AVAILABILITY OF FOLIC ACID FROM SELECTED GERMINATED CEREALS AND PULSES

By

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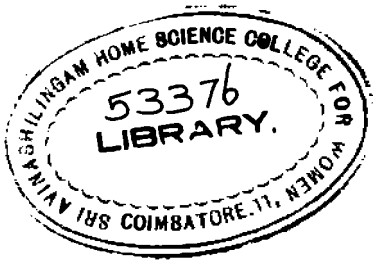

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I INTRODUCTION

Health is man's most precious possession, it influences all of his activities, it shapes the destinies of nations (Sopalan, 1979).

Nutrition is central to good health, it is in fact fundamental to all phases of human development and good food is the basis for good nutrition (WHO 1978, and Gomezatma, 1979).

Malnutrition emerges as a product of chronic *in*-sufficiency of food-both quantitatively and qualitatively, superimposed upon this is the ignorance of the value of certain foods for the maintainence of good health, (Devadas, 1979 and Mahler, 1979). Apart from the much talked about energy protein malnutrition, nutritional anemia, vitamin A deficiency and B-complex deficiencies exist as burning nutritional problems of India (Indian Council of Medical Research (ICMR), 1978). It is ironic that these diseases together take the toll of practically all age groups in India.

Anaemia though not fatal, results in reduced work efficiency, lethargy, sluggishness often mistaken for laziness and increased susceptibility to infection with low birth weight in the new born. It results from the inability of the erythropoietic tissue to maintain a normal haemoglobin concentration on account of an inadequacy , or lack of iron, folic acid, vitamin B12, proteins and ascorbic acid (Heaton, et al 1976 and WHO, 1978).

In India about 47 per cent of the pregnant women in general are anaemic, 40 per cent of these, suffer from folate deficiency (Gopalan, 1977). Every alternate woman in the third trimester of pregnancy and 50 per cent of children below the age of five years are anaemic (Gopalan, 1977 and ICMR, 1978).

Deficiency in the intake of foods like yeast, liver, fresh green vegetables, pulses as dals and in the whole form and sprouted cereals is one of the major causes ultimately resulting in folate deficiency anaemia. In an average Indian diet which is predominant in cereals, pulses following next (Pamikar, 1974 and Devadas, 1970, 1971, 1974, 1979), it would be very commendable if proper processing of these foods could enhance the intake of folate.

The commonly adopted methods of processing foods such as cooking, heating, roasting and dehydration, improve the palatibility and digestibility but result in considerable losses of nutrients particularly of the B-complex vitamins and ascorbic acid. Germination, on the other hand, is also a commonly employed method of processing and (Babu, 1976), has been reported to modify some vital constituents of the seed, such as the conversion of starch to simpler carbohydrates, fats to fatty acids, proteins to peptides and some acids, bound vitamins and minerals to their free form, increase in tocopherols and significant synthesis of many B-complex vitamins and ascorbic acid, (Bhagwat and Rao, 1942; Patwardhan et al. 1942; Chattopadhyay and Banerjee, 1953; Dhami, 1964; Aykroyd and Doughty, 1964; Jaya et al. 1975, and Rajalakshmi, 1976). Babu (1976) has reported significant increases in folate levels on germination.

Inspite of several such reports on the desirable changes during germination, very few comprehensive studies have been carried out to estimate the beneficial effects of these to the human system. The present investigation is an effort in this direction, with an intention of estimating the folate available from germinated cereals and pulses on college going adolescent girls. Attempt has also been made to throw light on the changes that take place in the concentration of folate in foods during germination. Since good nutrition is not always synonymous with the expensive items of food, the utilisation of locally available inexpensive foods has been considered for exploration in this study, as stressed by the WHO (1976) and Devadas et al. (1979). In the present study, ragi (Eleusine coracana), a cereal preferred by the poor people in Tamil Nadu for its cheapness and sustaining qualities (Pare and Nagar, 1976) and bajra (Pennisetum typhoideum), yet another nourishing cereal designated as the poor man's bread (Kurian et al. 1961; Desai and Zende, 1979) have been investigated for their folate availability.

The pulses chosen were Bengal gram (Cicer arietinum) and green gram (Phaseolus aureus Roxb), both commonly consumed in South India (Radhaswaran, 1969) and the germinated forms of which are popular (Protein Foods Association of India, 1973 Chandrasekar et al. 1978). It is hopeful that a thorough exploration of this subject would be invaluable in improving the dietaries of poor people and reducing the occurrence of nutritional anemia in India.

II REVIEW OF LITERATURE

The literature pertaining to the present study on "The availability of folic acid from selected germinated cereals and pulses," is reviewed under the following headings:

A. Chemistry of folic acid

B. Role of folic acid in human nutrition

C. Folate deficiency and the contribution of dietary sources towards its prevention and

D. Assessment of folate status and the availability from foods.

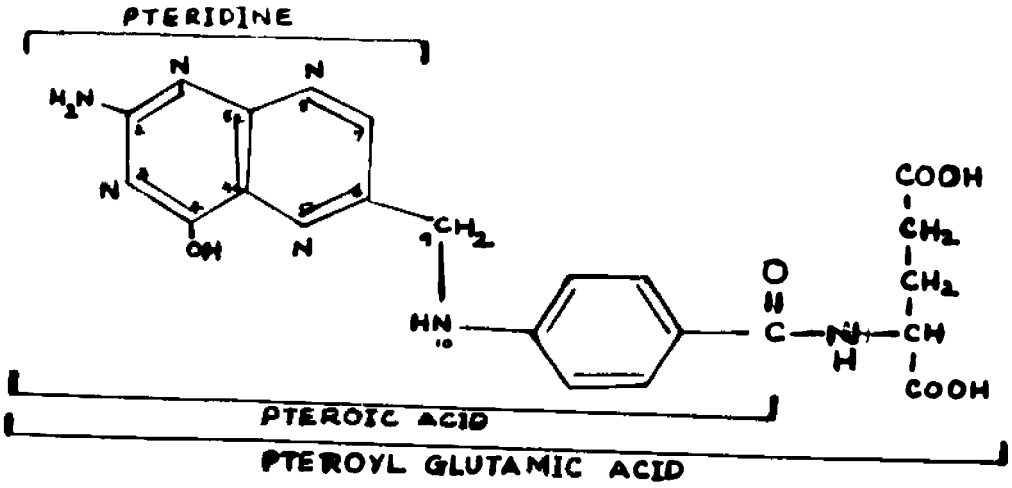
A. Chemistry of folic acid:

1. Structure:

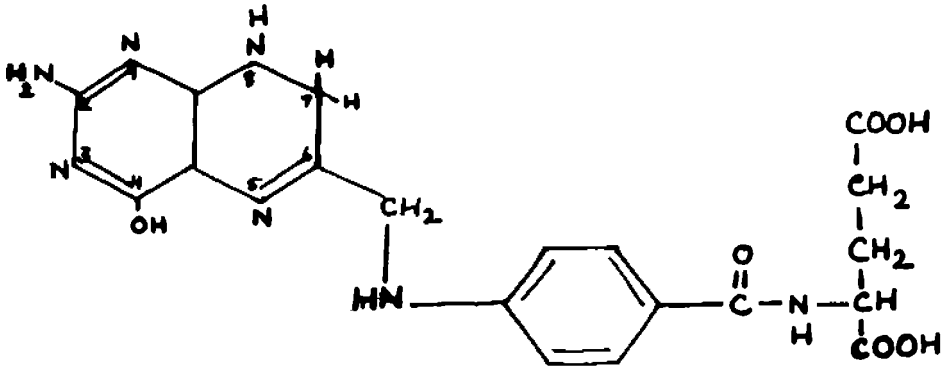
Folic acid is a pteridine derivative, linked through a methylene bridge to a molecule of para-amino benzoic acid. Folic acid becomes active only on reduction.

A very large number of folates occur and do occur in nature and in the cell; they are present in very low concentrations (Nanomoles/g.) Figure I presents the compounds with folate activity and Figure 2 reveals the chemical structure of folates.

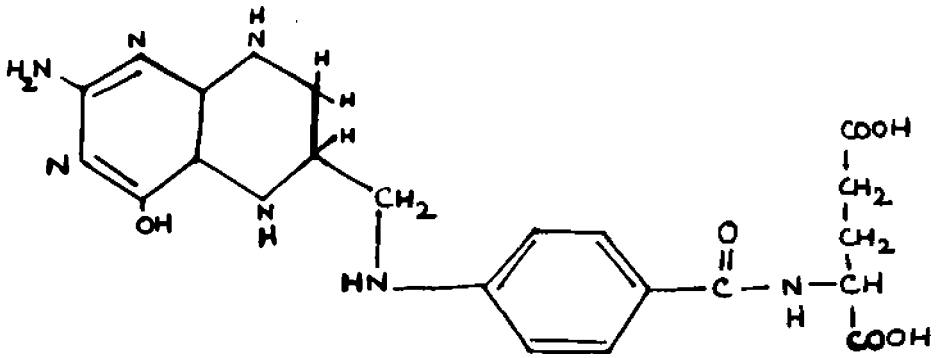
Some of the assay organisms can utilise petroyl polyglutamates having more than three glutamyl residues. To detect these higher forms, it is necessary to degrade them by digestion with enzymes called "conjugases". Conjugases are ubiquitous peptides that degrade the poly-γ-glutamyl chain completely or to a pteroyl-di-glutamate.



FOLIC ACID



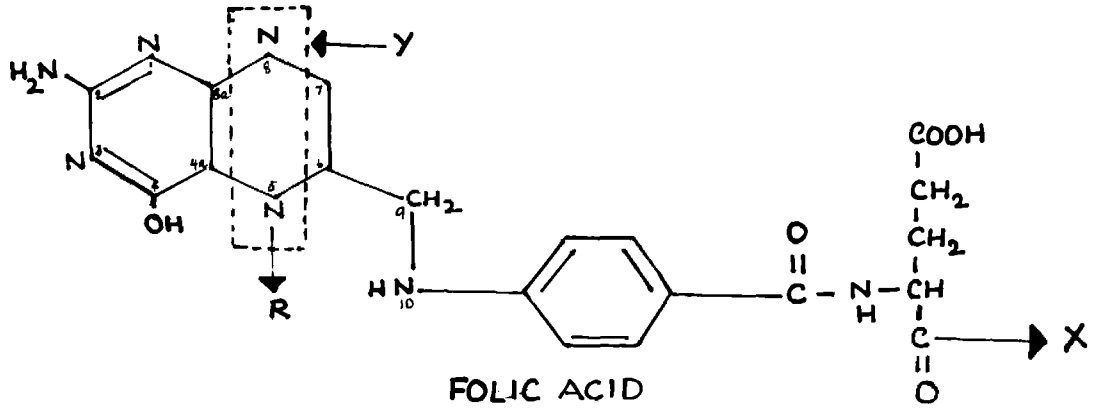
7,8 DIHYDRO FOLIC ACID



5,6,7,8 TETRAHYDRO FOLIC ACID

FIGURE 1

COMPOUNDS WITH FOLATE ACTIVITY.

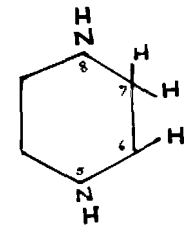


FOLIC ACID

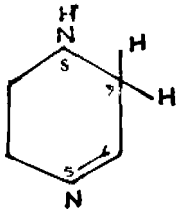
ATTACHMENT AT R

$-CHO$	at N^5 or N^{10}	FORMYL
$HC-$ (=)	at N^5 - N^{10}	METHENYL OR METHYLIDENE
$HC=NH$	at N^5	FORMIMINO
H_2C	at N^5 - N^{10}	METHYLENE
CH_3	at N^5	METHYL

ATTACHMENT AT Y

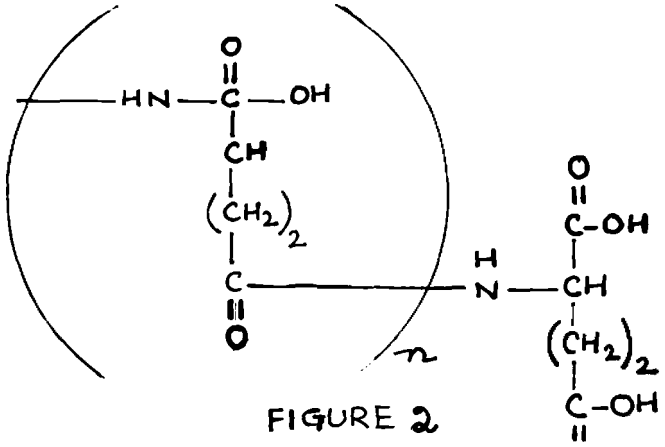


5,6,7,8 TETRAHYDRO FOLIC ACID



7,8 DIHYDRO FOLIC ACID

ATTACHMENT AT X



(n = 0 to 7 or more)

FIGURE 2

CHEMICAL STRUCTURE OF FOLATES.

2. Folic acid conjugase:

Chaldelin et al (1943) observed that the content of folic acid increased to several folds on treatment with "takadiastase", a crude enzyme preparation. Welch and Wright (1943) observed that enzymatic factors were necessary to convert natural folates to forms detectable by micro-organisms.

Folic acid conjugase is reported to occur in tissues like brain, bone, pancreas, intestinal mucosa, kidney, spleen, muscle, heart, liver, bone marrow, plasma, bile and leucocytes of several animals like hog, beef, rat, rabbit, dog, chick, poultry and man (Bird et al, 1946; Lesnowski et al, 1945; Minis et al, 1947; Wolff et al, 1949 and Bernstein and Gruetzum, 1949). Its presence in egg yolk has been reported by Tooter et al (1949).

B. Role of folic acid in human nutrition:

1. Absorption and transport:

Dietary folates occur mostly as polyglutamates which are not absorbed intact into the circulation. Studies show that orally administered pteroyl polyglutamates labelled with 14 C in the second (or higher) glutamyl residue, distal to the pteroyl moiety are cleaved to pteroyl glutamic acid during absorption. This is indicated by the appearance of carbon-di-oxide in breath and the absence of radio activity in the folates of serum. On the other hand, if the label forms a part of the folyl moiety, none is recovered in breath, and all appear in plasma as pteroyl glutamate. The site of cleavage of the polyglutamate chain seems to be the interior of

the intestinal epithelial cells (Baugh, 1969). There is no evidence of significant intraluminal conjugase activity nor is the enzyme found in isolated brush border fractions (Halsted et al, 1975). The conjugases seem to occur in lysosomal particles. The question of how dietary polyglutamates traverse the outer boundary of the intestinal lining and come in contact with the intracellular conjugases still remains unanswered. Later in 1977, Reisenauer et al identified two seperate types of conjugases in the human jejunum. Both exhibited different pH optima, molecular weights and inhibition characteristics. Folate conjugase in the brush border may accomplish the initial digestion of dietary polyglutamates.

Absorption is a carrier mediated process (Weir et al , 1973 and Chanarin, 1975) and man absorbs preferentially the natural (L,l.) form. Hepner and Hoffbrand (1967) reveal that folates are absorbed well in the jejunum and less in the ileum. Elsborg (1974) suggests that under physiological conditions like eating pH is lower in upper intestine and absorption rate increases in jejunum and not in ileum. Transport of folates is an active process and about 80 per cent free folate and 20 per cent polyglutamates are absorbed by man(Perry and Chanarin, 1968).

Zamkowski et al (1970) reveal that absorption of folates from food is quickest from yeast, slowest from liver hydrolysates and walnuts. 5 formyl THFA(Tetra Hydro Folic Acid) is less well absorbed, and most compounds appear in blood as methyl derivatives. Isak et al (1973) suggest that in man 5-methyl THFA is the major folasin of the liver and is also the one absorbed from the gut.

Kneeman and Noronha (1971) observe that X-radiation completly prevents the absorption of naturally occurring folates. Gerson et al (1971) show that glucose enhances the folate absorption as well as of sodium and water.

Rosenberg and Godwin, (1971) suggest that absorption of polyglutamates is better than monoglutamates while Perry and Chanarin report the opposite. Perry and Chanarin (1973) also conclud from studies carried out on healthy adults that folate polyglutamates from natural sources are not as well absorbed by man as a solution of folic acid.

Williams et al (1973) and Tamura et al (1978) postulate that zinc depletion in rat and man lead to decreased hydrolysis of pteroyl-polyglutamates and that such hydrolases are zinc dependent enzymes.

Elsborg (1972) indicate that folic acid is carried by β albumin in the body. Markkanen et al (1972) observe that about 1/10th of the folacin in the serum is carried by transferrin.

2. The functions of folic acid:

The functions of folic acid are listed below:

a. Folic acid is one of the important hematopoietic agents necessary for proper functioning in animals and man (Heaton and Mc Henry, 1964). It brings about reticulocytosis and haemoglobin regeneration in pernicious anaemia patients, however it does not correct the neurologic degradation due to B12 deficiency.

b. (PGA) plays a role in tyrosine oxidation. PGA deficient rats, as well as rats fed aminopterin (inhibits the maturation and proliferation of malignant cells), showed decreased ability of their liver to oxidise tyrosine.

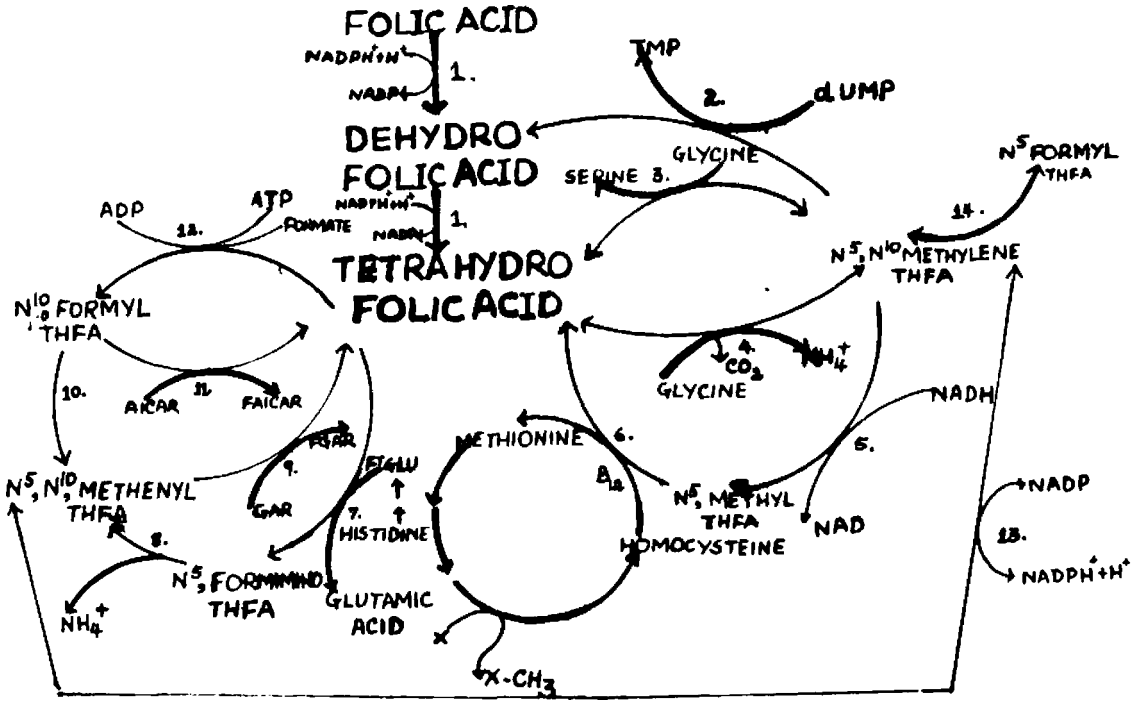
c. Folic acid is also implicated in tyrosine decarboxylation and subsequently in the formation of adrenaline. In very high concentrations methyl folic acid inhibits tyrosine decarboxylase. The inhibition can be reversed by folic acid.

d. Another function is its involvement in the synthesis of thymine required in nucleic acid synthesis.

e. Yet another role is its relation to certain hormones in animal nutrition. For instance oestrogens have no effect on the oviduct of young chicks until folic acid is given. Folic acid is also required for the maintenance of normal pregnancy in rats. Studies carried out by Tagbo and Hill (1978) showed that rats deprived of folic acid in pregnancy showed resorption of their foetuses.

f. Vitamin B_{12} deficiency results in the reduction of the ratio of DPNH to DPN which in turn brings about a decrease in the oxidation-reduction potential of the tissues. This effect reduces the capability of the animal in the conversion of folic acid to tetrahydro folic acid. Some authors believe that folic acid may effect the absorption of B_{12}.

g. Folic acid as a coenzyme is involved in several carbon 1 transfer reactions. The details are illustrated in Figure 3.



REACTIONS IN FOLATE METABOLISM

REACTION	ENZYME	REMARKS
1.	DIHYDRO FOLIC REDUCTASE	PREFERRED SUBSTRATE DHFA
2.	THYMIDYLATE SYNTHETASE	POLYGLUTAMATES
3.	SERINE HYDROXY METHYL TRANSFERASE	POLYGLUTAMATES
4.	GLYCINE CLEAVAGE ENZYME	
5.	N^5, N^{10} METHYLENE THFA REDUCTASE	
6.	N^5, METHYL THFA HOMOCYSTEINE METHYL TRANSFERASE.	2 KINDS a) B_{12} DEPENDENT b). B_{12} INDEPENDENT POLYGLUTAMATE DEPENDENT
7.	FORMIMINO GLUTAMIC THFA FORMIMINO TRANSFERASE.	
8.	N^5 FORMIMINO THFA CYCLODEAMINASE	
9.	GLYCINEAMIDE RIBOTIDE TRANSFORMYLASE	C^8 IN PURINE BIOSYNTHESIS
10.	N^5, N^{10} METHENYL THFA CYCLOHYDROLASE	
11.	5-AMINO, 4-IMIDAZOLE CARBOXIAMIDE	C^2 IN PURINE BIOSYNTHESIS
12.	N^{10} FORMYL THFA SYNTHETASE	POLYGLUTAMATES
13.	N^5, N^{10} METHYLENE THFA DEHYDROGENASE	
14.	N^5, FORMYL THFA CYCLOHYDRASE	

FIGURE 3

FOLATE REQUIRING REACTIONS.

6. Folate deficiency and the contribution of dietary sources towards its prevention:

1. Folate deficiency:

In India folate deficiency ranks only next to iron deficiency anaemia (Venkatachalam, 1969). However iron deficiency is usually associated with folate deficiency (NIN, 1975 and Ramakko et al, 1975).

Girdwood, (1971) and Banerjee and Chaterjee (1975), emphasise that even in apparently healthy individuals, low serum vitamin B_{12} and folate levels exist. Devi et al, (1975) suggest that in megaloblastic anaemia, B_{12} deficiency does not exist because of low requirements (2 to 1.8 µg.) and as bacterial contamination of food and water is its source.

Anaemias of various origins, mostly haemolytic, malignancy, parasitic infestation, infection and even aseptic abscesses also result in higher folate needs.

Herbert (1970) recognises five basic causes of all nutrient deficiencies, three are inadequacies of ingestion, absorption and utilisation and two are increases of requirements and losses. Any circumstance, physiologic or pathologic, leading to increased rates of cell multiplication will result in higher requirements. Thus pregnancy, lactation, early infancy and adolescence are physiologic states particularly vulnerable to the development of folic acid deficiency (Krishna Menon, 1967).

A number of substances interfere with the normal absorption of folate, eg. ethanol (Halsted etal 1967), which interferes with the delivery of N^5 methyl THFA from storage areas (Richmer et al, 1973).

There is evidence that anticonvulsants, in particular diphenyl hydantoin interfere with absorption of folic acid (Gerson, et al; Arakawa et al, 1974, and Reynold et al 1974). Methotrexate and other 4-amino-4-deoxy folate analogues owe their antitumour effect to the potent inhibition of dihydrofolic reductase which eventually leads to death of the cell. Other antifolates in common usage are antimalarial pyrimethamin (i, 2, 4, diamino pyrimidine) the antibacterial trimmterase, are also potential offenders (Clarke, 1976).

Several report point out that oral contraceptives alter folate metabolism (Shojania et al, 1971, Thomas, 1972 Nut. Rev., 1974 and Lynn, 1975), by increasing the clearance of absorbed folate from the plasma. Antituberculosis therapy with isoniazid and cycloserine may produce low serum folate concentration in 50 per cent of the patients (Klipstein, et al, 1967).

Important features of folate deficiency in man appear as accounted by Heaton and Mc Henry (1964).

1. Mucosal atrophy and inflammation of tongue (glossitis), mouth (stomatitis), and the pharynx (pharyngitis).

2. Degenerative lesions of the posterior and lateral columns of the spinal cord (Combined system disease) resulting in peripheral sensory disturbances, hyperactive reflexes, ataxia and paralysis.

3. The hematological picture could result from any of the following:

a. Nutritional macrocytic anaemia (dietary deficiency in FOA)

b. Megaloblastic anaemia of pregnancy (Mechanism unknown, relative FOA deficiency).

c. Megaloblastic anaemia of infancy (dietary FOA deficiency).

d. Megaloblastic anaemia after extensive intestinal resection (inadequate absorption).

e. Megaloblastic anaemia in sprue (inadequate absorption).

f. Macrocytic anaemia in liver disease (inadequate storage or conversion).

g. Macrocytic anaemia in infestation.

The main metabolic consequences of deficit of folate is a derangement of DNA synthesis producing arrest or prolongation of the cell cycle (Mannies et al, 1966). This is because of the participation of folates in biosynthesis of thymidylate and of purines. The result is a set of characteristic changes in nuclear morphology. Tissues having the highest rates of cell multiplication are affected first. These nuclear changes are referred to as "megaloblastic", a term applied to changes in the nucleated redcells of the bone marrow. Morphological changes are also seen in nuclei of leucocytes, as well as epithelial cells of stomach, small intestine, vagina and uterine cervix. Goolsch and Klipstein (1978) put forth, that folate deficiency of intestinal mucosa in rats alters the transport of water and electrolytes but not of such solutes like xylose, glucose and L-leucine.

Chida et al (1972) studied the effects of dietary folate deficiency on fatty acid composition of myelin cerebroside in growing rats. They found that in the folacin deficient rats the hydroxy fatty acids were decreased, suggesting that folic acid might play a role in desaturation and hydroxylation of long chain fatty acids in the brain of growing rats.

Martin et al (1965), Hibbard and Smithells (1965) and Hibbard and Jeffcoate (1966) believed in an association between folate deficiency and abnormalities in pregnancy, but this was denied by Giles (1966), Krishna Menon et al. (1966) and Scott et al (1970). It has been revealed by Rammelag et al (1970), Iyengar (1971) and Srikantia and Iyengar (1972) that folate supplements given to malnourished pregnant women resulted in increased birth weights. Also administering folic acid to low birth wt. infants, increased their weights (Samuel et al, 1973).

Tagbo and Hill (1978) revealed that pregnant rats with low folacin levels delivered litters whose brain weights and DNA content per gram of tissue was less than controls. Some of the rats showed resorption of their foetuses.

Studies on folate deficiency conditioned by pregnancy were reported by Salomons et al (1962) and by lactation by Mot, (1970). Following delivery maternal serum folate rises, but again goes down with lactation (Edelstein et al, 1966).

2. Contribution of dietary sources towards preventing deficiency:

Folates are present in almost all foods of animal and vegetable origin, but folic acid (pteroyl glutamic acid, PGA) constitutes only about five per cent of the total (Hurdle, 1975).

Folic acid is present in liver, kidney , mushrooms, lima beans, bananas, grass and other green leaves (Toepfer et al. 1951 Lakshmiah et al. 1969, and Jägerstad, et al 1974). Peng et al (1975) report that orange and grape fruit are excellent sources of folate, while Rosenberg, et al (1971) and Tamura et al (1973) report that orange juice and yeast nucleic acid might have some inhibitory factors which prevent folate utilization.

Roberts et al (1972) claim that the outer most and greenest leaves of cabbage are the richest in folate. Tamura et al (1973) report that the presence of conjugase in cabbage effects the availability of folate present. Swendseid et al (1947) note, that conjugase inhibitors in yeast preparation may decrease folate absorption. Goat's milk containing 6 mg/ml is a poorer source of folic acid than cow's or human milk which contain 50 mg/ml (Ford et al 1972 and Whitis 1970).

There is uncertainty about the actual intake of folate by people. This uncertainty arises because of appreciable differences which have been reported for the amount of vitamin in particular foods and the unknown extent of destruction of folate during processing and cooking of foods (Roberts and Wadsworth, 1972). The situation is complicated further by the varying extents to which different chemical forms of folate in foods are absorbed and utilised in the body.

The content of folic acid in foods varies with freshness, raw or cooked, manner of storage, presence of reducing agents to protect against oxidative destruction of reduced folate etc. Much of the folic acid initially present in the Asian diet is destroyed during cooking because of prolonged gentle heating of finely cut foods. (Hunt 1976). Pulses which are reported to be a good sources of folic acid for Asians

are unfortunately boiled for about an hour during which most of their activity is no doubt lost (Lakshmiah and Ramaswamy, 1969).

In India the improvement of diet in general will go a long way in improving intakes of folic acid by following proper cooking methods, adopting proper selection of foods and proper storage.

Processing is a prerequisite for several food stuffs, in order to improve their palatability and digestibility (Babu, 1976). Methods such as cooking, heating, roasting, dehydration, boiling, canning and storage lead to a loss of nutrients especially of the B-complex group (Chelchlin et al 1945, Herbert, 1963, Schweigert et al, 1948, Hurdle et al, 1968, Fardinge et al 1961, Taguchi et al, 1974, Kaul, et al and Reed et al, 1976).

Germination is also employed as a method of processing. The changes during germination involve the conversion of storage ingredients into simpler forms, which are utilised by the growing embryo. Thus starch is converted to simpler carbohydrates, fats to fatty acids, protein to peptides and amino acid, and the bound minerals and vitamins to their free form. In addition, appreciable synthesis of the water soluble vitamins and tocopherols takes place (Padwardhan, 1942, Burkholder 1943: Devadatta et al, 1951 Chattopadhyay and Banerjee, 1953 and Shastre et al 1975). These changes help to increase the nutritive value as well as their digestibility. Sprouted legumes take much less time to cook than the whole legumes and even precooked ones.

In several parts of India, Bengal gram, green gram and ragi are frequently germinated before they are consumed. The concentration of ascorbic acid, nicotinic acid and riboflavin have been reported to increase in these foods during germination. However information regarding another important vitamin, folic acid is scanty.

Miyamoto et al (1974) estimated the folic acid content of some fermented soya bean products and vegetables. Values of folacin extracted in the presence of ascorbic acid was generally higher than that extracted without ascorbic acid.

Colman et al (1975) Russel et al (1976) and Klebong (1975) reported that fortification of maize, rice, bread with folic acid resulted in an increment of the vitamin and prevented anemia.

Russel et al (1976) stated that dietary fiber from Iranian bread did not complex with folate and render it insoluble or prevent absorption.

B. Assessment of the folate status and the availability from foods:

1. Folate status:

Of the tests now available to assess the nutritional status of folate in individuals, the most widely used is the determination of serum or plasma folate. The predominant form of folate in plasma is N^5 methyl tetrahydrofolic acid, no polyglutamates being present. Until the advent of isotope dilution techniques, plasma folacin levels were determined by microbiological assay with L. casei, the only assay organism that responds to N^5 methyl THFA. Normal values in adults range from 3.0 to 12.0 ng/ml. with an average of about 6.0 ng/ml.

Average normal values of red cell folates are about 160 ng/ml. Zamberlich et al (1975) consider values lower than 140 ng/ml as deficient. According to Reshmilowits et al (1975), red cell folate is a better indicator of folate levels.

Folates can be estimated by chemical, bio-chemical, physio-chemical, bio-autographic, biological and microbiological methods. Recently a radioisotopic method of estimating folate in serum by using tritiated PGA has been suggested.

Assays based on the growth rate of chicks were first introduced by O' Dell and Hogan (1945) and Campbell et al (1944). One day old chicks were fed a folate free diet (Johns, 1955) and after eight days they were divided into groups of 10 each. A set of groups were given folate in the diet at different levels. Yet another set of groups were given the test diet. After 28 days the haemoglobin was estimated and the growth was estimated by comparing with the control groups. Rats were also used after immobilising the intestinal microbiological source.

The most useful method for the assay of small amounts of folate in biological material have proved to be microbiological assay methods. Teply and Elvehjem (1945) developed a method of folate analysis using titrimetric measurements of growth of *L. Casei*. This organism has become the most popular although the following have also been employed- Bacillus coagulans, Pediococcus cerevisiae, Leuconostoc citrovorum, Streptococcus faecalis, the ciliated protozoan, Tetrahymena geleii W, and the flagellate Crithidia fasciculata. (Chamarin, 1969).

2. Folate availability from foods, measured through urine:

Studies on the amounts of food folate actually available for absorption have been beset by methodological difficulties (Nut.Rev., 1974). Even ingestion of food stuffs rich in folic acid may be followed by small rises in excretion of folate in urine (Fleming 1973). A change in serum level is difficult to quantitate and depends on the previous folate status of the individual. Reisner (1969) used a method in which folate absorption from food was estimated from the subsequent urinary excretion of folate. However uncontrollable variations of urinary folate were observed. Tamura and Stokstad (1973) have attempted to minimise this variability by maintaining, the experimental subjects in a continuously saturated state with respect to folate. They observed that the excretion of a dose of 5 mgs. or less of folic acid was completed within 16 to 24 hrs., as the urine levels of folic acid after 24 hrs. was very low.

Subjects preloaded 24 hrs. before with 2 mg of folate, were given the smaller dose and then folate excretion over the subsequent 24 hours was measured. This was found to be approximately five times higher than that in subjects given a similar dose without preloading. It was therefore concluded that presaturating with folic acid increases the sensitivity of the urinary excretion method of estimating folate.

It has been noted by Tamura et al. (1973) that treatment of urine with folate conjugase did not increase folate value, revealing that in urine no poly glutamates are excreted. He also observed that serum folate values were unrelated to urinary folate values, presumably because subjects were fully saturated. Normal urinary excretion of folate per day for Indians is 1-12/mg/24 hours. (Babu, 1976).

Srikantia and Babu (1976) carried out a similar study and they revealed that the availability of folic acid from egg was 72 per cent, from liver was 70 per cent, from Bengal gram and green gram it was 70 per cent.

The availability of folate in food stuffs, is an important problem which must be resolved by reasonably accurate estimates of folic acid requirements, or the adequacy of diets to the folate content can be evaluated. The requirements of folic acid for different age groups in India as given by the Nutrition, Expert Group of the ICMR (1978) is presented in Table I.

TABLE I

REQUIREMENTS OF FOLIC ACID FOR VARIOUS AGE GROUPS IN INDIA.

Group	Age	Allowance (µg/day)
Man	Adult	100
Woman	Adult	100
Pregnancy		150-300
Lactation		150
Infants	upto 1 year	30
Children	1-12 years	30-50
Adolescents	13-18 years	30-50

However Banerjee et al., (1975) recommended a daily minimal requirement of 75 µg. for Indians, based on studies they conducted on human volunteers.

III EXPERIMENTAL PROCEDURE

The experimental procedure pertaining to the study on the availability of folic acid from selected cereals and pulses was designed under the following steps:

A. Selection of foods for the study

B. Estimation of folic acid in the non-germinated and germinated foods

C. Selection of subjects and

D. Determination of folic acid availability from foods.

1. Determination of initial urinary excretion of folic acid on a normal diet.

2. Saturation of subjects with folic acid and

3. Administering the foods and estimating the folate availability.

A. Selection of foods for the study:

For the present study, two cereals and two pulses were selected because cereals constitute the major item in Indian diets, pulses coming next in line (Devadas 1971, Pandhar, 1974 and Devadas, 1979).

Legumes are important sources of protein (Kappagoudy et al, 1978 and Patwardhan, 1961). The germinated ones constitute a fair portion of the total legumes consumed in several states of India (Protein Foods Association of India, 1973). Moreover they are more acceptable to consume in the sprouted form than any other food.

Among the cereals, ragi (Eleusine coracana) and bajra (Pennisetum typhoideum) were chosen, since they form the staples for the poor people in many parts of South India (Rao et al, 1976). People prefer these for their sustaining qualities and their inexpensiveness. Consumption of sprouted ragi is very common in South Indian whereas the trial with sprouted bajra was a new venture. The pulses selected were green gram (Phaseolus aureus Roxb) and Bengal gram (Cicer arietinum) because they are commonly consumed in South India, both in the germinated and non-germinated forms (Ranaswamy et al, 1969).

B. Estimation of folic acid content of the non-germinated and germinated foods:

For all the four selected foods, folic acid content was estimated in the nongerminated as well as germinated forms. Reports that folic acid content elaborates as germination proceeds, are revealed by Babu and Srikantia (1976). Hence it was decided to germinate the sample over periods of 24, 48, 72 and 96 hours and estimate the folic acid content of all the samples. From the outcomes of the analysis it was planned to find out the ideal period of germination for each food when folate content reached a maximum.

Estimation in the non-germinated samples were carried out by using the powdered unsoaked samples. Samples of all foods chosen for germinating the foods, were soaked in distilled water for 24 hours. They were then transferred to sterilised petri dishes containing moist filter paper. Requisite amount of sterile distilled water was added daily. The seeds were allowed to germinate at a temperature between 25° and 30° C as stated by Babu (1976). At intervals of 24, 48, 72 and 96 hours, samples were analysed for free and total folic acid concentration.

Folic acid was determined by microbiological assay using L.casei (ATCC 7469) organisms obtained from the National Chemical Laboratory, Poona. The procedure followed was as given by the National Institute of Nutrition, Hyderabad (NIN, 1971), the details of which are presented in Appendix A.

In foods, folate is present mostly as polyglutamates, which are not free. Hence analysis was carried out for free and total folic acid contents of germinated foods at intervals of 0, 24, 48, 72 and 96 hours. Total folate was estimated by incubating the food samples with blood plasma, as a source of conjugases.

The results of the analysis revealed, that the increase in the folic acid concentration of both the pulses was maximum at 72 hours of germination. In the case of cereals, ragi showed a maximum folate content at 96 hours and bajra at 72 hours.

Reliet, (1969); Tamura et al (1974) and Babu (1976) have reported the difficulty in testing the availability of folates from foods because of the huge quantities that are required to be ingested, for carrying out such a study.

To minimise this difficulty it was decided to carry out the availability studies of folic acid at 72 hours of germination for the pulses and bajra, and at 96 hours of germination for ragi, when their folic acid concentration reached a peak.

C. Selection of subjects:

For carrying out the folic acid availability study, 12 post adolescent subjects of the age 20 to 22 years from Sri Avinashilingam Home Science College hostel were selected. Since the subjects were in the hostel it was easy to contact them, control their diets and administer the test foods, more over they were co-operative for a scientific trial.

The subjects were divided into two groups of six each. One group was used to test the pulse foods and the other group was used to test the cereal foods.

D. Determination of folic acid availability from foods:

1. Determination of initial urinary excretion of folic acid on a normal diet:

Initially the day's urinary excretion of folic acid was determined for all the 12 selected subjects. This estimation was carried out to determine the amount of vitamin excreted through urine per day, when the routine hostel meals were consumed.

Twenty four hours urine was collected for all the subjects over toluene for three consecutive days. Every day, samples were analysed in triplicates for their folic acid concentration. One ml of the 24 hour urine sample was diluted suitably and taken directly for the assay. The procedure followed was as given by Babu (1976) and is annexed in Appendix A. The urine was analysed only for free folate as Tamura (1974) reported that there was no increase in folic acid content on treatment with conjugase.

2. Saturation of subjects before starting the availability studies:

Tamura and Stokstad (1973) found that presaturating subjects with folic acid increased the sensitivity of the urinary excretion method of estimating food folates. In the light of this finding all subjects in the present investigation were given an oral dose of five mg. of synthetic pteroylglutamic acid for a period of six days before the availability studies were started. The folate tablets with a trade name 'Folvite' were obtained from Cyanamid India Limited (Lederle Division) Bulsar. Each tablet contained five mg of folic acid.

After six days of initial saturation, the subjects were maintained in a saturated condition, through out the study by giving them a dose of two mg of the vitamin on every alternate day. The tablets were powdered and the quantity of powder supplying two mg of the vitamin was taken in small packets and distributed to all the subjects on alternate days.

3. Administering the food samples and estimating the folate availability:

a. Germination of foods:

From the results of the food analysis, it was decided to carry out the folic acid availability studies with 72 hours germinated Bengal gram, green gram and bajra, and 96 hours germinated ragi, because the folic acid content was maximum at these stages. The cereals and pulses were soaked in water for 24 hours and then allowed to sprout at room temperature by suspending them in a muslin cloth tied at the proximal end.

The samples were sprinkled with sufficient water every day and were allowed to germinate until each attained the maximum content of folic acid. These germinated food were used for feeding the subjects.

b. Administration of foods:

Baby (1976) reported that the ingestion of a minimum of 400 µg of folic acid was essential for determining the folate availability through urinary excretion, as the amounts below 400 µg led to inconsistent folate excretion through urine. In this study a test dose of 700 µg of folate per day was provided to the subjects as per the procedure adopted by Baby(1976).

Since it was not possible to ingest food, in amounts to supply this quantity of folate, a synthetic dose of 400 µg of pteroylglutamic acid (PGA) was supplemented along with 300 µg of folate from the food item under test. Thus a test dose of 700 µg of folate was given to study the availability of the vitamin from foods.

The supplementation of foods was carried out on alternate days when two mg of synthetic folic acid was not given. The tablets of PGA were powdered and distributed as before, to provide 400 µg (12.8 mg of tablet) of the vitamin along with the test food.

The sprouted grams were seasoned with chillies, salt, mustard and jeera and cooked for just one minute with the addition of lime juice to improve the taste. Lime juice was used also to prevent the oxidation of folic acid. Studies reveal that ascorbic acid activities folic acid (Krumdiek, 1974).

In the case of cereals, they were first crushed to remove the coarse fibre which was sieved off. They were then seasoned like the pulses and cooked with the addition of lime juice.

The quantities of different foods fed to subjects are presented in Table II.

TABLE II

QUANTITIES OF GERMINATED FOODS AND THE SYNTHETIC FOLATE FED TO SUBJECTS

Germinated Foods Fed	Quantity fed (µg)	Folate Content of Foods		Total folate present in the food fed (µg)	Synthetic folate given (µg)	Total folate ingested (µg)
		Free	Total µg/100			
Ragi	250	69.8	119.7	300.0	400	700.00
Bajra	270	60.8	110.9	300.0	400	700.0
Bengal Gram	90	68.2	341.0	307.0	400	707.0
Green Gram	120	115.3	250.6	300.7	400	700.7

The prepared foods were weighed and fed to the subjects at 9 am in the morning. Along with the test food a cup of coffee was served at breakfast. No food was allowed for three hours after the ingestion of the test dose. The subjects were not allowed to consume any folic acid rich foods such as green leafy vegetables, fruits, eggs or whole pulses during the experiment. (Babu, 1976).

The cereal diets were consumed with a little difficulty because of the large quantities served. The feeding trials of the six subjects in the cereal group was started by feeding one of the cereals and the six in the pulse group, with one of the pulses. The feeding was carried out for three consecutive alternate days. When the study with one food was over, the subjects were continued with the next food, (the pulse group with the next pulse and the cereal group with the next cereal) leaving a day in between when the subjects were given two mg folic acid for saturation. The same procedure as followed for administering the foods previously, was followed with the second food also.

c. Collection and analysis of urine samples:

On the days when the food was fed to subjects, 24 hour urine excretions were collected under toluene for all the subjects and the folic acid content was estimated microbiologically with L.Casei (ATCC 7469) organisms.

d. Calculation of the availability per cent of food folate:

The availability per cent of food folate was calculated using the following formula:

$$\text{Availability per cent} = \frac{\text{A } \mu g - 400 \mu g}{\text{B } \mu g} \times 100.$$

A = Value obtained for urinary excretion on a test dose.

B = Folate activity in food measured using L. casei.

IV RESULTS AND DISCUSSION

In this investigation, an attempt was made to evaluate the availability of folic acid from germinated cereals and pulses on post adolescent girls. The results obtained for this study were discussed under the following headings:

A. Changes in folic acid content during germination and

B. Availability of folic acid from germinated foods.

1. Availability from cereals and

2. Availability from pulses.

A. Changes in folic acid content during germination:

As a first step in this study, the folic acid content of all the four raw germinated foods namely ragi, bajra, Bengal gram and green gram, were analysed. Table III reveals the results of this analysis in comparison to the values reported by the NIMR (1978).

TABLE III

FOLIC ACID CONTENT OF RAW GERMINATED FOODS.

(µg/100 g sample)

Foods	Present study		NIMR values (1978)	
	Free folate	Total folate	Free folate	Total folate
Ragi	5.4	13.2	5.2	18.5
Bajra	10.2	15.1	14.7	45.5
Bengal Gram	18.0	197.0	34.0	186.0
Green Gram	25.5	120.5	*	*

* Values not available for whole green gram.

Food folate is present mostly as poly glutamates which are not free. Reddi (1969) confirms that free folate is not a good measure of the folate content of a food because ultimately all polyglutamates present in the food are split by conjugases to monoglutamates and absorbed (Streiff and Rosenberg, 1967; Perry and Chanin, 1968; Hoffbrand et al 1969 and Butterworth et al (1976)). Therefore the total folate is an important criteria for the content of folic acid.

Table III Presents the folic acid content of foods both in terms of free and total folate. In general pulses had a greater amount of folic acid, when compared to cereals. The analysed variety of ragi showed a folic acid content comparable to that reported by the ICMR(1978). A free folate of 5.4 µg/ 100 g. and a total folate of 13.2 µg/100 g was obtained in the laboratory, where as the ICMR reports a free folate of 5.2 and a total folate of 18.3 µg/100g.

The sample of bajra analysed had a free folate content of 10.2 µg/ 100 g and a total folate of 15.1 µg/100 g as against 14.7 for free folate and 45.5 µg/100 for total folate reported by the ICMR. Here the total folate content of bajra was very much below the values reported by the ICMR (1978).

Bengal gram showed a free folate of 18.0 µg/100 g and a total folate content of 197.0 µg/100 g. The values reported for total folate by the ICMR is much lower than the obtained in the present study. The reasons for the higher content could be due to a difference in the variety of the sample analysed. Green gram showed a free folate value of 25.5 µg/100g and total folate of 120.5 µg/100g. The ICMR values for folate content of whole green gram are not available.

The four selected foods were germinated over periods of 24, 48,72 and 96 hours. Figures 4 and 5 present a picture of the germinated cereals and pulses respectively. It was noticed that in all the samples as germination progressed the length of the sprout also increased.

The mean length of the sprouts at different stages of germination was obtained by measuring 25 randomly selected seeds. A piece of thread was used to trace the length of the sprout from the origin to the tip. The length was thus noted and read off on a scale. The mean value of 25 seeds of each food sample was thus arrived at.

Table IV and figure 6 illustrate the folic acid content of foods at different stages of germination. The details of individual values are presented in Appendix B. Figure 7 presents the percentage increase of folic acid content in different foods, during the process of germination.

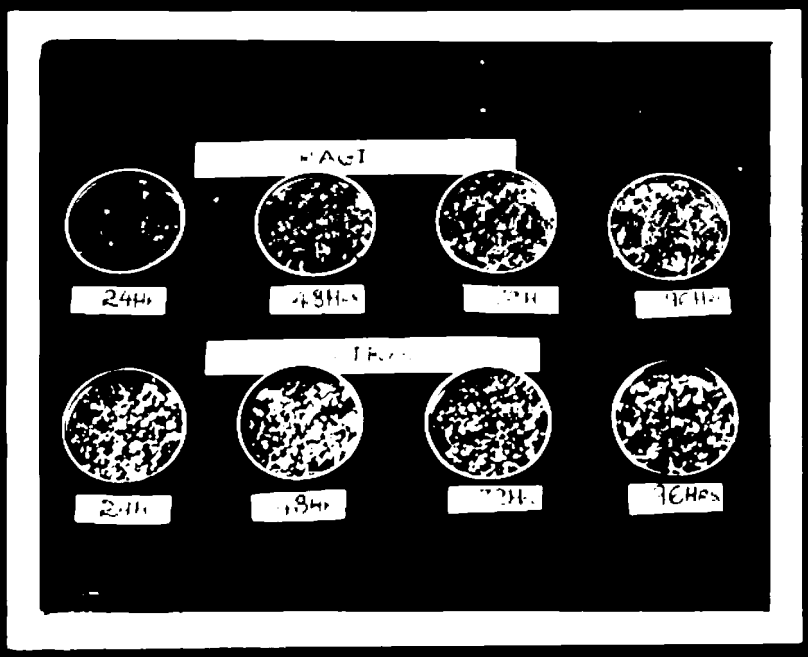


FIGURE 4

CEREALS AT DIFFERENT STAGES OF GERMINATION

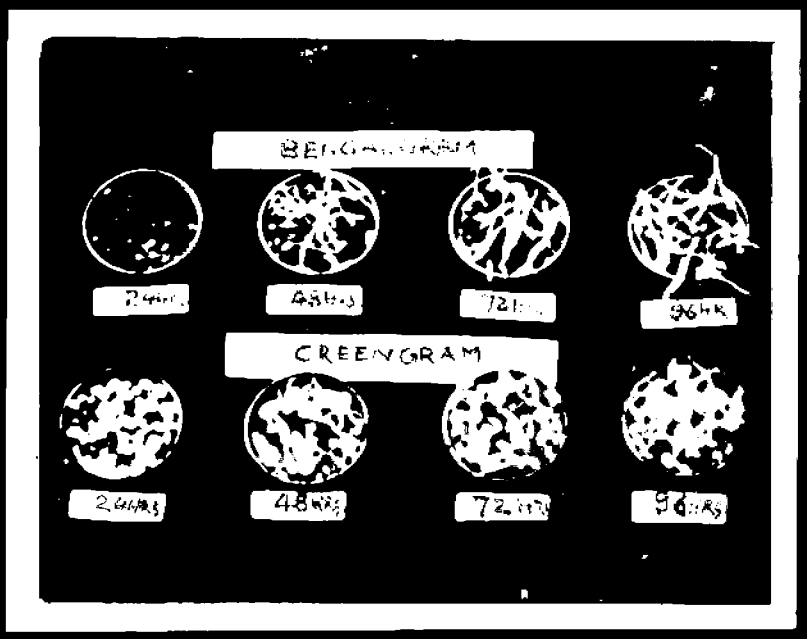


FIGURE 5

PULSES AT DIFFERENT STAGES OF GERMINATION

TABLE IV

FOLIC ACID CONTENT OF FOODS AT DIFFERENT STAGES OF GERMINATION (µg/100g)

Food samples	Non germinated (0 hours)		24 hours		48 hours		72 hours		96 hours	
	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Ragi	5.4	13.2	9.3	18.2	25.1	56.3	60.4	71.1	69.0	119.7
Bajra	10.2	15.1	18.0	20.2	21.4	50.2	60.8	110.9	70.3	105.2
Bengal gram	18.0	197.0	42.1	286.0	60.4	317.7	68.2	341.0	94.1	302.8
Green gram	25.5	120.5	48.9	150.3	80.8	196.1	115.3	250.6	130.4	245.5

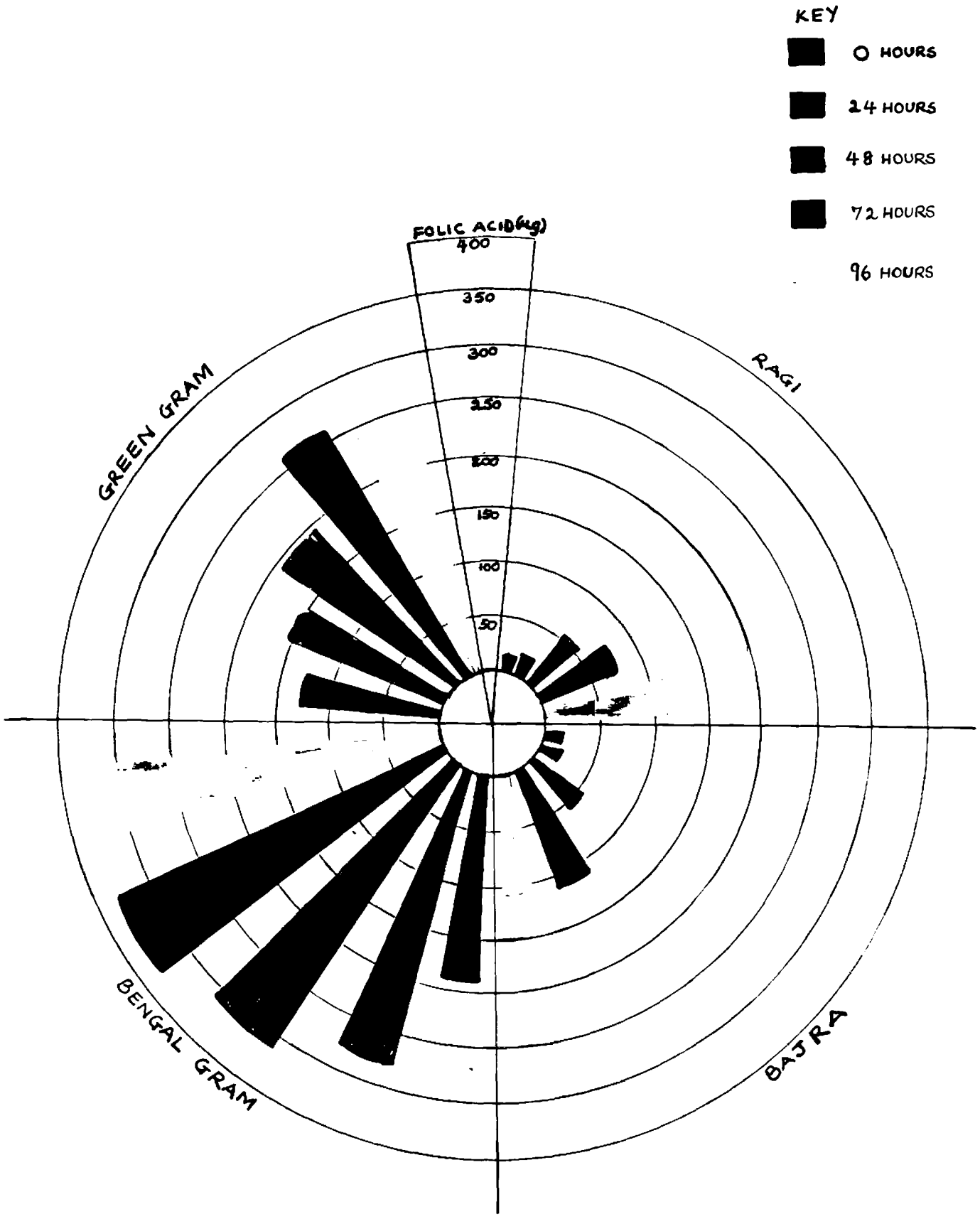


FIGURE 6

TOTAL FOLIC ACID CONTENT OF

THE FOODS AT DIFFERENT STAGES OF GERMINATION

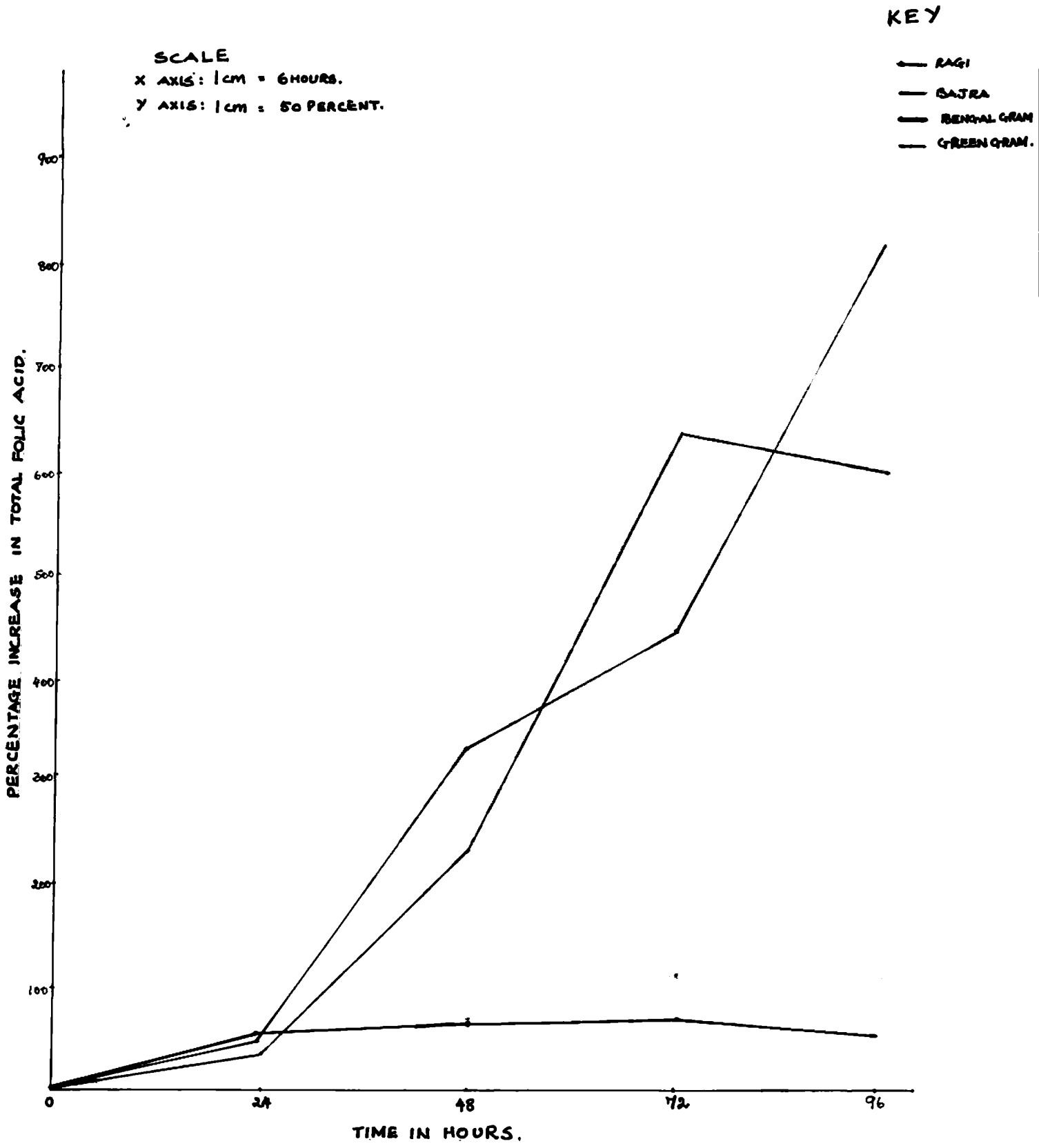


FIGURE 7

PERCENTAGE INCREASE IN FOLIC ACID AT DIFFERENT STAGES OF GERMINATION FROM THE INITIAL LEVEL.

The results revealed that on germination, foods registered an increase in the folic acid. This is in agreement with the results obtained by Shastri et al (1975) and Babu (1976) who report an increase in folic acid content while germinating Bengal gram, ragi, garden pea and field beans (bitter variety).

At 24 hours of germination the sprouts were visible only in green gram, the length of the sprout measured 0.2 cm. The total folic acid content of ragi, bajra, Bengal gram and green gram at 24 hours of germination was found to be 18.2, 20.2, 286.0, 190.5, µg/100g respectively and the increases observed were 37, 34.45, and 25 per cent over and above the initial values. However the increases reported in the present study are greater than those reported by Babu (1976) who observed 12 per cent and 44 per cent increases for ragi and Bengal gram respectively.

After 48 hours of germination the length of the sprouts were visible for all the samples. The sprout length, for ragi was 0.8 cm, for bajra 0.5 cm, for Bengal gram 0.8 cm and for green gram, 1.0cm. This stage of germination was marked by a further increase in total folic acid from the initial levels', 327 per cent increase in ragi (36.3µg/100g), 252 per cent increase in bajra (50.2 µg/100g), 61 per cent increase in Bengal gram (317.7 µg/100g) and 63 per cent increase in green gram (196.1 µg/100g) were noted. Babu (1976) reported 335 per cent and 62 per cent increase in ragi and Bengal gram respectively at 48 hours of germination.

The length of the sprouts at 72 hours measured 1.5 cm for ragi, 1.1 cm. for bajra, 2.2 cm for Bengal gram and 2.5 cm for green gram. At 72 hours of germination the increments in folic acid continued even further. Ragi showed an increment of 439 per cent (71.1 µg/100g), bajra showed 634 per cent (110.9 µg/100g), Bengal gram showed 73 per cent (341.0 µg/100g) and green gram showed 108 per cent (250.6 µg/100g) increase in folic acid content over and above the initial levels. It was noted that both the cereals showed tremendous increases in folic acid content against the initial levels, when compared with the pulses.

As germination proceeded the sprout length also increased. Ragi registered a sprout length of 1.8 cm, bajra 1.6 cm, Bengal gram 3.0 cm and green gram 3.3 cm at 96 hours of germination. The 96 th hour of germination revealed considerable changes in folate content. In the case of ragi the folate content continued to increase. An increase of 68 per cent from the content at 72 hours and 807 per cent from the level at 0 hours was noted in ragi. Bajra on the other hand showed a five per cent decline in folic acid content from that of the 72 hours. In both the pulses the folate content decreased. Babu (1976) reported similar results in Bengal gram. The content in Bengal gram decreased by about 11 per cent compared to the values at 72 hours. In green gram a two per cent decrease was noted. This decline in folic acid content of germinating pulses were reported by Banerjee et al (1952) to be due to the cleavage of citrovorum factor.

From the results obtained in the first part of the study it could be concluded that the sprouted pulses are better sources of folic acid than the cereals. However, since the sprouted cereals also evince tremendous increase in folic acid from the initial level, they also could serve as good sources of folate.

It was also noted that there was a low degree of correlation between the length of the sprout and folic acid content of the food grains (r=.4720). The details of the calculation are given in Appendix C.

B. Availability of folic acid from germinated foods:

The second part of the study consisted of supplementing the germinated food as a source of folate in the diets of the selected subjects and determining the folate availability.

The administration of food samples was preceded by saturating the subjects for six days with five mg of PGA every day.

The results obtained are presented below.

1. Availability from cereals:

Table V and Figure 8 show the availability of folic acid from germinated cereals. The results of statistical analysis are presented in Table VII and the individual values are in Appendix D.

TABLE V

FOLIC ACID AVAILABILITY FROM GERMINATED CEREALS

Food tested	Subjects	Dose of folate administered * (µg)	Mean urinary excretion of folate (µg/24 hrs.)	Mean availability per centage
Germinated ragi	1	700	546.4	48.8
	2	700	616.5	72.1
	3	700	447.9	16.1
	4	700	388.3	59.3
	5	700	648.6	82.7
	6	700	455.8	18.6
	Mean	700	555.6	49.6 ± 25.1
Germinated bajra	1	700	626.5	74.8
	2	700	552.8	50.6
	3	700	557.8	52.9
	4	700	489.3	29.8
	5	700	554.1	51.3
	6	700	590.3	65.4
	Mean	700	561.13	53.8 ± 13.7

* 400 µg from synthetic source and 300 µg from germinated foods.

The intake of folate by all the 12 subjects consuming the germinated cereals was similar namely 300 µg from food source and 400 µg from the synthetic source.

The mean urinary excretion of folate in the ragi group ranged from 447.9 to 648 µg for 24 hours with a mean value of 555.6 µg. In the case of bajra it varied from 489.3 to 634.5 µg with a mean value of 561.15 µg.

The mean folate availability from cereals was found to be lower than that from pulses. For germinated ragi the availability was found to be 49.6 per cent (16.1 to 82.7 per cent range) while for bajra it was 53.8 per cent (29.8 to 74.8 per cent range).

In the calculations on the availability of folate from foods, it was taken for granted that all the 400 µg of synthetic folate was absorbed and excreted through urine. This procedure was adopted as per the previous workers, (Tamura et al, 1974 and Babu and Srikantia, 1976). However studies carried out at NIN (1976) report that cereals and millets reduced the absorption of synthetic folate. When ingested alone most of the synthetic PGA was absorbed, but when ingested with cereals eg. rice, 97 per cent of the synthetic folic acid was absorbed, with wheat 46.1 per cent, with sorghum 44.70 per cent and with ragi 47.9 per cent of synthetic folate was absorbed.

3. Availability from pulses:

Table VI and Figure 8 present the availability of folic acid from germinated pulses with the individual values in Appendix .B.

TABLE VI

FOLIC ACID AVAILABILITY FROM GERMINATED PULSES

Food tested	Subjects	Dose of folate administered * (µg)	Mean urinary Excretion(µg/24 hours)	Mean availability per centage
Germinated Bengal gram	1	707.0	613.0	69.4
	2	707.0	634.5	92.6
	3	707.0	687.3	93.5
	4	707.0	476.0	24.7
	5	707.0	665.0	86.3
	6	707.0	586.0	60.6
	Mean	707.0	613.6	71.2± 25.97
Germinated Green gram	1	700.7	655.2	84.8
	2	700.7	679.6	93.0
	3	700.7	660.8	87.7
	4	700.7	492.3	30.7
	5	700.7	651.1	83.3
	6	700.7	535.8	45.2
	Mean	700.7	612.3	70.7 ± 25.54

* 400 µg from synthetic source and the rest from germinated foods.

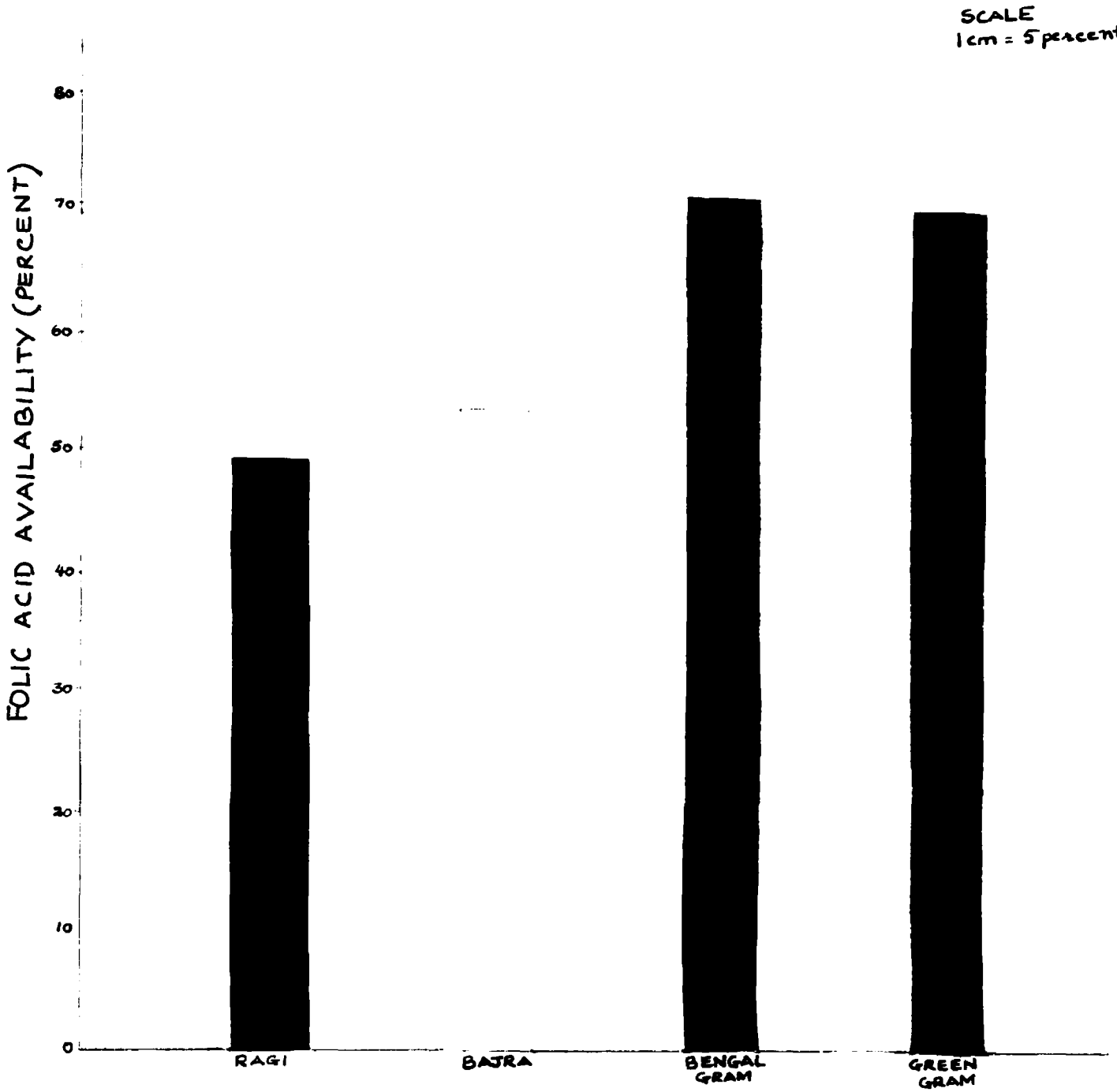


FIGURE 8

PERCENTAGE AVAILABILITY OF FOLATE FROM GERMINATED FOODS.

TABLE VII

STATISTICAL COMPARISION OF FOLATE AVAILABILITY

Groups compared	't' values
Ragi Vs bajra	0.35
Ragi Vs Bengal gram	1.39
Ragi Vs green gram	1.37
Bajra Vs Bengal gram	1.40
Bajra Vs green gram	1.39
Bengal Gram Vs green gram	0.05

The subjects in the Bengal gram group received 307.0 µg folate while the subjects in the green gram group received 300.7 µg folate through the consumption of the test food. Along with the test food, the subjects were given 400 µg of synthetic folate uniformaly.

The mean initial 24 hours urinary excretion of folates in the subjects ranged between 1.9 and 3.9 µg with a mean value of 2.9 µg/24 hours. This is in accordance with the reports of several workers. Rahm and Srikantia (1976) reported a value of one to twelve microgrems per day for Indians. However the administration of 700 µg of folate in the present study had increased the excretion of the vitamin (Chanarin, 1969).

The 24 hours urinary folate excretion in the Bengal gram varied from 476.0 µg to 687.3 µg with a mean of 618.6 µg. The mean availability of folic acid from germinated Bengal gram was 71.2 per cent, with a range of 24.7 to 93.9 per cent. Among the six subjects three had registered more than 80 per cent availability and only one had a very low availability of 24.7 per cent.

The mean availability of folic acid from green gram was 70.7 per cent. Among the six subjects, four had registered more than 80 per cent availability and the lowest availability was found to be 30.7 per cent. None of the subjects in both the pulse groups showed 100 per cent availability as had been reported by Babu (1976) and Tamura (1973).

Tamura and Stokstad (1973) suggested the presence of conjugases in some foods. The presence of such conjugases have been reported in foods like orange juice and yeast nucleic acid (Rosenberg, et al 1971 and Tamura et al, 1973). It is possible that such conjugases enhanced the availability of folates in pulses and some inhibitors might have prevented the availability from cereals.

However the difference in the availability of folate from cereals and pulses was not statistically significant (Table VII), showing thereby that cereals are also good sources of folic acid though the mean availability of the folic acid from pulses is much greater than that of the cereals. The great individual variations made the differences not appreciable statistically.

Statistical analysis of the data further revealed that there was no significant difference in the absorption of folic acid between the two pulses, as well as between the two cereals.

From the findings of the study, it could be concluded that both in pulses and cereals, the folic acid content increases during germination. The folic acid content reaches its maximum at 72 hours of germination in Bengal gram, green gram and bajra and in the case of ragi, the maximum concentration of folic acid was registered at 96 hours of germination. The difference in availability of folate from these four sources is not statistically significant. Though the germinated pulses are good sources of folate, germinated cereals can also contribute a fair amount of folate in the diet because they are consumed in larger quantities than pulses, especially in an average Indian diet.

V SUMMARY AND CONCLUSION

This study was carried out to investigate the availability of folic acid from selected germinated cereals and pulses on post adolescent girls.

The cereals selected for the study were ragi (Eleusine Coracana) and bajra (Pennisetum typhoideum) and the pulses selected were Bengal gram (Cicer arietinum) and green gram (Phaseolus aureus Roxb).

All these foods were analysed at different stages of germination namely 0 hours, 24 hours, 48 hours, 72 hours and 96 hours for their free and total folate content.

Bengal gram, green gram and bajra registered a maximum content of total folic acid at 72 hours of germination, and ragi had the maximum content at 96 hours of germination. The folate availability studies were carried out with these germinated foods at the maximum level of folate content.

For testing each food six post adolescent girls were included and their initial daily urinary folate excretion determined. The quantity of each food, administered to the subjects was determined in such a way as to supply 300 µg of folic acid. In order to increase the sensitivity of folate excretion 400 µg of synthetic folate was given along with test food. Urinary excretion was used as the criteria to find out the availability of folate from different foods.

The results of the study revealed the following:

1. During germination the folic acid content of cereals and pulses increased. The increase was observed only up to a particular stage for each food, after which the concentration began to decrease.

2. For Bengal gram, green gram and bajra, the maximum level of total folate namely 341.0 µg, 250.6 µg and 110.9 µg/100g respectively was reached at 72 hours of germination, as against the initial level of 197.0 µg, 120.5 µg, and 15.1 µg/100 g respectively. In the case of ragi the maximum folate concentration, that is 110.7 µg/100g was reached at 96 hours of germination from the initial level of 13.2 µg/100g.

3. The absorption of folic acid was found to be better from pulses than from cereals, but there were very wide individual variations. The mean absorption of folate from ragi and bajra were 49.6 per cent and 53.8 per cent respectively. The absorption from Bengal gram and green gram were 71.2 per cent and 70.7 per cent respectively. Because of the great individual variations in the folate availability, none of the differences in folate absorption among the four foods was statistically significant.

4. The mean initial urinary excretion of folate in the selected subjects on a normal diet was found to be 2.92 µg/24 hours.

5. A low positive correlation (r = 0.4720) was found between the sprout length and the folic acid content of foods.

A few problems and limitations were encountered by the investigator while undertaking this study. Since the microbiological assay was the first effort in the laboratories, obtaining all the chemicals, organisms and standardisation of the techniques had been a great challenge in this study. Because of the limitations of time, it was not possible to construct the dose response curve for every subject as was carried out in a few elaborate studies of the past. Hence the urinary excretion of the subject was arrived at by collecting 24 hours urine voided and analysing the folate content.

Avenues are wide open to throw more light on this subject through further studies in this direction. Some of them are as follows:

1. In view of the importance of folic acid in our diets, it would be valuable if the availability of folates from other commonly consumed germinated cereals and pulses are determined. More studies are required to reveal the causative factors for the different rates of increases in folic acid concentration during germination of different foods.

2. The folic acid losses while cooking and preservation would be another useful field for investigation.

3. Yet another area requiring further study is the effect of different food stuffs on the absorption of synthetic folate.

4. In the present study, it was taken for granted as the previous workers that all the 400 µg of synthetic folate given along with the food was absorbed and excreted in the urine. Since this may not be true in the real sense, the results obtained may be only the relative availability of folate from different foods. Hence there is a wide scope to explore the possibilities of improving this experimental procedure.

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APPENDICES

APPENDIX A

MICROBIOLOGICAL ASSAY OF FOLIC ACID

AIM: Estimation of folic acid by microbiological assay.

PRINCIPLE:

Microbiological methods are based on the observation that certain micro-organisms require specific nutrients for growth. Using a basal medium complete in all the respects, except for the nutrient under test, growth responses of the organism, are compared quantitatively in standard and unknown solutions.

Either the acid or the turbidity produced by the organism is measured to determine the extent of growth and there by the amount of nutrient in the test solution.

Organism used:

Lactobacillus casei ATCC 7469.

Reagents required:

1. **Salt solution A:** Dissolve 25 g each of KH_2PO_4 and K_2HPO_4 in distilled water and make up the volume to 250 ml.

2. **Salt solution B:**

Dissolve the following in distilled water, add 5 drops of concentrated hydrochloric acid and make up the volume to 250 ml.

$MgSO_4 \cdot 7H_2O$ - 10.0g.

NaCl - .5g

$FeSO_4 7H_2O$ - .5g

$MnSO_4 \cdot H_2O$ - .5g

3. Vitamin solution:

Thiamine hydrochloride	20 mg
Nicotinic acid	20 mg
Para amino benzoic acid	10 mg
Calcium pantothenate	20 mg
pyridoxine hydrochloride	50 mg
Biotin solution (100 µg/ml)	1 mg

Dissolve the above in about 100 ml distilled water and add to the solution a solution of 20 mg. riboflavin in distilled water prepared with the use of a few drops a acetic acid and gentle warming if necessary. Finally make up the volume of the vitamin solution to 200 ml.

4. L. Tryptophan solution (.2%)

Dissolve 1 gm. of L.tryptophan in distilled water using a few drops of concentrated hydrochloric acid and make up the volume to 500 ml.

5. L. cystine solution (.5%)

Dissolve 4g of L-cystine in distilled water using a few drops of concentrated hydrochloric acid and make up the volume to 500 ml.

6. D.L. alanine solution (2%)

Dissolve 2 gm. of D.L. alanine in distilled water and make up the volume to 100 ml.

7. Xanthine solution (.2%)

Dissolve 200 mg. of xanthine in distilled water using a few drops of concentrated ammonia and make up the volume to 100 ml.

8. Adenine, guanine and uracil solution(.2%)

Dissolve 200 mg. each of adenine hydrocholoride, guanine sulphate and uracil in distilled water, using a few ml. of concentrated hydrochloric acid and make up the volume to 100 ml.

9. Peptone solution 4%.

Dissolve 4g of 'Difco' peptone in distilled water and adjust the PH of the solution to 3.0 using concentrated hydrochloric acid and make up the volume to 100 ml. Add to the solution 2 g of activated charcoal and filter. Repeat the treatment with charcoal and filter two more times.

10. Protein hydrolysate solution: (10% vitamin free).

Dissolve 100 g of 'Hydroprotein' (a protein hydrolysate available from Bengal immunity co. ltd. Calcutta), in about 800 ml distilled water with the aid of heat of necessary. Adjust the PH of the solution of 3.5, using 40% sodium hydroxide solution and make up the volume to 1 litre. Stir the solution with 20 g of activated charcoal for 30 minutes and filter. Repeat the charcoal treatment three more times (If the blank value i.e. the inoculated blank value is high the charcoal treatment may be done once more).

The protein hydrolysate solution suggested is an alternate for the casein hydrolysate normally used.

For preparation of the conventional Casein hydrolysate solution, see for example Methods of Vitamin Assay by Freed Nyer (1966). Published by Association of Vitamin chemists. Acid hydrolysed casein: Stir 100 g of 'Vitamin free' casein with 250 ml of 95 per cent ethyl alcohol for 15 minutes in an 800 ml beaker and filter with suction. Repeat using another 250 ml portion of alcohol. If 95 per cent ethyl alcohol is unavailable commercial brands of denatured alcohol can be used with satisfactory results. It has been found that some commercial brands of "Vitamin free" Casein contain enough niacin to give appreciable growth in the blanks, provided this extraction is not made. The alcohol treatment may be omitted if the casein is not to be employed in the niacin assay.

Transfer the alcohol-washed casein into a round-bottom flask of at least 1-litre capacity, preferably one having two neck ground to standard taper. Mix well with 500 ml of constant boiling HCl. Fit the flask with a glass stopper and a water cooled condenser and reflux over a low flame or hot plate for 8-12 hours. A mixture of one volume of concentrated HCl (37 per cent) with one volume of water gives a 20.1 per cent HCl which is satisfactory for the hydrolysis.

Since casein tends to froth during the initial stages of hydrolysis, heat carefully and gradually. Mix the content of the flask occasionally by shaking and have a wet towel ready to cool the flask if the reaction becomes too vigorous. After refluxing fit the flask with a condenser and receiving flask suitable for vacuum distillation and remove as much HCl as possible by concentrating the hydrolysate to a thick paste under reduced pressure. Air introduced through a bleeder tube placed well into the bottom of the flask will minimize bumping during the final stages of the concentration. The temperature at which the distillation is carried out should not exceed that of a boiling water bath. Temperature of 70°-80° have been recommended. To get rapid and complete distillation at this low temperature, it is necessary to reduce the pressure considerably. This may be possible with a water aspirator as a vacuum can be used. Care must be taken to trap HCl fumes effectively, especially with a ~~a~~ vacuum pump.

It is the usual custom to redissolve the paste in approximately 200 ml of water and repeat the concentration to remove additional amounts of HCl, however a satisfactory hydrolysate can be attained with a single concentration to a rather thick paste. In any case the acid concentration should be low enough so that subsequent neutralization will not yield enough salt to retard bacterial growth on the basal medium.

Dissolve the hydrolysate paste in about 700 ml. of water and adjust the PH to 3.5 with 40 per cent NaOH. Decolourise by stirring with 20g of activated charcoal. (eg. Norite A or Darco G-60) at room temperature. Stir until a small test filtrate is light straw coloured. The decolourisation may be complete in 5 minutes or may require more than an hour depending upon the charcoal used. This step removes any niacin which may have remained in the alcohol-washed casein. Some workers omit the preliminary alcohol wash and still obtain low blanks. Filter through a large fluted filter or by suction as preferred. The decolourisation treatment also removes residual folic acid.

Adjust the PH of the filtrate to 6.8, dilute to 1 litre and store under toluene and over chloroform in the refrigerator. Occasionally, a precipitate will form in this solution on standing. This is mainly tyrosine. It is a good practice to shake up this solution and use the suspended material as well as fluid portion. The insoluble material will dissolve when the entire medium is prepared.

11. Standard folic acid solution (stock):

Dissolve 10 mg of pure pteroyl glutamic acid (folic acid) in 100 ml of .8% sodium bi-carbonate solution. Store at 2-4°c.

12. Folic acid working standard:

(to be prepared on the day of use)

Dilute the stock standard folic acid solution to give a folic acid concentration 100 pg per ml.

0.1 ml of stock standard is diluted to 100 ml with distilled water. Then 0.1 ml of this solution is again diluted to 100 ml to give a concentration of 100 µg/ml.

13. Sodium phosphate buffer: 2M pH 6.1

Solution 1: 31.2 g of $NaH_2PO_4 \cdot 2H_2O$ in 1000 ml.

Solution 2: 24.8g of Na_2HPO_4 in 1000 ml. Mix 85 ml of solution 1 and 15 ml of solution 2.

14. Ascorbic acid phosphate buffer:

(to be prepared on the day of use)

Prepare a 5 per cent solution of ascorbic acid in water and stir the solution with activated charcoal (about 400 mg per 10 ml) for 15 minutes and filter. (An approximately 4 per cent solution would be obtained by this procedure.) Add to the filterate an equal volume of 0.2 M sodium phosphate buffer PH 6.1 with 10 per cent NaOH. This solution is further diluted two fold to obtained approximately 1 per cent ascorbic acid in 0.05 M phophate buffer.

Maintenance of culture:

The culture is maintained by fortnightly transfer in agar stands prepared as follows:

Dissolve:

Anhydrous glucose	: 10g
Sodium acetate $3H_2O$	: 17g
'Difco' peptone	: 5g
'Difco' yeast extract	1g

Salt solution	A	:	2.5 ml
Salt solution	B	:	2.5 ml

in about 400 ml distilled water. Adjust the solution to PH 6.8 using 40 per cent NaoH and make up the volume to 500 ml. To the above solution add 7.5 g agar and dissolve the agar by heating the mixture. While the solution is still hot, dispense approximately 10 ml of it to different test tubes (6½"). Plug the tubes with cotton and sterilise them by autoclaving for 15 minutes at 15 lbs pressure. Store the tubes at 2° - 4°c when not in use.

Preparation of Inoculum:

The inoculum is to be prepared on the day prior to the day of assay, by transferring cells from the stock culture tube to a sterile inoculum tube which is prepared in the same way as above but omitting addition of agar.

Preparation of Washed inoculum:

In order to wash the organism free of contamination with folic acid, transfer the incubated inoculum aseptically to a sterile 50 ml centrifuge tube plugged with cotton. The cotton should be placed and held in position by a rubber band so that it is not sunked into the tube during centrifugation.

Alternatively sterile screw capped centrifuge tubes can be used.

Centrifuge the inoculum and discard the supernatant. Suspend the cells in 10ml of sterile normal saline and centrifuge again. After repeating this process of washing two more times, finally suspend the cells in sufficient amount of sterile normal saline to yield a slightly opalescent suspension.

Preparation of basal medium (Double strength):

Glucose	:	4g
Sodium acetate 3 H_2O	:	6.6g
Protein hydrolysate solution	:	10 ml
L-tryptophan solution	:	10 ml
L-cystine solution	:	5 ml
DL-alanine solution	:	1 ml
Peptone solution	:	1 ml
Salt solution A	:	5 ml
Salt solution B	:	1 ml
Adenine, guanine, uracil solution	:	1 ml
Xanthine solution	:	1 ml
Vitamin solution	:	2 ml

Dissolve in about 70 ml of distilled water adjust to pH 6.8 using 40 per cent NaOH, make up the volume to 100 ml and filter.

PROCEDURE:

Preparation of food samples for assay:

The method of food folate assay was based on that of Lakshmiah and Ramakrishnan (1969) with slight modifications. Each food sample (5-10g) was homogenised in 40 ml of 0.2 M phosphate buffer.

Preparation of 2M Phosphate buffer:

$Na_2HPO_4 \cdot 2H_2O$ = 35.59g/lit

$NaH_2PO_4 \cdot 2H_2O$ = 31.20g/lit

Mix 720 ml of $Na_2HPO_4 \cdot 2H_2O$ + 280 ml of $NaH_2PO_4 \cdot 2H_2O$.

Add to this 1000 ml water

adjust PH to 7.2

Add 10 g of ascorbic acid to this.

PH 7.2, containing .5 per cent ascorbate. The volume was made upto 50 ml with the buffer. The homogenate was autoclaved at a pressure of 15 lbs. per inch for 15 minutes. After cooling to room temperature, the homogenate was adjusted to PH 4.5. The final volume of this extract was made upto 100 ml with distilled water and filtered.

A portion of this was used directly for the estimation of free folate activity after suitable dilution.

Determination of total folate in foods

For the determination of total folate activity, 1.0 ml of the above filtrate was diluted with 3.5 ml of 0.2 M acetate buffer PH. 4.5.

Preparation of Acetate buffer:

Sodium acetate anhydrous (Mol wt 82.05) 16.4 g/lit.

Glacial acetic acid 11.55 ml/lit

	Acetic acid	Sodium acetate
For pH 4.4	71.0 ml	39.0 ml.
pH 4.6	51.0 ml	29.0 ml.

Mix 710 ml of acetic acid and 390 ml of Sodium acetate. Dilute with 1000 ml distilled water. Adjust PH to 4.5.

and .5ml of 100 m mercaptoethanol (or 2 per cent ascorbic acid phosphate buffer). Finally .2ml of human plasma was added as a source of conjugase to hydrolyse polyglutamates to give total folate concentration of the test sample. The mixture was layered with a few drops of toluene and incubated overnight at 37°C. After incubation, the samples were diluted suitably before folate determination.

Assay procedure:

Standards

Set up eight duplicate sets of test tubes and to each add 1 ml of 0.25 per cent ascorbic acid phosphate buffer (prepared by diluting 4 fold the 1 per cent ascorbic acid phosphate buffer). Two sets of tubes serve as zero blanks (one as the uninoculated blank and one as the inoculated blank). To the other tubes add successively in duplicate, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 ml of the folic acid working standard (equivalent to 50,100, 150, 200, 300 and 400 pg of folic acid respectively per tube). Make up the volume in each of the tubes (including zero blank tubes to a total of 5 ml with distilled water).

Test solutions:

Five tubes would be required (singly or in duplicate) for each of the sample to be analysed. Add to these tubes successively 0.5, 1.0, 2.0, 3.0 and 4.0 ml of the diluted test food extract prepared as described earlier for urine, dilute suitably and use directly. Add 1 ml of .2% ascorbic acid phosphate buffer to all the tubes and make up the total volume to 5 ml with distilled water.

To all the tubes (standards and tests) add 5 ml of the double strength basal medium, plug the tubes with cotton and autoclave, then for 12 minutes at a pressure of 12 lbs per square inch. Cool the tubes to room temperature and inoculate each of the tubes, (except the set of tubes which served as uninoculated blanks) with a loop full of washed inoculum prepared as described earlier. Incubate the tubes at 37°C for 12-18 hours and at the end of this period measure the turbidity in a colorimeter using 660 mμ filter and setting up with the uninoculated blank. Plot the optical density the instrument to zero optical density against the concentration of folic acid in the standard and read off from the standard curve the concentration of folic acid in the test solution.

Calculation:

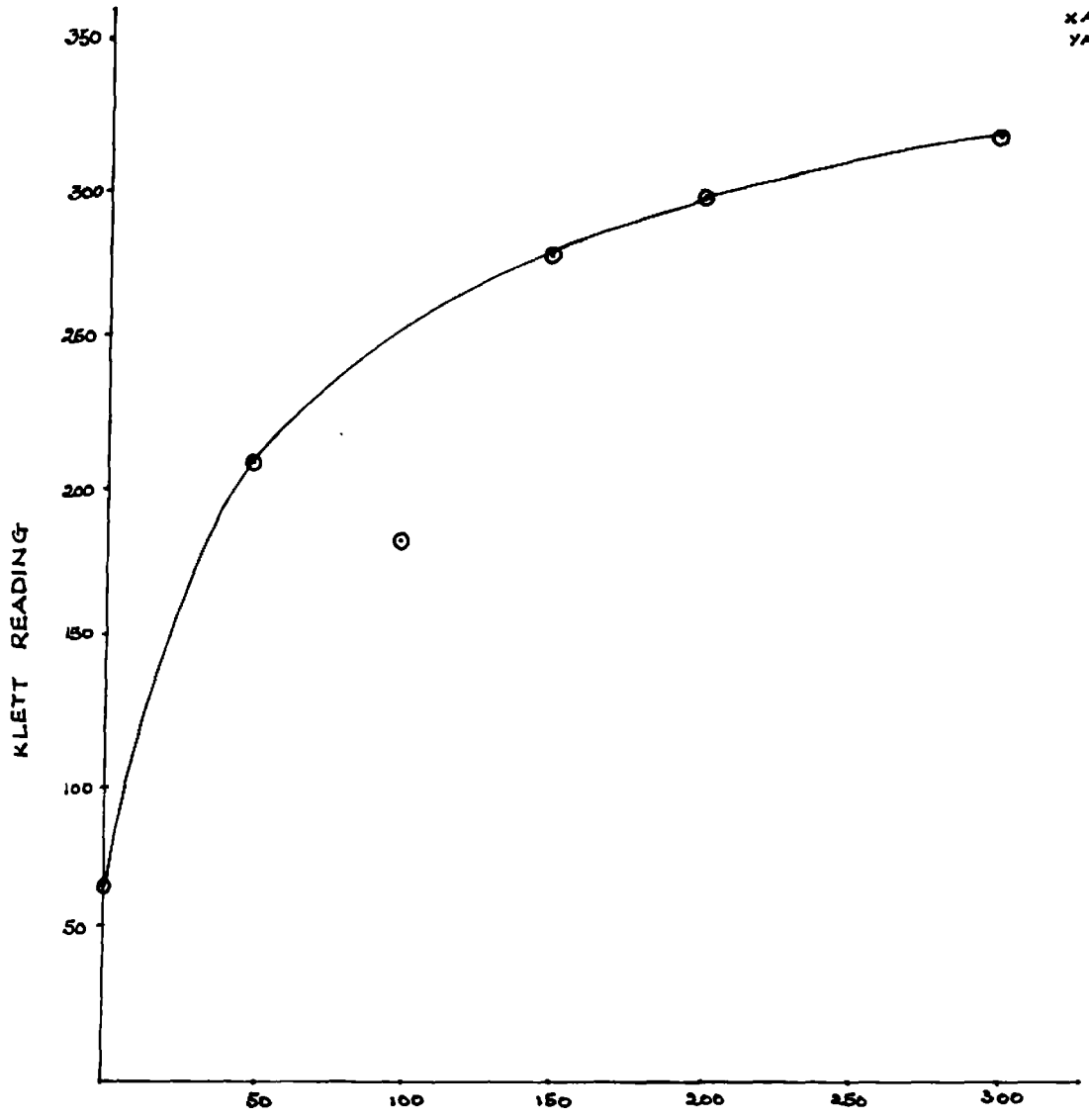
For a valid assay, the concentration per ml of the test solution at different levels should agree within 10-15 percent. Average the values obtained with different levels of the test solution and calculate folic acid concentration.

Folic acid content in 1g of food stuff = Concentration per ml of test solution X dilution factor X 100

Assay of folic acid in urine:

The samples were suitably diluted with distilled water for determination of urinary folate. No differences in urinary folate activity were found after treatment with plasma conjugase (Babu 1976).

Folic acid content in 1 ml of urine : Concentration per ml of test solution X dilution factor.



STANDARD CURVE FOR FOLIC ACID USING L. CASEI.

APPENDIX B

FOLIC ACID CONTENT OF FOODS AT DIFFERENT STAGES OF GERMINATION

(µg/100g)

Food Sample	Raw ungerminated		24 hours		48 hours		72 hours		96 hours	
	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Bengal Gram	18.6	197.9	41.1	282.8	56.4	321.7	55.8	386.0	112.4	320.5
	17.4	196.1	43.1	290.0	60.4	313.7	80.6	296.0	75.8	285.1
Green Gram	36.7	135.8	63.3	172.6	93.2	206.6	107.8	280.3	156.3	221.9
	14.3	105.2	34.5	130.0	78.4	185.6	122.8	220.9	104.5	268.1
Ragi	6.9	10.3	11.5	20.6	15.9	32.5	34.1	41.9	45.8	112.5
	3.9	6.1	7.1	15.8	30.3	80.1	86.7	100.3	92.2	126.9
Bajra	7.7	13.2	11.7	14.3	16.5	40.9	45.6	96.4	48.1	101.2
	12.7	17.0	26.3	26.1	26.3	59.5	76.2	125.4	92.5	109.2

APPENDIX C

CORRELATION TABLE FOR SPROUT LENGTH AND FOLIC ACID CONTENT.

			Total Folic acid Content in µg									
		MIDX	25	75	125	175	225	275	325	Σy	$v\Sigma y$	$v^2\Sigma y$	Σnv.
V_{xp}	1.75 / .50	MIDY	Length of sprout	0-50	50-100	100-150	150-200	200-250	250-300	300-350			
-3	.25	MIDY	0- .5	4	-	1	2	-	1	+ 8	-24	72	33
-2	.75	MIDY	.5-1.0	-	2	-	-	-	-	1 3	- 6	12	2
-1	1.25	MIDY	1.0-1.5	-	-	1	1	-	-	- 2	-2	2	1
0	1.75	MIDY	1.5-2.0	-	1	-	-	-	-	1 2	0	0	0
1	2.25	MIDY	2.0-2.5	-	-	-	-	-	-	- -	0	0	0
2	2.75	MIDY	2.5-3.0	-	-	-	-	-	1	- 1	2	4	4
3	3.25	MIDY	3.0-3.5	-	-	-	-	-	-	- -	0	0	0
	U_{xp}=175 / 50			-3	-2	-1	0	1	2	3 16	-30	90	40
		f_x		4	3	2	3	4	2	2 16			
		uf_x		-12	-6	-2	0	1	4	6 -9			
		u^2f_x		36	12	2	0	0	8	18 76			
		f_{xu}		36	8	4	0	0	-2	-6 40			

$$r_{xy} = \frac{n\sum xy - \sum x \sum y}{\sqrt{n\sum x^2 - (\sum x)^2 \cdot n\sum y^2 - (\sum y)^2}}$$

$$= \frac{16 \times 10 - (-9) \cdot (-30)}{\sqrt{16 \times 76 - (-9)^2 \cdot 16 \times 90 - (-30)^2}}$$

$$= \frac{640 - 270}{\sqrt{(1216 - 81)(1440 - 900)}}$$

$$= \frac{370}{\sqrt{1135 \times 540}}$$

$$= \frac{370}{\sqrt{612900}}$$

$$= \frac{370}{782.87}$$

$$= 472$$

APPENDIX D

PERCENTAGE FOLIC ACID AVAILABILITY FROM GERMINATED CEREALS

Food Under Test	Subjects	Urinary excretion of folate µg 24 hours			Mean folate excretion 50µg/24 hrs.	Availability per cent			Mean availability per cent
		1st day	2nd day	3rd day		1st day	2nd day	3rd day	
Germinated Ragi	1	536.9	511.7	548.6	546.4	62.5	57.2	46.9	48.8
	2	655.0	610.1	584.3	616.5	85.0	70.7	61.4	72.1
	3	486.9	415.8	470.9	447.9	19.0	5.4	25.7	16.0
	4.	562.1	586.4	556.4	568.3	54.0	62.0	52.1	59.3
	5	600.8	660.7	684.3	648.6	66.9	86.8	94.6	82.7
	6	419.8	446.5	501.3	455.8	6.7	15.5	33.7	18.6
				Mean=	547.0			Mean =	49.58±25.1
Germinated Ragi	1	608.8	648.5	616.3	624.5	69.6	82.8	72.1	74.8
	2	520.6	584.0	547.9	550.8	40.2	61.5	49.3	50.6
	3	580.6	554.5	538.4	557.8	60.2	51.4	46.1	52.9
	4	501.1	470.7	496.2	489.3	33.7	25.6	32.1	29.8
	5	535.4	546.5	580.7	554.1	45.1	48.8	60.2	51.3
	6	597.8	572.9	600.1	590.3	65.9	57.6	66.7	63.4
				Mean =	561.1			Mean =	53.8± 13.7

APPENDIX B

PERCENTAGE FOLIC ACID AVAILABILITY FROM GERMINATED PULSES

Food under Test	Subjects	Urinary Excretion of folate µg 24 hours			Mean folate excretion µg/24 hrs.	Availability per cent			Mean availability per cent
		1st day	2nd day	3rd day		1st day	2nd day	3rd day	
Germinated Bengal Gram	1	614.9	620.4	604.3	613.0	70.0	71.7	66.4	69.4
	2	690.5	702.0	660.9	684.5	94.6	98.3	84.98	92.6
	3	696.4	675.1	690.4	687.3	96.5	89.6	94.5	93.5
	4	425.4	478.9	520.6	475.96	9.25	25.7	39.2	24.7
	5	656.7	690.8	648.4	665.0	83.6	94.5	80.9	86.3
	6	502.4	576.8	598.9	586.0	59.4	57.6	64.78	60.6
					Mean = 618.63				Mean= 71.18± 23.97
Germinated Green Gram	1	650.4	699.8	655.0	655.2	83.1	86.4	84.9	84.8
	2	680.9	676.0	682.9	679.8	93.4	91.8	94.0	93.0
	3	638.4	654.1	690.9	660.8	79.3	84.5	96.4	86.7
	4	456.8	499.4	520.8	492.3	18.9	33.1	40.2	30.7
	5	689.2	614.4	649.6	651.1	96.2	71.3	83.0	83.5
	6	510.9	575.6	520.8	535.8	36.9	58.5	40.3	45.2
					Mean = 612.5				Mean= 70.6±25.54