# PROTOCOL FOR STUDIES OF IRON ABSORPTION IN HUMAN VOLUNTEERS

BACKGROUND: For the past 4 years we have been involved with studies on the physiology of iron absorption. Most of the work has been done with animals and much worthwhile information has resulted[1-3]. These studies have given information on the site, rate, and control of iron absorption in the gastrointestinal tract in rats. Similar information is not available for humans. In keeping with our mission to conduct basic and clinical research projects in the areas of nutrition and gastrointestinal physiology we propose to conduct this study in humans.

In order to carry on this investigation we will need to study normal human volunteers. The procedures to be employed have been used by others[4,5] and are accepted as being quite safe. The study does require use of radioisotopes of iron but in amounts well below the maximum permissable body burdens. It is anticipated that the experiments will yield valuable information on the physiology of iron absorption.

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## PLAN OF EXPERIMENT

The Study Involves 2 Parts

### I SITE OF ABSORPTION

#### A. DESCRIPTION

The basis for this part of the study is the method for comparative studies on iron absorption in man using two radioiron isotopes described by Brise and Hallberg[4]. Iron absorption from different compounds, or, different sites in the gastrointestinal tract can be compared in the same individual with this method. The method is based on the fact that $iron^{59}$ and $iron^{55}$ can be assayed separately in the same blood sample. We are currently using this technique successfully[6].

In the proposed study we will give on alternate days, for 6 days, $iron^{55}$ and $iron^{59}$ mixed with an identical carrier of non-radioactive iron. One isotope will be given by mouth, the other by a fine tube placed in the small intestine, specifically to bypass a selected area of gut. Two weeks after the last dose of iron, a blood sample will be taken and the ratio: orally administered isotope - tube administered isotope will be determined. This ratio will reflect the effect, on iron absorption, of bypassing the particular intestinal site selected. Since the absorption of each isotope actually results from tests done on 3 separate days, day to day variation in iron absorption for each individual is greatly reduced. Since each individual is a complete study, the large variation between individuals is eliminated.

B. DETAILED METHOD

Day 1. Routine blood counts, serum iron and unsaturated iron binding capacity are done. A #160 polyethylene tubing with Crosby cup attached is passed. Assure the position of the cup by fluoroscopy or X-ray and then tape it to the skin to secure tubing when position is satisfactory. Flush tubing with saline and air, and seal it with heat to prevent backflow.

Day 2, 4, 6. Either $Fe^{55}$ or $Fe^{59}$ is given orally or by tube. The isotope and the route of administration will be varied to avoid systematic error. Carrier iron - 5 mg of Ferrous iron.

Day 3, 5, 7. Either $Fe^{55}$ or $Fe^{59}$ is given orally or by tube depending on what was given and by what route on Day 1. Carrier iron - 5 mg of Ferrous iron. Tube is removed on Day 7.

Day 21. Fifty (50) ml of blood are taken to determine ratio of orally administered isotope to isotope given through the tube.

C. NUMBER OF SUCCESSFUL STUDIES TO BE DONE.

1 study/person

5 - Bypassing stomach.

5 - Bypassing duodenum.

5 - Bypassing upper 1/2 of small intestine.

5 - Bypassing entire small intestine.

20 volunteers

D. AMOUNT OF ISOTOPE TO BE USED IN EACH EXPERIMENT AND BODY RADIATION DOSE.

Total $Iron^{55}$ - 45 microcuries.

Total $Iron^{59}$ - 15 microcuries.

Using a 5 milligram carrier, only 20 per cent or less of the dose is usually absorbed[7,8]. This will result in a total body dose of

approximately 120 millirads which is within the accepted safe limit[9-13]

Estimated iron$^{55}$ to be absorbed - 9 uc.

Estimated iron$^{59}$ to be absorbed - 3 uc.

## II RATE OF ABSORPTION

### A. DESCRIPTION

Our previous work in rats strongly suggests that iron is absorbed by a regulated process with characteristics compatible with an enzymatic process[3]. With what we regard to be physiological amounts of iron, the absorptive mechanism demonstrates a maximum transport capacity which seems capable of regulating iron absorption. Information of this type should be obtained in humans and this is the second aim of the proposed protocol.

By use of a double lumen tube placed in selected intestinal sites, the kinetics of iron absorption can be studied. The proximal lumen is used for infusion of the test material, and the distal lumen located 30 cm below the proximal is used to siphon fluid from that site. When a non-absorbable, water soluble marker such as polyethylene glycol is used in the infused test material containing iron$^{59}$, changes in concentration of the iron$^{59}$ due to intestinal fluid shifts can be determined. This allows calculation of movement of the iron into absorptive cells of the intestinal site being investigated. When the test material is infused in a known concentration at a known rate, an accurate calculation of iron absorption kinetics per area of intestine can be made. This method has been used by others[14] and is regarded as being safe.

From previous work[2] we know that all iron entering absorptive cells from the intestinal lumen is not transported to blood but a variable portion is ultimately sloughed back into the lumen. Consequently, simply determining disappearance of iron from material infused into the intestinal tract does not accurately describe overall iron absorption. To compensate for this, one must, in addition, determine rate of entry of absorbed iron into blood. If samples of blood are taken during the time of intestinal iron infusion, one can detect entry of radioiron into peripheral plasma. Since the level of radioiron in plasma, at any time, is a resultant of the rate at which iron is entering plasma (inflow) and the rate at which iron is leaving plasma (outflow), the outflow rate must be determined during the absorptive period. This has been done by others[5] and we will use a similar method. It consists of giving intravenously a different iron isotope than the one being infused into the intestine. For these studies $iron^{55}$ bound to the volunteer's plasma will be given. When serial blood samples are taken each isotope is assayed simultaneously in the same sample[6].

Since we want to study absorption from a limited segment of intestine, and the distal tube will not siphon off all the infused iron, some absorption could occur below the site being investigated. In order to prevent this, a third tube, extending slightly beyond the siphon tube, will be used to infuse desferrioxamine. This material combines with and prevents the absorption of iron. Therefore, absorption should be confined to the area being investigated.

B. DETAILED METHOD

1. Subject is fasting.

2. Pass tubing, locate desired site by fluoroscopy or X-ray.

3. Put indwelling needle in right antecubital vein to obtain baseline blood samples for serum iron and unsaturated iron binding capacity.

4. Inject auto plasma labelled with 25 microcuries of $Fe^{55}$.

5. Thirty (30) minutes later begin infusion of iron solution into intestine solution to contain varying amounts of carrier ferrous iron but not to exceed a total dose of 15 microcuries of $iron^{59}$.

6. At same time as #5, begin infusion of desferrioxamine into intestine just distal to site of study.

7. Siphon off samples of intestinal fluid every 15 minutes for determination of $iron^{59}$ and polyethylene glycol concentration.

8. Draw blood samples every 15 minutes for simultaneous determination of $iron^{55}$ and $iron^{59}$, serum iron and unsaturated iron binding capacity. Begin sampling after #4.

9. Discontinue study after 2 hours.

C. NUMBER OF SUCCESSFUL STUDIES TO BE DONE - 10

D. AMOUNT OF ISOTOPE TO BE USED -

Total $Iron^{55}$ - 25 microcuries I.V.

Total $Iron^{59}$ - Not in excess of 15 microcuries.

Since the $iron^{59}$ will be given into the intestine, it is estimated that not more than 20 per cent will be absorbed (3 uc). This will result in a total body dose of 245 millirads, which is within the accepted safe limit[9-13].

For both Part I and Part II only males over 20 years of age will be studied and, only one study will be done on any one subject.

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