ETRICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. A.N. Alam

Trainee Investigator (if any)

Supporting Agency (if Non-ICDDR,B)

Project status:
- New Study
- Continuation with change
- No change, do not fill out rest of form

Source of Population:
- I ill subjects
- Non-ill subjects
- Minors or persons under guardianship

Does the study involve:
- Physical risks to the subjects
- Social risks
- Psychological risks to subjects
- Discomfort to subjects
- Invasion of privacy

Are the study involve:
- Use of records, (hospital, medical, death, birth or other)
- Use of fetal tissue or abortus
- Use of organs or body fluids

Are subjects clearly informed about:
- Nature and purposes of study
- Procedures to be followed, including alternatives used
- Physical risks
- Sensitive questions
- Benefits to be derived
- Right to refuse to participate or withdraw from study
- Confidential handling of data
- Compensation &/or treatment where there are risks or privacy is involved in any particular procedure

Will signed consent form be required:
- From subjects
- From parent or guardian

Will precautions be taken to protect anonymity of subjects

Check documents being submitted herewith to Committee:
- Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies)
- Protocol (Required)
- Abstract/Synopsis (Required)
- Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or with drawn (Required)
- Informed consent form for subjects
- Informed consent form for parent or guardian
- Procedure for maintaining confidentiality
- Questionnaire or interview schedule

If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

Agree to obtain approval of the Ethical Review Committee for any changes giving the rights and welfare of subjects before making such change.

Principal Investigator

Trainee
SECTION 1 - RESEARCH PROTOCOL
(Pilot protocol)

1. TITLE: BIOAVAILABILITY OF IRON FROM BANGLADESHI MEALS.

2. PRINCIPAL INVESTIGATOR: Dr. A.N. Alam

   CO-INVESTIGATORS:
   Mr. M.A. Wahed
   Dr. M.A. Rashid
   Professor of Haematology, IPGM&R.
   Dr. M.M. Rahaman

   CONSULTANT:
   Dr. J.D. Cook
   (International Center for Control of Nutritional Anaemia, Kansas University Medical Center, Kansas, U.S.A.).

3. STARTING DATE: August 1985

4. COMPLETION DATE: January 1986

5. TOTAL INCREMENTAL COST: US $ 1,550.00

6. SCIENTIFIC PROGRAMME: This protocol has been approved by the Nutrition Working Group.

Signature of Scientific Programme Head: __________________
Date: 28/7/1985
7. **ABSTRACT SUMMARY:**

Iron deficiency anaemia is a major public health problem in both Bangladesh and elsewhere although diet in most countries contains large amount of iron. The present study proposes to determine the bioavailability of iron from different types of Bangladeshi meals, tagged extrinsically with a small amount of radioactive iron, in ten healthy adult males and five anaemic adult male subjects. This will help in suggesting ways to improve iron nutrition in our population.

8. **REVIEWS:**

(a) Research involving human subjects: -------------------------------

(b) Research Review Committee: -------------------------------------

(c) Director: --------------------------------------------------------
SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objectives:

The aim of this study is to determine the bioavailability of iron from whole Bangladeshi diet.

2. Background.

Most nutritional anaemias in a population are due to iron deficiency\textsuperscript{1}. This is attributed to a marginal intake of iron or more importantly to its low bioavailability. Dietary iron as it relates to iron uptake by the intestinal mucosal cell is divided into two distinct compartments: nonhaeme and haeme iron. Nonhaeme iron derived from cereals, fruits, vegetables etc. constitute the larger component of dietary iron. Assimilation of this form of iron is determined largely by the extent to which it remains soluble as reduced ionic iron within the upper intestinal lumen. Because foods differ substantially in their content of factors that promote or inhibit iron solubility, the absorption of nonhaeme iron is greatly influenced by the nature of the meal\textsuperscript{2}. In contrast, haeme iron derived from haemoglobin and myoglobin gains entry to the mucosal cell as an intact iron porphyrin complex and is relatively independent of other components of the meal. It forms a relatively minor part of total iron intake. Even in Western diets with high meat content, haeme iron usually accounts for only 10 to 15 per cent of the total daily iron intake.
In most developing countries, dietary iron intake may range from 15 to 30 mg\(^3,4\). However, people eat less of meat and fish, because of economic restraints. Although absorption of non-haeme iron is greatly affected by the iron status of the subject, this lack of dietary haeme iron could partly account for iron deficiency anaemia. The latter results in reduced work performance and thereby impose economic limitations on people whose livelihood depends on physical work. The main source of dietary iron in rural Bangladesh is from rice and only 1 to 2% of the iron in rice has been shown to be absorbed by normal subjects\(^5\). Dietary fibre with a modest inhibitory effect on iron assimilation \(^6\) may partly contribute to low availability from Bangladeshi diet. Associated hookworm infestation may also create additional requirements for iron \(^7\). Iron absorption in the adult amounts to about 0.5-2 mg/day (equalling the daily loss) and this occurs in the small intestine (largely duodenum), apparently in the ferrous state. Iron may combine with intracellular storage proteins called ferritin and mucosal transferrin in the intestinal mucosal cells before being absorbed.

National Nutrition Surveys conducted in 1975-76 \(^8\) and 1981-82 \(^9\) revealed a high prevalence of anaemia (7 out of every 10 people are anaemic) in Bangladesh. A recent study \(^10\) conducted in a rural area has shown anaemia to be present in about 35 to 50% of the population depending on age and sex groupings as classified by WHO and majority of them are iron deficient as determined by serum ferritin estimation. Low bioavailability of dietary iron may be responsible for the high prevalence of iron deficiency anaemia in this region. This, in turn, may decrease the work capacity of our economically underprivileged population.
An important recent advance in studies of food iron assimilation was the demonstration that iron absorption can be measured by simply adding a small amount of inorganic radioiron with the administered food (extrinsic tag) rather than biosynthetically incorporating the label. It was possible to show that a common pool of nonheme or haeme iron in formed in the intestinal lumen by several foods ingested in the same meal and that absorption from this pool can like-wise be measured by extrinsic tagging. Thus food iron absorption depends not on the food source but on the composite effect of factors in a meal that either block or promote iron availability, largely by affecting its solubility.

It is usually unnecessary to measure the absorption of haeme iron in Third World countries because it constitutes only a small portion of dietary iron and is little affected by the composition of the diet. Non-haeme iron absorption measurements are therefore usually sufficient specially in nutritional studies. The effect of subject-to-subject differences in iron status can be eliminated by performing as many as four separate absorption measurements using dual radioisotopes in the same individual. This will also allow comparisons to be made between different meals. Moreover, the inclusion of a radioiron reference dose (containing 3 mg ferrous iron, which represents the final common pathway by which all forms of dietary iron with the exception of haemoglobin are assimilated, and 30 mg ascorbic acid) measurements to characterize iron status of a subject is particularly important when evaluating iron availability from regional diets.
By measuring absorption in each subject both from meals and the reference dose, the results can be expressed as a regression line between the two absorption measurements. A difference in iron absorption between two meals can then be assessed by comparing the slopes of the regression lines relating food iron absorption to absorption of the reference dose. This implies that the ratio or mean absorption from a meal and from the reference dose is a measure of relative bioavailability of non-haeme iron in the meal. Because of the highly skewed distribution of percentage absorption, analysis is performed on the logarithmic scale using geometric mean 14.

B. **SPECIFIC AIMS:**

Inspite of relatively large intake of iron in the diet, a high incidence of iron deficiency anaemia has been observed in Bangladesh. The present study will aim at evaluating the possible inhibitory role of locally available diet in causing anaemia in the population.
C. METHODS AND PROCEDURE:

Ten apparently healthy iron-replete adult males and five anaemic subjects (iron deficiency will be confirmed by serum ferritin determination prior to selection for the study) between 18 - 50 years of age and without history of G.I. disorders, acute or chronic infection, or hematologic disease will be selected for the study. Informed consent will be obtained in every case explaining the risk and discomfort involved in the study. To avoid any imprecision when determining absorption from a single test meal, comparison will be made between absorption of iron from four separate test meals. Five ml of venous blood will be taken from the study subjects on the previous day for background radioactivity and conventional hematologic measurements, such as, haematocrit serum iron, TIBC and serum ferritin. Height and weight will also be taken. After an overnight fast, they will be given test meals (according to the meal plan) on two successive mornings tagged with either $^{59}$Fe or $^{55}$Fe. The subjects will be allowed to resume their normal diet 3 hours after the test meal. Absorption from meals tagged extrinsically with ferric chloride will be determined from the radioactivity (circulating red cell radioactivity) incorporated into blood obtained 14 days after the administration of the test meals. A further two absorption tests will be performed using $^{59}$Fe or $^{55}$Fe in the test meals offered on day 15 and 16 after overnight fast. Absorption of these test meals will be determined by measuring the rise in radioactivity in blood sample obtained on day 30.
The test meals will be as follows:

### Meal Plan:

<table>
<thead>
<tr>
<th>Day - 1</th>
<th>Day - 2</th>
<th>Day - 15</th>
<th>Day - 16</th>
</tr>
</thead>
</table>
| 200 gm rice  
100 gm lentils  
35-40 gm vegetables (potatoes, cauliflower, and green bean, carrots)  
Water ad libitum to drink | 200 gm wheat chapatti and 300-40 gm vegetables as on day 1  
Water ad libitum to drink | 250 g samilina  
120 ml whole milk  
24 g sugar  
14 g butter | Reference dose:  
3 mg FeSO$_4$ 7H$_2$O is dissolved in 1 ml 0.01 N HCl for each volunteer. The isotope is then added is to the solution.  
18.9 mg ascorbic acid is dissolved in 50 ml water |

+ 1.5 μci $^{55}$FeCl$_3$  
+ 0.5 μci $^{59}$FeCl$_3$ with 0.1 mg carrier FeCl$_3$ | + 1.5 μci $^{55}$FeCl$_3$ with 3 mg carrier FeCl$_3$ | + 0.5 μci $^{59}$FeCl$_3$ | One ml of isotope solution will be added to ascorbic acid solution prior to administration to the subject. |

Radioiron will be added to one of the food items immediately before it is offered to the subject.  
Will be given as a drink.

The total dose of radioactivity administered to each subject will be 3 μci $^{55}$Fe and 1 μci $^{59}$Fe and a total of 15 ml of venous blood will be drawn from each subject. Assuming a maximum mean absorption of 10 percent of the administered test dose, the above radiation doses will be less than one percent of the maximal permissible doses for occupationally exposed persons.
Calculation of iron absorption from incorporated blood radioactivity requires (i) an estimate of total blood volume from height and weight using the following formula:

\[ B.V. = 28.5 \times \text{Ht (in cm)} + 31.6 \times \text{Wt (in kg)} - 2820 \]

and an (ii) estimate of the proportion of absorbed radioactivity incorporated into circulating red cells (normally 80-90%).

Measurement of blood radioactivity will be done at the Hematology Research Laboratory, Kansas University Medical Center, USA. In principle, the samples of whole blood are pipetted into Kjeldahl flasks and are then prepared by wet digestion for liquid scintillation counting using the method of Eakins and Brown. For each administered dose of radio-iron absorption is calculated as follows:

\[ \text{Absorbed counts (X)} = \text{CPM blood} \times \text{blood volume} \]
\[ \text{Administered counts (X)} = \text{CPM standard} \times 0.500 \]
\[ \text{Absorption (\%)} = \frac{\text{Absorbed counts (X)}}{\text{Administered counts (X)}} = 100 \times F \]

where 'F' is the factor to adjust for incomplete incorporation of absorbed radioactivity into circulating whole blood.

D. COLLABORATIVE ARRANGEMENTS:

This will be part of a multinational study on iron absorption to be done in collaboration with the International Centre for Control of Nutritional Anaemia (ICCNA) at the University of Kansas Medical Center. Iron absorption study was carried out in healthy American volunteers using Bangladeshi diet as the first part of the collaborative study.
E. SIGNIFICANCE:

The proposed study will help us in establishing ways to improve iron nutrition either by institution of remedial measures such as, supplementation, fortification or by adding substances to the diet that would render the iron more available for absorption.
REFERENCES:


10. Alam AN et al. Unpublished observation


## SECTION III - BUDGET

### 1. Personnel services:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>% effort</th>
<th>Taka</th>
<th>Dollar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. A.N. Alam</td>
<td>Principal Investigator</td>
<td>20%</td>
<td>144,000</td>
<td>5760.00</td>
</tr>
<tr>
<td>Mr. M.A. Wahed</td>
<td>Co-Investigator</td>
<td>10%</td>
<td>36,000</td>
<td>1440.00</td>
</tr>
<tr>
<td>Dr. M.A. Rashid (Prof. of Haematology, IFGM&amp;R)</td>
<td>do</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr. M.M. Rahaman</td>
<td>do</td>
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<td></td>
</tr>
<tr>
<td>Dr. J.D. Cook</td>
<td>Consultant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sub Total = US $ 7,200.00

### 2. Supplies and materials

- $^{55}$Fe Cl$_3$
- $^{59}$Fe Cl$_3$

Source: Amersham, U.K.

Biochemical tests:
- Ferritin
- Serum iron
- TIBC
- Haematological test: HCT

### 3. Equipment
- nil

### 4. ICDDR,B transport
- nil

### 5. Patient hospitalisation 5 X 6 X 200
- 6,000 240.00

### 6. Outpatient care
- nil

### 7. Transportation of patients and things
- 280.00

### 8. Travel
- nil

### 9. Rent, communication, utilities
- nil

### 10. Printing and publication
- nil

### 11. Other contractual services
- nil

### 12. Construction
- nil

GRAND TOTAL = $ 8383.00

Incremental cost = $ 1183.00

31% overhead cost = $ 367.00

TOTAL, US $ = 1550.00
ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

1. Two groups of adult male subjects, ten apparently health volunteers and five anaemic subjects will be studied. Healthy volunteers will be selected from amongst ICDDR,B employees and five anaemic subjects will be selected from patients reporting to IPGM&;R.

2. Blood will be obtained by venepuncture which may cause little discomfort. Small amount (total of 4 μci) of radioisotopes in the form of ferric chloride will be offered with food. This amount is far less than the recommended dose for human experimentation (a copy of relevant certificate is enclosed).

3. All possible care will be taken and trained personnel will draw venous blood to cause minimal pain.

4. Only hospital identification numbers of the study patient will be used.

5. Informed written consent will be obtained from study subjects.

6. The study will involve obtaining information about clinical history only and it will take less than 5 minutes to take the interview of either the volunteer subject or the patient.

7. The study patients will be treated adequately for anaemia at the IPGM&;R.

8. This protocol requires the use of hospital records and body fluid.
This is to certify that the use of 3 microcurie radioiron – 59 and 1 microcurie of radioiron – 55 for iron absorption studies in human being is much below the maximum permissible level and is within safety limit recommended by I.C.R.P.

( Kamaluddin Ahmed )
Director.
CONSENT FORM

A STUDY OF FOOD IRON BIOAVAILABILITY

There is high incidence of iron deficiency anaemia in Bangladesh in spite of abundance of iron in the Bangladeshi diet. It may be due to low bioavailability of iron from those diets. ICDDR,B in collaboration with Kansas Medical Center, USA has undertaken a study to investigate the causes of such low bioavailability in healthy and anaemia adult males. This study is expected to reveal useful information to initiate improvement of iron nutrition in the population either by iron supplementation to the diet or possibly by adding substances to the diet that should render the iron more available for absorption. We would like you to participate in the study.

You will be offered diets after overnight fast according to the following schedule:

First phase (on two successive days):
Day - 1. Rice, lentils, vegetables The meals will contain isotopes of iron (1.5 μci and 0.5 μci respectively).
Day - 2. Chapati and vegetables

Second phase (on two successive days)
After 14 days of 1st phase, the following diet with isotopes of iron will be offered:
Day - 3. Samolina, milk, sugar with 1.5 μci $^{55}$FeCl$_3$
Day - 4. Ascorbic acid with 0.5 μci $^{59}$FeCl$_3$

For laboratory examinations 5 ml of venous blood before first phase and at the end of each phases will be drawn. This amount of blood (a total of 15 ml) drawn and the dose of isotope will not cause any problem to you.

You may not have to stay in the hospital. But we will like you to come to the hospital in the morning after overnight fast for required investigations. We will provide you with proper treatment if anaemia is found. To protect your personal social status, we will maintain the confidentiality of data.

If you agree to participate in the study, please put your signature or thumb impression below. You have every right to ask any question about the study. Moreover, you may withdraw your consent at any time during the study.

Signature of the investigator

Signature/Left thumb impression of the volunteer.

Date: --------------------------
লেখা নিবন্ধ

বাংলাদেশ সরকারের নৌক্রিগত পদ্ধতি প্রচুর সত্যা সৃষ্টিতে নৌক্রিগত অভাব বেহুল রওঁ শুন্যতা বিরাজ করে। আমেরিকার ক্যাম্পাস মেডিকেল সেন্টারের সর্বোচ্চ আই, রিপল্স, মাইক্রোসকোপের চেয়ে এই গবেষণার পরিকল্পনা করেছে। এই গবেষণায় মূলধন সরকার এবং রওঁ শুন্যতা উপহার এর পরিকল্পনা অংশ গবেষণা আন্তর্জাতিক। এই গবেষণা নিবন্ধিতকরণের দায়ের রওঁ শুন্যতা উপহারের অভাব ব্যবস্থা 

নির্ধারণের শিষ্যভাবে সাহায্য করে। 

গবেষণায় অংশ হিসাবে নিরূপিত মোতাবেক আগ্রহে বার দেওয়া হবে।

প্রথম পর্যায়:

শেষে ০.৫ অথবা ১.৫ মাইক্রোকিউবি 
লেখ আইনোটেন দেওয়া হবে।

দ্বিতীয় পর্যায়:

গবেষণার পর্যায়ের ১৫ দিন পর আবার পাঠিয়ে থেকে পর পর দুটি পাঠার ২ সাই- ক্রকিউইফি আইনোটেন দেওয়া হবে। পরিকল্পনা হয় গবেষণার ৫ সি, সি, রুঁ এবং উই পর্যায়ে আরও ১০ সি, সি, পরিমাণ রুঁ নেওয়া হবে। তাতে আবার পরিচয়ের কোন রক্ত হবে না।

আবার হাসপাতালে অবশয়ে কোন বাধ্যবাধ্য না হয়। গবেষণা চলাকালে আবার পরিচয়ের থাকা সত্যিকর। এখানে নেওয়া হবে। গবেষণার তথ্যদির গোষ্ঠীর অবশয় রক্ত করা হবে যাতে করে আবার ব্যবস্থার কোন ভাবে কুন্ত না হয়।

এখানে উল্লেখ করা যাকে, আবার গবেষণা পদক্ষেপ যে কোন বিষয় জানার এবং গবেষণা থেকে যে কোন সময়ে সন্ত্র ভ্রয়বাসার অধিকার রাখে।

আবার এই গবেষণায় অংশ গ্রহণ কালীর যাবতে নিজে সৃষ্টি/সমাধি করব।

______________________________
গবেষনারকারীর শ্রদ্ধার

______________________________
গবেষনায় অংশ গ্রহণ কালীর সৃষ্টি/সমাধি

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tারিখ:

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tারিখ: