chment 1.
EE SHEET)

Date July 16, 1990

ETHICAL REVIEW COMMITTEE, ICDDR,B.

cipal Investigators: DR.K.Jahan & Mr.S.Barua

Trainee Investigator (if any) _____

ication No: PCC/07/90

Supporting Agency (if Non-ICDDR,B) PCC

e of Study DEVELOPMENT OF A HODOLOGY FOR ASSESSMENT OF LIVER AMIN-A STORAGE.

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

No Man human subjects are involved

le the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
(a) Ill subjects — Yes (No)
(b) Non-ill subjects — Yes (No)
(c) Minors or persons under guardianship — Yes (No)

Does the study involve:
(a) Physical risks to the subjects — Yes (No)
(b) Social Risks — Yes (No)
(c) Psychological risks to subjects — Yes (No)
(d) Discomfort to subjects — Yes (No)
(e) Invasion of privacy — Yes (No)
(f) Disclosure of information damaging to subject or others — Yes No

Does the study involve:
(a) Use of records, (hospital, medical, death, birth or other) — Yes (No)
(b) Use of fetal tissue or abortus — Yes (No)
(c) Use of organs or body fluids — Yes (No)

re subjects clearly informed about:
(a) Nature and purposes of study — Yes No
(b) Procedures to be followed including alternatives used — Yes No
(c) Physical risks — Yes No
(d) Sensitive questions — Yes No
(e) Benefits to be derived — Yes No
(f) Right to refuse to participate or to withdraw from study — Yes No
(g) Confidential handling of data — Yes No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes No

5. Will signed consent form be required:
   (a) From subjects — Yes No
   (b) From parent or guardian (if subjects are minors) — Yes No
6. Will precautions be taken to protect anonymity of subjects — Yes No
7. 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 Summary (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 withdraw (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.

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

Sajarmay Barua

Principal Investigator

Trainee

SECTION - I

1. Title : Development of a methodology for assessment of liver Vitamin-A storage.

2. Principal Investigator(s) : Dr. K. Jahan, INFS, DU
Mr. S. Barua, INFS, DU

3. Co-Investigator(s) : Mr. M. A. Wahed, ICDDR,B
: Dr. K. Ahmad, BIHM

4. Starting date : September 1990

5. Completion date : June 1991

6. Total direct cost :

7. Recommendations :

17/7/90
Director, INFS, DU

Associate Director, LSD
ICDDR,B

Research Director, BIHM
17.7.90

Collaborating institutions: (a) Institute of Nutrition & Food Science(INFS), Dhaka University

(b) Bangladesh Institute of Herbal Medicine(BIHM), Dhaka

(c) International Centre for Diarrhoeal Disease Research, Bangladesh(ICDDR,B)

8. Abstract Summary : (250 words or less)

Liver Vitamin A Storage is the true index of Vitamin A status in a population group, since serum vitamin A level tends to be within normal range until the liver storage is largely depleted.

Thus population at risk with respect to vitamin A nutriture can only be identified through the determination of liver vitamin A storage.

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In absence of a simple method for the determination of liver vitamin A storage suitable for field condition, the present animal study aims at establishing a simple device for the estimation of liver Vitamin A storage. As hemoglobin level is dependent upon serum vitamin A which, in turn, is dependent upon liver vitamin A storage upto a considerable range, attempts will be made to find if /rise in hemoglobin level/ with administration of additional Vit.A could be adopted to the determination of liver vitamin A storage. Animals will be made deficient in vitamin A. Vitamin A deficient rats in five groups will be repleted with five varying doses of vitamin A for a period of two weeks. After that, hemoglobin level of all rats belonging to all groups will be determined by amputating the tailtip. Then representative number of rats from each group will be sacrificed. Serum Vitamin A liver storage/ serum vitamin A and liver iron will be determined. Rest of the animals belonging to each group will be provided with a single dose of Vitamin A (60 µg). After two weeks, rise in hemoglobin level in each group will be determined by collecting blood from tailtip. After that all rats will be sacrificed and liver vitamin A, liver iron and Serum vitamin A will be determined. Relationship between rise in hemoglobin level and liver vitamin A storage of respective groups of rats will be determined.

9. Reviews :

i) Scientific Review Committee(SRC) of PCC _____ (Approved/Not approved)

ii) Ethical Review Committee(ERC) of ICDDR,B _____ (Approved/Not approved)

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SECTION II : RESEARCH PLAN

A. INTRODUCTION

1. Objectives : There is no satisfactory method for estimating liver vitamin A storage in human population. Recently however, a method has been suggested by Barbara Underwood that in absence of vitamin A iron arrested in its depot, resulting in poor supply of iron for hemoglobin-synthesis in erythroid cells. In another experiment (23) we also observed varying levels of hemoglobin in rats induced to varying degrees of vitamin A nutriture. Hemoglobin levels of (10.43 $\pm$ 0.76), (12.57 $\pm$ 0.13) and (14.06 $\pm$ 1.07) g/d corresponded to serum vitamin A levels of 20 μg, $<$20-30 μg and $>$ 30 μg per dl respectively. Since serum or plasma vitamin A is a function of liver vitamin A Storage (28-29), there is every reason to support that hemoglobin level might have a good association with liver - vitamin A storage.

2. Rationale : Vitamin A deficiency continues to be one of the major nutritional catastrophies world wide. Not only developing nations but also developed nations are afflicted with the problem. The number of children with nutritional blindness throughout the world is more than 10 million. In Bangladesh about 30,000 children go blind every year due to vitamin A deficiency and the number suffering from undefined consequences of vitamin A deficiency may be many more. Average Bangladeshi diet caters only 35 percent of recommended amount of vitamin A. In this context, a correct evaluation of vitamin A status amongst our population is all the more important for any

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remedial action programme. Since traditional methods for the determination of vitamin A status such as the estimation of serum or plasma vitamin A is not suitable for field study, elucidation of a simpler device for the purpose would go a long way to the solution of the problem, not only in our country but also in other developing countries of the world.

3. Specific Aims : To devise a simple method for the determination of liver vitamin A storage in a population group, based on the determination of hemoglobin level.

4. Methods and Procedure : Post weaning rats of Long-Evans strain will be used in the experiment. Animals will be made deficient in vitamin A by putting them on vitamin A free synthetic diet. Vitamin A deficient diet will contain all other hematopoietic nutrients such as iron, copper and vitamin B_{12} inadequate amount. After the rats are made deficient in vitamin A, rats will be divided on a random basis into five groups- Gr.A, Gr.B, GR.C, GR.D and Gr.E; and will be provided respectively with 10%, 25%, 50%, 75% and 100% of recommended amount of vitamin A. Normal dose of vitamin A approximates to be retinol equivalent per rat per day. Vitamin A will be administered by intubation. During repletion period rats of all groups will be provided with Vitamin A deficient based diets similar in all respects except Vitamin A. After a repletion period of 2 weeks, hemoglobin levels of all rats belonging to all groups will be determined by collecting blood from tail tip. After that, representative number of rats /selected on random basis/ from all groups will be sacrificed by decapitation. Serum vitamin A, liver-vitamin A storage and liver-iron also will be determined. This will be done as a base line study for the 2nd phase of the experiment. Rest of the animals belonging to

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each group will be provided with a single dose of vitamin A (60 µg). After two weeks of this dose, hemoglobin will be determined by collecting blood from tailtip. After that, all rats will be sacrificed by decapitation and circulating vitamin A (Serum Vitamin A), liver vitamin A storage and liver iron will be determined. During the determination of liver vitamin A-storage and hemoglobin, the technicians will be blinded to the diets the rats were provided with. Relationship between rise in hemoglobin levels following the single dose of vitamin A and the liver vitamin A storage of the respective group of rats will be studied thereafter.

Liver vitamin A (29) and serum vitamin A (30) will be determined by HPLC. Hemoglobin level will be determined by cyanme-hemoglobin method (31), liver iron will be determined colorimetrically by using bathophenenthroline as chromogenic reagent (32).

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PART III - DETAILED BUDGET

A. Personnel	Percent month	Salary/ honorarium per month	Total amount (In taka)
Dr. K. Jahan, PI	30%	Tk.3,000	Tk. 30,000
Mr. S. Barua, PI	30%	Tk.3,000	Tk. 30,000
Research Assistant	100%	Tk.3,500	Tk. 35,000
Lab. Attendant	100%	Tk.2,000	Tk. 20,000
Mr. M. A. Wahed, Co-Investigator	5%	-	-
Dr. K. Ahmad, BIHM, Consultant	10%	-	-
B. Equipments			Tk.1,50,000
C. Chemicals			Tk. 50,000
D. Transportation			Tk. 5,000
E. Animal, Animal feed and cages			Tk.1,00,000
F. Stationary and printing			Tk. 15,000
G. Contingency			Tk. 10,000
		Total	: Tk.3,30,000

Approximately US$ 9,500/-