	34
			69	57			74	53
P779	28	24	69	51	20	16	52	53
			27				32	
S192	-	-	39	40	-	-	27	13
			60	24			35	8

* Test done when dog was anaemic.

Absorption and utilisation of 10 micrograms ferric iron given intragastrically in 4 dogs showing the effect of removal of the duodenum from continuity with the rest of the alimentary tract.

Dog	Utilisation of Iron % Dose Absorbed			
	No Vit C		With Vit C	
	Preop.	Postop.	Preop.	Postop.
P455	100	90	89, 31	46, 73*
P777	35	91	97, 107	93, 55
P779	72	67	84, 75	104
S192	-	-	69, 58	54, 20

* Test done when dog was anaemic.

Percentage Utilisation of iron absorbed from 10 micrograms ferric iron given intragastrically in 4 dogs showing the effect of removal of the duodenum from continuity with the rest of the alimentary tract.

Percentage absorption of 10 micrograms ferric iron in varying test solutions from isolated duodenal loops in 4 dogs.

Dog	Absorption of 10 micrograms ferric iron % Dose							
	pH 2	Ascorbic Acid 100 mg. at pH 7	Ascorbic Acid at pH 2 mg. 100	20	10	5	2	1	Gastric Juice
P779	12.8 8.8	12.0 8.5	25.0 23.9 25.7 28.3 32.1 31.9		26.3 37.8	25.0 25.0	25.0 18.7	12.5 6.8	21.2 14.3
Mean	10.8	10.2	27.8		32.0	25.0	21.8	10.1	17.7
P455	16.7 20.9	- -	25.6 30.0 32.2 37.1 30.0 31.2	33.1 32.1	-	27.4 19.8	20.0 16.7	10.0 11.5	7.8 11.8
Mean	18.8		31.0	32.6	-	23.6	18.3	10.7	9.8
P777	4.0	-	17.4 14.0 19.2 16.5						
Mean	3.0	-	16.8						
S192	5.4 5.3	-	18.9 14.8 11.2 11.6	14.3 9.1	-	12.5 10.1	-	9.8 8.0	-
Mean	5.3	-	14.1	11.7	-	11.3	-	8.9	-

Each group of two readings represents the results from 2 scintillation counters at one test.

Dog	Weeks from Beginning Tests	Movement of Water ml/15 min. Insorption	Exsorption	Net.
P455	0	18.6	17.9	+ 0.7
	2	28.0	30.2	- 2.2
	4	16.8	16.9	- 0.1
	6	22.4	24.8	- 2.4
P779	0	15.1	21.1	- 6.0
	2	19.6	20.6	- 1.0
	4	15.0	13.5	+ 1.5
	6	16.2	18.9	- 2.7
	8	15.5	14.3	+ 0.8
	9	18.9	20.3	- 1.4
P777	0	16.3	20.5	- 4.2
	2	17.4	21.3	- 4.1
S192	0	22.6	21.6	+ 1.0
	2	23.5	26.7	- 3.2
	4	24.7	26.5	- 1.8

Movement of water in isolated duodenal loops (Thiry Vella) after giving radioactive iron in 25 ml. Tyrode's solution at pH2.

RESULTS OF STUDIES ON ABSORPTION OF IRON

IN RATS.

Site of injection	Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose 5th day	7th day	Mean	Mean
Duodenum 1	1	200	84.3	88.2	86.2	
2	"	195	76.6	82.0	79.3	
3	"	190	68.2	72.4	70.3	83.2±3.0*
4	"	180	89.1	88.3	88.7	
5	"	185	82.8	86.5	84.6	
6	"	180	91.8	88.3	90.0	
Jejuno-ileum 1	"	190	20.0	21.4	20.7	
2	"	180	23.4	21.9	22.1	
3	"	200	18.4	19.7	19.0	19.7±1.22*
4	"	190	23.0	22.3	22.6	
5	"	175	18.1	18.9	18.5	
6	"	185	14.1	14.7	14.4	
Ileum 1	"	210	3.6	3.4	3.5	
2	"	170	4.9	5.4	5.1	
3	"	190	2.9	3.0	2.9	4.9±1.26*
4	"	195	10.6	10.5	10.5	
5	"	180	1.8	1.8	1.8	
6	"	175	5.8	5.6	5.7	

* S. E. M.

Percentage absorption of 1 microgram ferric iron with 5 mg. ascorbic acid injected into the duodenum or jejuno-ileum of ileum of 18 normal rats.

Site of injection		Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose			Mean
				5th day	7th day	Mean	
Duodenum	1	10	200	46.4	50.9	48.6	
	2	"	180	49.9	53.6	51.7	
	3	"	190	50.0	50.2	50.1	43.6±6.95*
	4	"	230	33.7	35.8	34.7	
	5	"	185	13.3	14.6	13.9	
	6	"	210	60.6	64.5	62.5	
Upper Jejunum	1	"	200	53.4	58.5	55.9	
	2	"	200	49.2	53.1	51.1	
	3	"	230	17.8	18.0	17.9	43.9±9.21*
	4	"	180	36.0	37.4	38.7	
	5	"	200	78.4	77.6	78.0	
	6	"	210	20.3	23.5	21.9	
Duodenum	1	100	195	30.8	33.2	32.0	
	2	"	200	28.0	26.1	27.0	
	3	"	230	24.4	31.7	28.0	27.2±2.29*
	4	"	205	16.9	18.5	17.7	
	5	"	190	25.0	25.6	25.3	
	6	"	180	33.1	33.9	33.5	
Upper Jejunum	1	"	210	18.5	11.0	14.7	
	2	"	180	24.2	25.0	24.6	
	3	"	205	19.0	17.9	18.4	17.0±2.94*
	4	"	210	8.4	8.5	8.4	
	5	"	190	9.9	10.8	10.3	
	6	"	230	25.2	26.3	25.7	

* S. E. M.

Percentage absorption of 10 or 100 micrograms ferric iron with 5 mg. ascorbic acid injected into the duodenum or the upper jejunum of 24 normal rats.

Site of injection		Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose 5th day	7th day	Mean	Mean
Duodenum	1	1000	170	14.6	14.3	14.4	
	2	"	185	23.0	24.2	23.6	
	3	"	190	18.6	18.4	18.5	18.1±1.42*
	4	"	180	19.4	19.5	19.4	
	5	"	210	18.4	18.6	18.5	
	6	"	190	15.0	13.5	14.3	
Upper Jejunum	1	"	200	11.2	10.6	10.9	
	2	"	175	12.0	12.2	12.1	
	3	"	170	9.1	9.9	9.5	10.9±0.70*
	4	"	180	8.2	8.6	8.4	
	5	"	175	11.9	11.1	11.5	
	6	"	215	13.3	12.9	13.1	

* S. E. M.

Percentage absorption of 1000 micrograms ferric iron with 5 mg. ascorbic acid injected into the duodenum or the upper jejunum of 12 normal rats.

Site of injection	Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose			Mean
			5th day	7th day	Mean	
Intragastric 1	10	180	76.2	77.1	76.6	
2	"	170	71.2	71.4	71.3	
3	"	185	83.4	82.8	83.1	
4	"	170	72.7	72.0	72.3	
5	"	175	77.8	82.3	80.0	
6	"	170	81.3	79.7	80.5	77.2±1.44*
7	"	170	77.7	77.7	77.4	
8	"	175	71.6	70.9	71.2	
9	"	180	81.5	75.8	77.6	
10	"	170	75.5	77.4	76.4	
11	"	175	84.6	90.2	87.4	
12	"	170	75.0	70.5	72.7	

*S. E. M.

Percentage absorption of 10 micrograms ferric iron with 5 mg. ascorbic acid given via an intragastric tube to 12 normal rats.

Site of injection		Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose 5th day	7th day	Mean	Mean
Duodenum	1	10	170	40.9	40.8	40.8	
	2	"	185	78.5	70.3	74.4	
	3	"	190	78.1	78.9	78.5	69.8±6.05*
	4	"	200	80.0	83.9	81.9	
	5	"	175	62.5	61.8	62.1	
	6	"	170	81.6	80.4	81.0	
Mid Jejunum	1	"	170	45.2	43.6	44.4	
	2	"	180	33.7	32.0	32.8	
	3	"	200	15.5	14.8	15.1	28.6±4.08*
	4	"	195	30.9	31.5	31.2	
	5	"	195	24.5	23.7	24.1	
	6	"	170	23.5	24.4	23.9	

* S. E. M.

Percentage absorption of 10 micrograms ferric iron with 5 mg. ascorbic acid injected into the duodenum or mid jejunum in 12 normal rats.

Site of injection		Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose			Mean
				5th day	7th day	Mean	
Duodenum	1	10	230	53.6	59.0	56.3	
	2	"	215	45.3	41.1	43.2	
	3	"	190	42.5	40.4	41.9	50.4±2.81*
	4	"	185	47.9	48.1	48.0	
	5	"	200	56.5	55.2	55.8	
	6	"	200	59.5	54.6	57.0	
Ileum	1	"	220	1.8	1.4	1.6	
	2	"	180	0.3	0.4	0.3	
	3	"	210	3.2	3.0	3.1	2.1±0.64*
	4	"	190	4.5	4.2	4.3	
	5	"	195	2.9	3.1	3.0	
	6	"	210	0.7	0.5	0.6	
Duodenum	1	10	230	43.8	45.3	44.5	
	2	"	205	61.3	62.8	62.0	
	3	"	205	44.2	39.9	42.0	53.1±3.32*
	4	"	210	54.0	56.4	55.2	
	5	"	185	56.5	52.7	54.6	
	6	"	200	60.2	59.9	60.0	
Jejuno-ileum	1	"	190	7.5	7.6	7.5	
	2	"	195	1.6	1.6	1.6	
	3	"	185	10.3	9.7	10.0	9.2±3.08*
	4	"	195	1.5	1.4	1.4	
	5	"	200	14.0	13.0	13.5	
	6	"	185	21.6	20.9	21.2	

* S. E. M.

Percentage absorption of 10 micrograms ferric iron with 5 mg. ascorbic acid injected into the duodenum or ileum or jejuno-ileum of 24 normal rats.

Site of injection		Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose 5th day	7th day	Mean	Mean
Duodenum	1	10	205	83.6	89.9	86.7	
	2	"	205	70.0	65.3	67.6	
	3	"	180	52.4	48.5	50.4	74 ± 7.71*
	4	"	170	70.1	71.5	70.8	
	5	"	175	97.5	91.5	94.5	
Mid Jejunum	1	"	200	31.4	30.6	31.0	
	2	"	180	27.1	27.8	27.4	34.3±3.05*
	3	"	195	39.9	39.7	39.8	
	4	"	170	41.7	36.6	39.1	
Jejuno-ileum	1	"	200	5.9	5.7	5.8	
	2	"	175	25.7	24.7	25.2	
	3	"	190	9.2	9.8	9.5	17.8±6.01*
	4	"	170	31.7	29.7	30.7	
Ileum	1	"	180	20.0	18.6	19.3	
	2	"	175	17.5	16.5	17.0	11.1±4.12*
	3	"	185	5.4	5.0	5.2	
	4	"	175	3.0	2.9	2.9	

* S. E. M.

Percentage absorption of 10 micrograms ferric iron with 5 mg. ascorbic acid injected at various sites in the intestine of 17 rats after bleeding.

Site of injection		Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose 5th day	7th day	Mean	Mean
Duodenum	1	100	205	41.2	43.4	42.3	35.7±2.07*
	2	"	200	36.5	36.7	36.6	
	3	"	185	31.0	29.3	30.1	
	4	"	200	34.1	33.7	33.9	
Mid Jejunum	1	"	205	18.7	22.5	20.6	20.5±1.26*
	2	"	180	20.3	19.9	20.1	
	3	"	210	17.5	17.9	17.7	
	4	"	200	23.4	24.3	23.8	
Jejuno-ileum	1	"	205	12.2	14.2	13.0	15.4±1.80*
	2	"	190	18.9	19.3	19.1	
	3	"	180	10.5	12.7	11.6	
	4	"	205	17.9	17.3	17.6	
Ileum	1	"	190	10.3	11.7	11.0	13.1±0.79*
	2	"	180	17.2	12.1	14.6	
	3	"	205	11.7	13.9	12.8	
	4	"	205	14.2	13.9	14.0	

* S. E. M.

Percentage absorption of 100 micrograms ferric iron with 5 mg. ascorbic acid injected at various sites in the intestine of 16 rats after bleeding.

Site of injection		Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose 5th day	7th day	Mean	Mean
Duodenum	1	1000	175	-	20.5	-	
	2	"	185	-	16.9	-	17.9± 0.87*
	3	"	170	-	16.4	-	
Mid Jejunum	1	"	180	-	25.4	-	
	2	"	185	-	7.6	-	18.6± 5.55*
	3	"	170	-	22.8	-	
Jejuno-ileum	1	"	190	-	11.8	-	
	2	"	170	-	15.3	-	14.7± 1.55*
	3	"	170	-	17.1	-	
Ileum	1	"	175	-	8.5	-	
	2	"	170	-	8.0	-	9.4± 1.19*
	3	"	185	-	11.8	-	

* S.E.M.

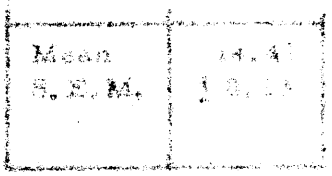
Percentage absorption of 1000 micrograms ferric iron with 5 mg. ascorbic acid injected at various sites in the intestine of 12 rats after bleeding.

Milk Diet for Rats.

Dried milk	90%
Casein	10%

To each 1 Kg were added

Thiamine	5 mg.
Pyridoxine	5 mg.
Pantothenate	50 mg.



Normal	
Rat No.	Hb G%
1	14.6
2	14.8
3	15.2
4	13.8
5	13.4
6	13.9
7	14.7
8	14.3
9	14.1
10	14.9
11	15.5
12	14.1
13	13.8
14	14.2
15	15.0
16	14.5
17	14.3
18	14.3
Mean	14.41
S.E.M.	± 0.13

Milk Diet	
Rat No.	Hb G%
1	10.5
2	11.5
3	10.2
4	10.6
5	11.8
6	11.9
7	12.0
8	12.1
9	11.2
10	11.4
11	11.9
12	10.9
Mean	11.33
S.E.M.	± 0.18

Haemoglobin levels in the blood of normal rats and rats on a milk diet.

Site of injection		Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose 5th day	7th day	Mean	Mean
Duodenum	1	100	190	20.1	23.6	21.8	
	2	"	185	38.1	36.6	37.8	30.1± 3.67*
	3	"	175	35.2	33.8	34.5	
	4	"	180	26.8	25.8	26.3	
Jejuno-ileum	1	"	195	14.9	14.0	14.4	
	2	"	195	29.0	29.8	29.4	31.3± 7.13*
	3	"	190	30.7	34.0	32.3	
	4	"	180	51.6	47.9	49.2	
Ileum	1	"	185	13.4	13.3	13.3	
	2	"	190	22.6	21.4	22.0	16.7± 2.37*
	3	"	180	12.3	12.3	12.3	
	4	"	175	19.2	19.3	19.2	

* S.E.M.

Percentage absorption of 100 micrograms ferric iron with 5 mg. ascorbic acid injected into various sites in the intestine of 12 rats on an iron-deficient milk diet.

Site of injection		Dose of Iron ug	Wt. of Rat gm.	Absorption of Iron % Dose 5th day	7th day	Mean	Mean
Duodenum	1	100	205	27.3	27.9	27.6	
	2	"	200	39.2	39.8	39.5	
	3	"	180	14.9	15.5	15.2	28.7±3.36*
	4	"	185	29.5	23.0	26.2	
	5	"	185	31.6	27.1	29.3	
	6	"	190	34.8	34.1	34.4	
Jejuno-ileum	1	"	195	40.4	41.1	40.7	
	2	"	185	27.7	28.6	28.1	
	3	"	200	10.6	6.5	8.7	19.0±6.49*
	4	"	210	29.2	30.5	29.8	
	5	"	205	2.7	3.7	3.2	
	6	"	180	3.6	3.6	3.6	
Ileum	1	"	185	1.5	1.7	1.6	
	2	"	195	1.9	1.3	1.6	
	3	"	200	2.9	1.6	2.2	2.6±1.12*
	4	"	205	1.1	0.2	0.6	
	5	"	190	2.2	1.7	1.4	
	6	"	180	8.6	8.0	8.3	

* S. E. M.

Percentage absorption of 100 micrograms of ferric iron with 5 mg. ascorbic acid injected at various sites in the intestine in 18 normal rats.

ABSORPTION OF IRON IN MAN.

Patient	Hb G%	P. C. V.	Serum Iron ug%
1	14.7	46	125
2	13.9	43	108
3	15.2	47	140
4	14.2	44	110
5	14.4	44	121

Haemoglobin, packed cell volume and serum iron levels in 5 patients in whom absorption of iron from isolated segments of bowel was studied.