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THE COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH, NEW DELHI

J. sci. industr. Res., Vol. 21C, No. 7, Pp. 167-192

JULY 1962

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Journal of

Scientific & Industrial Research

Vol. 21C, No. 7, JULY 1962

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J. sci. industr. Res., Vol. 21C, No. 7, Pp. 167-192. July 1962

Annual Subscription — All Sections (A, B, C and D): Rs 30 (Inland), £ 4 or $ 12.00 (Foreign). Individual Sections: Rs 10 (Inland), £ 1 or $ 3.00 (Foreign). Single Copies (Individual Sections): Re 1 (Inland), 2 sh. or 30 cents (Foreign)

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The Influence of Iron & Magnesium on Cobalt & Zinc Toxicities in Germinating Seedlings of *Phaseolus radiatus*

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Manuscript received 7 *February* 1962

The toxicity of zinc and cobalt at $10^{-3}M$ levels to young seedlings of green gram (*Phaseolus radiatus*) and the influence of iron and magnesium on the counteraction of toxicity have been studied. Magnesium has been shown to reverse the growth inhibition completely in zinc and partially in cobalt toxicities. Iron does not have a similar beneficial effect in the short-term experiments. The influence of iron and magnesium on the accumulation of zinc and cobalt has also been studied by the use of Zn^{65} and Co^{60}, in leaves, cotyledons and the stem.

THE prolonged administration of extra-physiological levels of a variety of heavy metals such as zinc, cobalt, nickel, manganese, molybdenum and copper has been shown to cause severe toxicity symptoms, closely resembling iron deficiency chlorosis, in plants[1,2]. In affected plant tissues, under such conditions, low iron and magnesium concentrations have been encountered[3]. Many of these investigations have been carried out in solution culture with long experimental periods extending up to 40 days. The leaf chlorosis has also been found to disappear when the leaves were painted with ferrous sulphate, indicating that some, at least, of the toxic effects are directly related to deranged iron metabolism[4]. Recent work with several lower organisms like *Neurospora crassa*[5], *Aspergillus niger*[6] and *Corcyra cephalonica* St.[7] has shown that the ability of iron and magnesium to counteract toxicities of zinc, cobalt and nickel is not only more generalized than recognized hitherto, but is also subject to species variation in finer details of mechanism. Since much of the data obtained with plants hitherto has been derived from long-term experiments, it has not been known how far such phenomena are prevalent in germinating seedlings. The present study was undertaken to examine trace element interrelationships in zinc and cobalt toxicities in germinating green gram seedlings, from the standpoint of growth and toxic metal accumulation.

Experimental procedure

Materials — Healthy green gram seeds (*Phaseolus radiatus*) were used throughout this investigation. Iron was supplemented as ferric ammonium citrate (Fischer Scientific Co., U.S.A.), zinc as zinc sulphate (Merck), cobalt as cobalt chloride (B.D.H.) and magnesium as magnesium sulphate (Merck). All chemicals were of analytical grade. Glass distilled water was used for making all solutions.

Germination technique — Seeds (2 g.) were sterilized and allowed to grow for 72 hr in 9 cm. petri dishes, containing 10 ml. of culture solution. Control seedlings were grown on water alone. Details of germination technique were identical with those employed in earlier studies[8].

Assay of growth — After 72 hr of germination, seedlings were removed, washed thoroughly with water till the washings were free of radioactivity when the latter was present in the medium and

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quickly dissected by snipping off the cotyledons at the point of attachment to the hypocotyl. The hypocotyl itself was then cut into two portions at the base of the leaves. The three parts of the seedlings were separately pooled, allowed to dry to constant weight at 60°C. in an oven and weighed accurately. In all experiments, growth was determined on the basis of dry weight of material.

Radioactive technique — The accumulation and distribution of toxic metals was determined by conducting the growth of the seedlings in culture solutions in which radioactive Co^{60} or Zn^{65} was included. In such experiments 1 μc. each of Co^{60} (tracer-free) or Zn^{65} (100 mc./g.) was provided in 10 ml. of culture solution. Both the isotopes were purchased from the Radiochemical Centre, Amersham, Bucks, U.K. Allowance was made for the amount of metal added along with the isotope where necessary.

After the growth period, seedlings were extensively washed till free of radioactivity and individual tissues dried and weighed as described earlier. The dried samples were ground to a fine powder, weighed aliquots transferred to pyrex test tubes of uniform thickness and counted in a DSS-5 well type scintillation counter attached to a Nuclear-Chicago decade scaler (model 161A, Nuclear-Chicago Corporation, U.S.A.). Counting of samples was always carried out for adequate time intervals, depending on the count rate of the sample, to an accuracy of ± 2 per cent.

Results and discussion

The present study was primarily directed to examine the influence of iron and magnesium on cobalt and zinc toxicities in germinating seedlings grown for a total period of 72 hr. Under these conditions, it is well known that the mechanism of growth involves a mobilization of reserve food materials stored in the cotyledons for the growth and metabolism of the growing plant[9]. It was desired to find out whether metal interrelationships of the nature encountered by earlier workers[1-4] in the case of plants grown for prolonged periods in solution culture were also prevalent under the present experimental conditions. All experiments were repeated a minimum of four times and typical data are recorded in Tables 1 and 2. The results indicate the patterns of growth obtained in zinc and cobalt toxicities respectively. The levels of cobalt and zinc employed were arrived at from preliminary studies and were found to cause pronounced toxicity during the experimental period as reflected by general appearance and size of the seedlings. Similarly, the level of magnesium employed herein was

TABLE 1 — INFLUENCE OF IRON AND MAGNESIUM ON GROWTH OF *P. RADIATUS* SEEDLINGS IN ZINC TOXICITY

Supplement	Dry wt, *mg.*			Total wt *g.*
	Leaves	Cotyledons	Stem	
None (control)	96	789	463	1·348
Zinc ($10^{-3}M$)	80	862	425	1·367
Zinc ($10^{-3}M$) + magnesium ($10^{-3}M$)	96	757	491	1·344
Zinc ($10^{-3}M$) + iron ($10^{-3}M$)	63	899	408	1·370

TABLE 2 — INFLUENCE OF IRON AND MAGNESIUM ON GROWTH OF *P. RADIATUS* SEEDLINGS IN COBALT TOXICITY

Supplement	Dry wt, *mg.*			Total wt *g.*
	Leaves	Cotyledons	Stem	
None (control)	91	792	475	1·358
Cobalt ($10^{-3}M$)	54	1022	312	1·388
Cobalt ($10^{-3}M$) + magnesium ($2{\cdot}5 \times 10^{-3}M$)	78	883	406	1·367
Cobalt ($10^{-3}M$) + iron ($10^{-3}M$)	55	1003	337	1·395

the most optimal one judged by similar criteria, and in both metal toxicities, the magnesium-supplemented seedlings were nearly indistinguishable from control seedlings. Iron was tested at $10^{-3}M$, though this was possibly not the most optimal one, since it has been reported by Crooke[10] that in metal toxicities a 1:1 ratio between toxic metal and iron was the one demonstrating best antagonism. An analysis of the growth data (Tables 1 and 2) shows a decrease in growth of the hypocotyl and a parallel increase in the weight of the cotyledons in both zinc and cobalt toxicities, when the values of the toxic seedlings are compared with controls. It may also be seen that in both cobalt and zinc toxicities, supplementation with magnesium restores the weights of individual tissues to values close to controls. Iron does not have a similar effect in either toxicity. Another point of interest is that the changes observed are much more pronounced when individual weights of leaves, cotyledons and stems are compared rather than when total growth is examined, pointing to the inhibitory effect of toxic metals on the utilization of seed reserves for plant growth.

The distribution and overall uptake of toxic metals by the seedlings under present experimental conditions are shown in Tables 3 and 4. The data presented in Table 3 show that, even when magnesium reverses the growth picture of zinc toxicity

TABLE 3 — INFLUENCE OF IRON AND MAGNESIUM ON Zn^{65} DISTRIBUTION IN *P. RADIATUS* SEEDLINGS IN ZINC TOXICITY

Supplement	Leaves*			Cotyledons			Stem			Total uptake	
	Counts/min. $\times 10^3$	Zinc $\mu g.$	Deviation from toxic %	Counts/min. $\times 10^3$	Zinc $\mu g.$	Deviation from toxic %	Counts/min. $\times 10^3$	Zinc $\mu g.$	Deviation from toxic %	Counts/min. $\times 10^3$	Zinc $\mu g.$
None (control)†	19·00	—	—	14·40	—	—	14·60	—	—	48·00	—
Zinc ($10^{-3}M$)	18·00	32·28	—	13·40	24·20	—	13·00	23·79	—	44·40	80·27
Zinc ($10^{-3}M$) + magnesium ($10^{-3}M$)	17·00	30·07	−6·78	11·80	21·34	−11·80	15·30	27·69	+12·19	44·10	79·10
Zinc ($10^{-3}M$) + iron ($10^{-3}M$)	16·70	30·20	−6·64	18·10	32·20	+33·05	11·10	20·07	−15·30	45·90	82·47

*All data on the basis of values calculated per 100 mg. dry weight.
†Control medium contained Zn^{65} isotope only at a level of 1 μc. ($3{\cdot}61 \times 10^5$ counts/min.); in all other cases, carrier zinc was provided to achieve the concentration of metal indicated.

TABLE 4 — INFLUENCE OF IRON AND MAGNESIUM ON Co^{60} DISTRIBUTION IN *P. RADIATUS* SEEDLINGS IN COBALT TOXICITY

Supplement	Leaves*			Cotyledons			Stem			Total uptake	
	Counts/min. $\times 10^3$	Cobalt $\mu g.$	Deviation from toxic %	Counts/min. $\times 10^3$	Cobalt $\mu g.$	Deviation from toxic %	Counts/min. $\times 10^3$	Cobalt $\mu g.$	Deviation from toxic %	Counts/min. $\times 10^3$	Cobalt $\mu g.$
None (control)†	17·10	—	—	16·90	—	—	16·30	—	—	50·30	—
Cobalt ($10^{-3}M$)	28·90	45·50	—	15·80	23·05	—	18·80	29·00	—	63·50	97·55
Cobalt ($10^{-3}M$) + magnesium ($2{\cdot}5 \times 10^{-3}M$)	23·70	37·06	−15·00	14·80	22·80	−1·07	15·40	23·74	−18·00	53·90	83·60
Cobalt ($10^{-3}M$) + iron ($10^{-3}M$)	30·00	46·20	+3·82	16·00	24·60	+6·70	18·90	29·10	+0·30	64·90	99·90

*All data on the basis of values calculated per 100 mg. dry weight.
†Control medium contained Co^{60} isotope only at a level of 1 μc. ($3{\cdot}82 \times 10^5$ counts/min.); in all other cases, carrier cobalt was provided to achieve the concentration of metal indicated.

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completely, the total uptake by the whole seedling is not markedly affected and that the control is again manifest more significantly in the individual values shown for leaves and cotyledons. The small decreases observed are probably indicative of the existence of a control process which might be more marked when examined at an intracellular level than is apparent from the gross picture. It may be pointed out in this connection that, in metal toxicities, localized decreases of magnesium concentration are more pronounced in necrotic areas of plant tissues[3]. Accordingly, the trend of values, rather than their magnitude, would appear to have greater significance. The data given in Table 4 again reflect a similar phenomenon in cobalt toxicity. The effect of magnesium is seen to be reflected almost entirely in the leaves and stem regions than in cotyledons, which show little deviation from corresponding toxic tissues. Support for this interpretation is provided by the absence of such an effect of iron in cobalt toxicity, in line with its inability to ounteract symptoms of toxicity.

Another feature brought out by these studies is that the responses of plants to iron and magnesium are markedly different in nature in short-term experiments than from those observed when the metals are supplied at lower concentrations for extended periods of time. The significance of this difference is probably related to the dependence of metal interactions on the nature of the metabolic processes occurring in the plants. As already pointed out, under the conditions of the present study, the growth is primarily at the expense of seed reserve nutrients which are translocated to the hypocotyl. On the other hand, in extended growth periods in solution culture, different mechanism of cell material formation is involved.

It would also appear that, under the present experimental conditions, magnesium is a more powerful antagonist of cobalt and zinc, than iron, which probably acts more slowly. More extensive investigations would be required to elucidate the differences in the mechanism of action of these two elements.

Acknowledgement

Thanks are due to the Council of Scientific & Industrial Research, New Delhi, for financial assistance during the course of these investigations.

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Effect of Rice Bran Oil on Cholesterol Metabolism in Albino Rats

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Manuscript received 7 *February* 1962

Rice bran oil has been found to be effective in preventing the accumulation of cholesterol in the tissues of rats fed hydrogenated fat at 25 per cent level. The mechanism of action of rice bran oil appears to be similar to that reported for corn oil, in that it stimulates both synthesis and degradation of cholesterol.

THE biochemical aspects of atherosclerosis have been the subject for extensive investigation in recent years. The implication of cholesterol as the primary factor for the causation of atherosclerosis is borne out by (i) predominance of it in atherosclerotic plaque and (ii) the inducement of arterial lesions resembling human disease in a variety of animals by simply elevating serum cholesterol by dietary and other means. Moreover, most atherosclerotic patients have high serum cholesterol and conditions such as myxoedema, nephrosis, Cushing's syndrome and familial hypercholesteremia, characterized by elevated serum cholesterol, are associated with high incidence of atherosclerosis. It has been generally recognized that hypercholesteremia and its duration are the two main factors determining the gradual development of this disease.

The studies of Keys and Anderson[1,2] demonstrated the correlation between prevalence of coronary heart disease and fat content of diet. Both the quality and quantity of fat appear to be partly, if not wholly, responsible for the plasma cholesterol concentration. Rutstein *et al.*[3] have shown that stearic acid promoted an increase in the intracellular deposition of lipid by tissue cultures of human aortic cells when added to the culture medium containing cholesterol, whereas linoleic acid inhibited the lipid deposition. In general, fats with high iodine number promoted a fall in plasma cholesterol and fats with low iodine number enhanced serum cholesterol level, thereby creating an environment favourable for the onset of atherosclerosis[4,5]. There is strong experimental evidence that fats like corn oil arrest the increase, due to dietary defects, of serum cholesterol level because of the unsaturated fatty acid and the anticholesterogenic factor that they contain[6]. Rice bran oil on an average contains 33 per cent linoleic acid and may act in a manner similar to that of corn oil.

The present investigation was, therefore, undertaken with a view to ascertaining whether (i) feeding of hydrogenated fat at 25 per cent level would enhance the level of cholesterol in the various tissues of albino rats and (ii) to study the effect of supplementing such a diet with rice bran oil.

Materials and methods

Young male albino rats weighing 30-40 g. were divided into three groups, each consisting of ten rats. The rats were caged individually and were fed the different experimental diets *ad libitum*, for 8 weeks. The various experimental diets were as follows: Group I: Basal diet plus 8 per cent hydrogenated fat. (The basal diet had the composition: sugar, 70; casein, 18; and salt mixture[7], 4 per cent. To 1 kg. batch of the diet were added the following vitamin supplements: thiamin hydrochloride, 12·5 mg.; inositol, 250 mg.; pyridoxine hydrochloride, 12·5 mg.; riboflavin, 25 mg.; calcium pantothenate, 62·5 mg.; niacin, 250 mg.; *p*-aminobenzoic acid, 125 mg.; vitamin K, 12·5 mg.; and vitamins A and D as adexolin, 1 ml.). Group II: Basal diet plus 25 per cent hydrogenated fat. Group III: Basal diet plus 25 per cent hydrogenated fat plus 10 per cent rice bran oil.

The diets were made isocaloric by substituting the fat for carbohydrates. The hydrogenated fat used was a gift from Messrs Hindustan Lever Ltd, Bombay, and rice bran oil was a gift from the Director, Central Food Technological Research Institute, Mysore. The percentage fatty acid composition of rice bran oil as given by the Central Food Technological Research Institute, Mysore, is as follows: myristic, 0·2; palmitic, 12-17·3; stearic, 1·2-2·6; arachidonic, 0·5; behenic, 0·5; lignoceric, 0·7; oleic, 41-45·6; linoleic, 27·6-36·7; and unsaponifiable matter, 4-5·7.

At the end of the experimental period, the animals were ether anaesthetized[8] and blood was withdrawn by heart puncture. Liver and carcass were saved for analysis. Liver lipid was extracted by the method of Handler[9] and carcass by the method of McHenry and Gavin[10]. Cholesterol was estimated according to the method of Sperry and Webb[11].

In vivo incorporation of acetate-1^{14}C into liver cholesterol and fatty acids — At the end of the experimental period two of the ten rats in each group were administered 20 μc. of acetate-1^{14}C (purchased from Radio Chemical Centre, Amersham, U.K.) by intraperitoneal injection. The animals were sacrificed after 4 hr, livers were removed and cooled immediately in a mixture of salt and crushed ice. Cholesterol was isolated and purified by methods described previously[7]. The cholesterol was plated using a stainless steel planchet, and radioactivity was determined in a shielded Geiger tube connected to a scaler of Nuclear Instruments, Chicago.

Results

The data obtained on liver weight, level of cholesterol in serum, liver and carcass and fatty acid in the liver are presented in Table 1. A slight elevation of serum cholesterol level was observed in rats in group II, as well as an accumulation of cholesterol in the liver and carcass. The cholesterol levels in rats of group III were found to be the same as in the control group which indicated that rice bran oil added in the diet of these rats had restored the cholesterol concentration to normal in all the three tissues tested.

The results obtained on the *in vivo* incorporation of acetate-1^{14}C into liver cholesterol and fatty acid are given in Table 2. The specific activity values for both cholesterol and fatty acid of group II were lower than in group I suggesting an inhibition of synthesis of these compounds in the liver. The inhibition of fatty acid synthesis was quite clear and significant as both specific activity and total incorporation were decreased. However, in the case of cholesterol, the total incorporation was not decreased. It may be that the synthesis was not suppressed to such an extent as to affect the total incorporation. Nevertheless, there was a significant increase in both specific activity and total incorporation in rats of group III when compared with either group I or group II indicating an accentuation of cholesterol biosynthesis.

Discussion

Hepatic synthesis of cholesterol and fatty acid from acetate was considerably influenced by the nutritional status of the animal. Hydrogenated fat, at a level of 25 per cent, not only elevated cholesterol

TABLE 1 — EFFECT OF HYDROGENATED FAT AND RICE BRAN OIL ON LIVER WEIGHT AND CHOLESTEROL CONTENT OF SERUM, LIVER, CARCASS AND LIVER FATTY ACID*

Group	Diet	Serum total cholesterol *mg./100 ml.*	Liver wt *g.*	Total cholesterol *mg. % wet liver*	Total fatty acid *% wet liver*	Carcass total cholesterol *mg. % wet wt*
I	Basal diet + 8% hydrogenated fat	75±2·1	5·46±0·5	257·0±11·8	6·01±1·0	222±5·2
II	Basal diet + 25% hydrogenated fat	95±6·8	6·46±0·9	449·0±15·1	8·40±1·5	300±8·9
III	Basal diet + 25% hydrogenated fat + 10% rice bran oil	80±3·2	6·00±0·6	316·6±11·1	6·44±1·1	186±2·1

*Values are the average of ten rats.

TABLE 2 — *IN VIVO* INCORPORATION OF ACETATE-1^{14}C INTO LIVER CHOLESTEROL AND FATTY ACID OF RATS FED HYDROGENATED FAT AND RICE BRAN OIL*

Group	Diet	Cholesterol			Fatty acid		
		Mg./liver	Sp. activity *c.p.m./mg.*	Total counts per liver	Mg./liver	Sp. activity *c.p.m./mg.*	Total counts per liver
I	Basal diet + 8% hydrogenated fat	14·05	30	421	328·2	77	25270
II	Basal diet + 25% hydrogenated fat	29·00	25	725	542·5	21	11400
III	Basal diet + 25% hydrogenated fat + 10% rice bran oil	19·00	45	855	386·5	78	30150

*The activity values are corrected for self-absorption and background. The values are the average of two rats. A similar pattern was obtained when the above experiments were repeated.

and fatty acid concentrations in the tissues, but also depressed hepatic synthesis of these compounds, notably that of fatty acid. Perhaps the elevation itself was partly responsible for the depression of the synthesis in an attempt by the organism to maintain normal levels of lipids in the tissues. It is known that inclusion of cholesterol in the diet has a pronounced effect in suppressing the rate of cholesterol synthesis. The rate of synthesis of cholesterol has been reported to be depressed in proportion to the quantity of dietary cholesterol[12]. Similar observations were made in starvation and in feeding a diet devoid of fat[13]. Data presented in this investigation for hydrogenated fat were in good agreement with those reported for coconut oil by Mukherjee and Alfin-Slater[13]. Rice bran oil was able to arrest the accumulation and correct the suppression of hepatic synthesis produced by hydrogenated fat.

The effect of rice bran oil on carcass cholesterol level was particularly remarkable. Though the level of cholesterol in tissues was maintained at a normal level, higher specific activity for liver cholesterol than for the control rat suggested accentuation of cholesterol biosynthesis. The maintenance of normal lipid level, despite enhanced synthesis, would be possible only when the catabolism of cholesterol was also increased. It appeared as though rice bran oil stimulated both catabolism and anabolism of cholesterol in the rat. The studies of Mukherjee and Alfin-Slater[13], Avigan and Steinberg[8] and Merrill[14] demonstrated that corn oil and other unsaturated fatty acids like linoleic acid capable of lowering serum cholesterol level increased both the incorporation of acetate into liver cholesterol as well as faecal excretion of sterols and bile acids[14,15]. It is quite probable that rice bran oil which contains significant amounts of linoleic acid also exerted, in a similar manner, its cholesterol-depressing action in tissues by stimulating both the synthesis and degradation of cholesterol. Whether the action of rice bran oil would be solely attributable to its linoleic acid content, or whether any other lipotropic factor is present in it, is yet to be elucidated.

Acknowledgement

The authors are thankful to the Vanaspati Manufacturers' Association of India, Bombay, for financial assistance.

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Terramycin & Growth: Part V—A Study of Amino Acid Utilization from a Rice-Legume Diet

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Manuscript received 4 *March* 1960†

The influence of terramycin and/or vitamin B_{12} and PGA on liver, plasma and urinary amino acids has been studied in rats reared on a rice-Bengal gram diet at 10 per cent protein level. The addition of terramycin or vitamin B_{12} and PGA to the diet increases the concentration of essential amino acids in the liver and decreases it in plasma; together they enhance this effect. Feeding of terramycin and vitamin B_{12} and PGA significantly decreases the urinary excretion of amino acids, the effect being somewhat additive.

IN a study with various antibiotics fed to rats, Sauberlich[1] found that they spared the requirements for amino acids such as isoleucine, valine, threonine and lysine. A sparing action of antibiotics for methionine[2,3] and tryptophan[4] in rats and in chicks[5] was also reported by others. The growth response obtained on addition of terramycin or vitamin B_{12} and PGA to a rice-legume diet reported earlier[6] would also indicate improvement in amino acid utilization. This has been ascertained by a more direct study of liver and plasma and urinary amino acid patterns in these animals.

Experimental procedure

Male weanling rats (laboratory-bred; Wistar origin), weighing about 40 g., were used. They were divided into four groups of six animals each and were fed *ad libitum* a basal rice-legume diet consisting of 60 per cent rice and 30 per cent Bengal gram (*Cicer arietinum*) with other additions as described earlier[6]. There were groups receiving supplements of terramycin (250 mg./kg.) and/or vitamin B_{12} (200 μg./kg.) and PGA (1 mg./kg.) as detailed previously[7]. The animals were weighed twice weekly and a record of daily food intake was maintained.

Urine samples were collected during the seventh and eighth week of experiments under toluene with usual precautions. Two separate 24 hr collections were made during each of the two-week period from individual rats.

At the end of eight weeks, all rats were sacrificed under ether anaesthesia. Livers were quickly removed, chilled in cracked ice and homogenized to give a 20 per cent suspension in ice-cold distilled water. Blood was withdrawn by a syringe from the portal vein, heparinized and centrifuged immediately in the cold to separate the plasma.

For determination of free amino acids, protein-free liver and plasma samples were obtained by the methods described earlier[8]. The urine samples were diluted as necessary and adjusted to *p*H 7·0.

Total amino acids in the diet were assayed in neutralized samples after acid hydrolysis; enzymic (pepsin and trypsin) hydrolysates were used for tryptophan assay[9].

The amino acids were assayed microbiologically using the media, organisms and procedures described by Barton-Wright[9]. The L-forms of the amino acids were used as standards. Where these were not available, synthetic DL-forms were used. The values are reported in terms of the L-amino acids and have been halved where racemic standards were used. Results reported are averages (± standard error of the mean) from determinations with each of the six animals in each group.

Results

Growth data, along with values for average daily food intake, are given in Fig. 1. These are consistent with observations reported earlier[6].

The incorporation of terramycin or PGA and vitamin B_{12} into the basal diet generally caused increase in free essential amino acids in the liver (Table 1); the plasma concentrations were decreased (Table 2). Supplementation with both antibiotic and vitamins enhanced these effects.

Values for urinary excretion of essential amino acids are reported in Table 3 and in Table 4 are summarized the data on amino acid composition of

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†Revised manuscript received 17 April 1962.

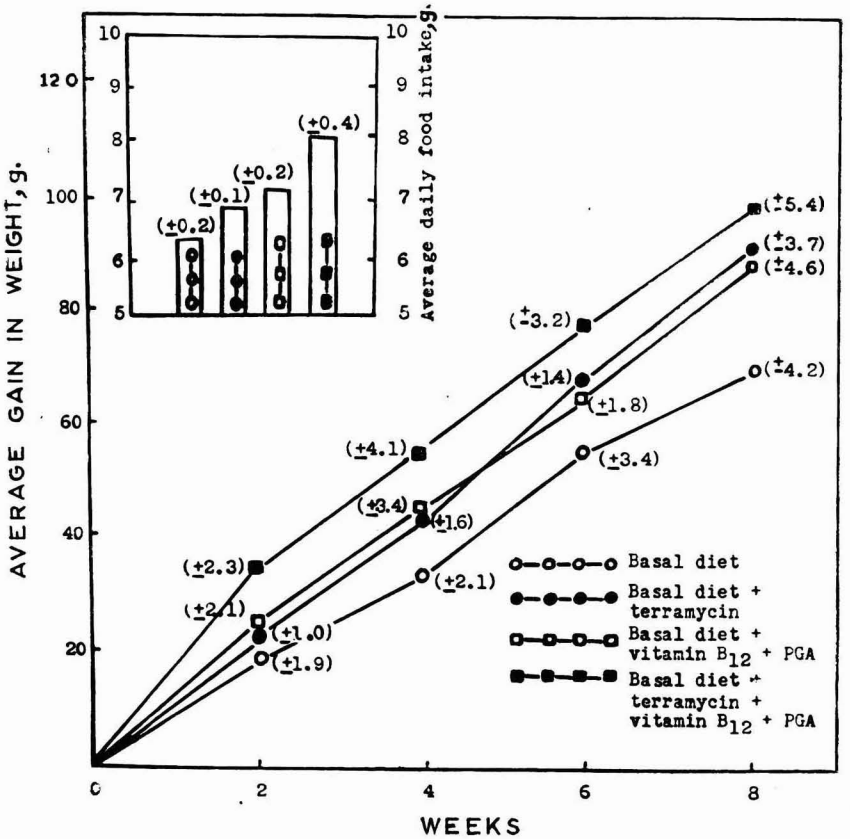

Fig. 1 — Growth differences and food intake of rats fed on different diets

basal diet, their average daily ingestion by animals in the different groups and the percentages of ingested amino acids excreted in urine. The results show that there is a marked decrease in the urinary excretion of amino acids in the groups fed the antibiotic or vitamins as compared to the control group; this excretion is reduced still further in the groups with combined feeding of terramycin and vitamin B_{12} and PGA.

Discussion

It was shown earlier[8] that addition of terramycin and/or vitamin B_{12} and PGA to a legume (Bengal gram) diet resulted in increased liver level of methionine and a decrease in this amino acid in plasma. Similar results observed with all the essential amino acids (Tables 1 and 2) are in conformity with this finding.

A marked decrease in amino acids excretion is observed in groups fed antibiotic in the diet as compared with the control group. This could possibly be due to higher intake of protein in this group. However, earlier experiment revealed an improvement in nitrogen efficiency on terramycin feeding. Inclusion of both antibiotic and vitamins reduces the urinary excretion of amino acids still further. The decrease, however, was not significant in the case of threonine, phenylalanine and leucine. Although no attempt was made to study the absorption of amino acids in different groups, there is reason to feel that in view of the thinning of the intestinal wall as a result of antibiotic feeding observed in earlier experiments[7] and by other workers[10], there could be greater absorption of amino acids on antibiotic feeding. Baliga and Rajagopalan[11] observed that the concentration of amino acids in the urine per gram of protein intake was lower in rats receiving vitamin B_{12} in the diet as compared to the deficient group. The decreased urinary excretion in the groups fed the vitamins may be due in part to the higher protein intake in the

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TABLE 1 — CHANGES IN CONCENTRATION OF FREE AMINO ACIDS IN LIVER

(*Amino acids expressed in μg. per g. of fresh liver*)

Supplements to basal diet	Lysine	Histidine	Methionine	Tryptophan	Threonine	Phenylalanine	Leucine	Isoleucine	Valine	Arginine
None	136·8 ±3·82	101·4 ±2·80	59·6 ±1·22	15·1 ±0·25	60·1 ±1·43	71·1 ±1·94	119·5 ±2·52	58·6 ±0·84	74·0 ±1·86	42·3 ±0·65
Terramycin	151·7 ±2·63	117·6 ±3·12	68·4 ±0·91	18·2 ±0·32	71·4 ±1·21	82·7 ±2·31	131·4 ±3·12	65·5 ±0·87	87·4 ±2·62	49·7 ±0·74
Vitamin B_{12} + PGA	154·1 ±2·84	124·2 ±4·15	70·5 ±1·10	19·4 ±0·35	74·6 ±1·86	85·6 ±1·47	136·2 ±2·34	67·8 ±0·91	86·5 ±1·64	51·2 ±0·71
Terramycin + vitamin B_{12} + PGA	162·6 ±3·61	127·6 ±3·36	73·3 ±0·95	21·1 ±0·45	79·4 ±2·12	91·7 ±2·92	142·5 ±3·06	72·1 ±1·12	95·4 ±2·92	54·4 ±0·78

L-Forms of lysine, histidine and arginine, and DL-forms of methionine, tryptophan, threonine, phenylalanine, leucine, isoleucine and valine were used as standards. Values expressed are, however, in terms of the L-amino acids (50 per cent in the case of racemic standards).

TABLE 2 — CHANGES IN CONCENTRATION OF FREE AMINO ACIDS IN PLASMA

(*Amino acids expressed in μg. per ml. of plasma*)

Supplements to basal diet	Lysine	Histidine	Methionine	Tryptophan	Threonine	Phenylalanine	Leucine	Isoleucine	Valine	Arginine
None	60·4 ±0·15	16·8 ±0·08	7·4 ±0·06	14·7 ±0·09	59·5 ±0·17	15·6 ±0·17	35·4 ±0·62	48·7 ±0·74	24·9 ±0·21	12·2 ±0·08
Terramycin	51·1 ±0·22	11·2 ±0·10	5·8 ±0·05	9·4 ±0·06	40·2 ±0·45	13·1 ±0·12	29·2 ±0·41	43·4 ±0·55	21·7 ±0·18	9·8 ±0·03
Vitamin B_{12} + PGA	46·6 ±0·24	9·4 ±0·06	5·5 ±0·06	8·2 ±0·12	38·6 ±0·52	13·2 ±0·08	28·6 ±0·23	40·1 ±0·52	19·4 ±0·19	9·2 ±0·05
Terramycin + vitamin B_{12} + PGA	42·4 ±0·13	8·6 ±0·05	4·2 ±0·02	6·2 ±0·08	35·2 ±0·32	11·6 ±0·04	24·1 ±0·34	38·1 ±0·48	18·5 ±0·16	8·1 ±0·02

L-Forms of lysine, histidine and arginine, and DL-forms of methionine, tryptophan, threonine, phenylalanine, leucine, isoleucine and valine were used as standards. Values expressed are, however, in terms of the L-amino acids (50 per cent in the case of racemic standards).

TABLE 3 — CHANGES IN URINARY EXCRETION OF AMINO ACIDS

(*Amino acids excreted expressed in mg. per day*)

Supplements to basal diet	Lysine	Histidine	Methionine	Tryptophan	Threonine	Phenylalanine	Leucine	Isoleucine	Valine	Arginine
None	0·20 ±0·03	0·42 ±0·06	0·29 ±0·04	0·15 ±0·02	0·68 ±0·03	0·79 ±0·07	1·24 ±0·04	0·69 ±0·05	0·77 ±0·06	0·58 ±0·01
Terramycin	0·16 ±0·01	0·23 ±0·03	0·21 ±0·02	0·09 ±0·01	0·64 ±0·02	0·48 ±0·05	0·83 ±0·03	0·48 ±0·02	0·45 ±0·03	0·56 ±0·02
Vitamin B_{12} + PGA	0·17 ±0·01	0·26 ±0·02	0·18 ±0·01	0·11 ±0·01	0·61 ±0·02	0·41 ±0·01	0·89 ±0·01	0·50 ±0·01	0·48 ±0·03	0·55 ±0·01
Terramycin + vitamin B_{12} + PGA	0·14 ±0·02	0·18 ±0·02	0·15 ±0·02	0·08 ±0·01	0·62 ±0·03	0·41 ±0·02	0·88 ±0·01	0·46 ±0·02	0·33 ±0·04	0·52 ±0·01

L-Forms of lysine, histidine and arginine, and DL-forms of methionine, tryptophan, threonine, phenylalanine, leucine, isoleucine and valine were used as standards. Values expressed are, however, in terms of the L-amino acids (50 per cent in the case of racemic standards).

TABLE 4 — CHANGES IN AMINO ACID UTILIZATION

Amino acid	Amino acid composition of basal diet %	Supplements to basal diet							
		None		Terramycin		Vitamin B_{12} + PGA		Terramycin + vitamin B_{12} + PGA	
		Amino acid ingested per day *mg.*	Ingested amino acid excreted %	Amino acid ingested per day *mg.*	Ingested amino acid excreted %	Amino acid ingested per day *mg.*	Ingested amino acid excreted %	Amino acid ingested per day *mg.*	Ingested amino acid excreted %
Lysine	0·56	31·8±0·26	0·63	37·1±0·34	0·43	38·1±0·22	0·44	42·4±0·56	0·33
Histidine	0·25	13·2±0·15	3·18	15·4±0·21	1·81	15·8±0·18	1·74	17·6±0·23	1·02
Methionine	0·23	16·2±0·12	1·79	18·9±0·35	1·11	19·4±0·27	0·93	21·6±0·32	0·69
Tryptophan	0·11	6·0±0·05	2·50	7·0±0·08	1·28	7·2±0·06	1·52	8·0±0·11	1·00
Threonine	0·47	29·4±0·28	2·34	34·3±0·41	1·86	35·2±0·36	1·73	39·2±0·51	1·57
Phenylalanine	0·55	33·6±0·32	2·35	39·2±0·16	1·25	40·3±0·25	1·01	44·0±0·64	0·91
Leucine	0·87	51·6±0·72	2·40	60·2±0·42	1·37	61·9±0·25	1·43	68·8±0·81	1·28
Isoleucine	0·70	37·8±0·46	1·82	44·1±0·35	1·09	45·3±0·42	1·10	50·4±0·56	0·91
Valine	0·68	39·0±0·23	1·97	45·5±0·37	0·98	46·8±0·28	1·02	52·0±0·42	0·63
Arginine	0·79	46·8±0·62	1·23	54·6±0·33	1·02	56·1±0·21	0·99	60·4±0·44	0·86

diets. It is known that both in rats and mice there is increased excretion of amino acids when dietary protein is deficient in essential amino acids than when the protein is biologically adequate[12,13] and that the percentage of ingested amino acid excreted on a high protein diet is invariably lower than on a low protein diet[14].

Vitamin B_{12} has been shown to enhance the biological value of poor protein diet (wheat-peanut)[15] and even casein diet[16]. An improvement in biological value of autoclaved soyabean meal[17] and in rice diets[18] was observed on aureomycin and vitamin B_{12} feeding, the effect being enhanced by combined feeding of the antibiotic and vitamin. The effect of vitamin B_{12} is due more to its participation in the synthesis of methyl group and possibly also in the metabolism of amino acids such as glycine, serine, histidine and threonine, rather than to any direct influence on protein utilization[19].

It would seem from the foregoing that improved utilization of amino acids on terramycin feeding could be due to increased protein intake as well as increased availability of vitamin B_{12} and PGA observed earlier[8]. The action of terramycin may partly be due to its effect on intestinal wall leading to conditions for increased absorption of nutrients.

Acknowledgement

The authors are thankful to the Council of Scientific & Industrial Research, New Delhi, for a research grant and to Chas. Pfizer & Co. Inc. for the gift of crystalline terramycin.

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Nutritive Value of Field Bean (*Dolichos lablab*): Part II—Effect of Feeding Raw, Autoclaved & Germinated Beans on the Growth of Rats & Nitrogen Balance Studies

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Manuscript received 7 *November* 1961

The effect of feeding raw and autoclaved field bean, casein and field bean mixtures and germinated and cooked field bean on the growth of young albino rats has been investigated. Raw field bean retards the growth of rats and ultimately leads to their death. The deleterious effect of feeding is more pronounced as the amount of field bean in the diet is increased. Autoclaving, germination or cooking does not overcome the growth inhibition of rats. Nitrogen balance studies indicate that biological value, true digestibility and net protein utilization of raw field bean are less than those of casein. These values are increased by autoclaving the pulse for 5 min. at 15 lb./sq. in. pressure, but the values are still lower than those for casein. Excretion of essential amino acids in the case of rats fed raw field bean is more than that of rats fed casein.

PULSES form the main source of proteins in Indian diets and as such it is of interest to know the nutritional quality of proteins they supply. Work on this aspect has been carried out by some laboratories in the country[1-4]. In this paper, the results of studies aimed at finding out the nutritive value of field bean using albino rats are reported. This study is of special interest in view of our previous findings that the digestibility of field bean *in vitro* is low and it is deficient in some of the essential amino acids[5].

Experimental procedure

Young male albino rats of original Wistar strain, obtained from the Haffkine Institute, Bombay, were housed individually in screen-bottomed cages. Water was supplied to rats *ad libitum*. The composition of their diets was: protein (casein or bean flour), 10; sucrose, 31·2; salt mixture[6], 4 and groundnut oil, 6. The total was made to 100 per cent by addition of corn starch. Before feeding the diets, 0·06 g. vitamin mixture[7] was fed to each rat. Vitamin A (1200 I.U.) and vitamin D (220 I.U.) were supplied to each rat along with the diet in the form of adexoline (Glaxo). The daily consumption of the diet by rats and their weights at weekly intervals were recorded. Casein was used as the standard reference protein.

In the first experiment, 12 animals were divided into two groups and were fed *ad libitum* (i) casein diet and (ii) raw field bean diet. A marked variation in the consumption of the diets was observed. In all the subsequent experiments, pair-feeding method was resorted to. The above experiment was repeated using pair-feeding technique.

The effect of feeding autoclaved beans on the growth of rats was investigated. Raw field bean flour was spread in the trays to a depth of about 0·5 cm. and was autoclaved for 5 min. and 4 hr at 15 lb./sq. in. pressure. A batch of 24 rats was divided into 4 groups which received the following diets respectively: (1) casein, (2) raw beans, (3) beans autoclaved for 5 min. and (4) beans autoclaved for 4 hr. At the end of the experiment, the pairs of rats were sacrificed and liver, heart, kidney, spleen, pancreas, adrenals and testes were dissected out and weighed.

The effect of feeding the different diets at 18 per cent level, instead of the usual 10 per cent protein level, was tried. A batch of 15 rats was divided into three groups. The first group received casein diet, the second, raw bean diet and the third, autoclaved (5 min.) bean diet.

It has been generally observed that a mixture of proteins exhibits a better biological value than a single protein. To see whether this is so in the

case of field bean proteins too, different amounts of bean flour (raw and autoclaved for 5 min.) were mixed with different amounts of casein and the mixtures were fed to rats. The experiment was carried out in three different sets.

The effect of feeding germinated and cooked beans was next investigated. Dry bean seeds were soaked in water for 18 hr and germinated in air for 48 hr. The seeds with the sprouts were dried in the sun, ground and used for feeding. A part of the germinated seeds was cooked in a beaker with twice the amount of water. When almost all the water was evaporated, the cooked beans were dried in the sun, ground and incorporated in the diet. A batch of 20 rats was divided into four groups which received the following diets respectively: (i) casein, (ii) raw bean, (iii) germinated bean and (iv) germinated and cooked bean.

In order to study the biological value, true digestibility and net protein utilization of raw bean and bean autoclaved for 5 min., the nitrogen balance technique[8-10] was followed using adult (100-125 g.) male albino rats. The rats were fed the control and experimental diets at 10 per cent protein level. The biological value, true digestibility and net protein utilization were calculated according to the formulae of Chick *et al.*[8,9]. Urine and faeces were hydrolysed with acid (with alkali for the estimations of tyrosine and tryptophan) and the amounts of essential amino acids were determined using microbiological method[11].

Results and discussion

The results given in Table 1 and Fig. 1 indicate that the field bean is inadequate to support the growth and normal maintenance of rats. Rats fed raw bean lost weight from the commencement of the experiment (8-10 g. within 7 days) and died within two to three weeks. The fur on their body was disfigured. The forehead and paws turned black and black scales were observed on the tail. The rats had a hunched back appearance. The inadequacy of different legumes to support the normal growth of rats has been reported by a number of workers[4,12-14].

The growth-depressing effect of raw bean is somewhat lessened by autoclaving the beans for 5 min. (Table 1 and Fig. 2). However, when beans autoclaved for 4 hr are fed to rats, the depression of growth is almost comparable to that obtained with raw bean (Table 1). Several workers have reported an increase in the nutritive value of legumes by autoclaving[15-18]. Riesen *et al.*[19] observed that soya-

TABLE 1 — EFFECT OF FEEDING FIELD BEAN ON THE GROWTH OF YOUNG RATS

Group No.	No. of rats	Source of protein in diet	Av. wt of diet consumed per rat†	Change in wt of rats g.
		Exp. I*		
1	6	Casein	173·0	+28·9
2	6	Raw beans	111·0	−15·6
		Exp. II‡		
1	6	Casein	81·1	+10·3 ±1·2
2	6	Raw beans	81·1	−12·3 ±1·0
		Exp. III		
1	6	Casein	80·0	+8·0 ±1·5
2	6	Raw beans	80·0	−13·0 ±1·7
3	6	Autoclaved beans (5 min.)	80·0	−5·0 ±1·0
4	6	Autoclaved beans (4 hr)	80·0	−11·2 ±0·7
		Exp. IV§		
1	5	Casein	47·6	+11·0 ±0·4
2	5	Raw beans	47·6	−16·4 ±1·8
3	5	Autoclaved beans (5 min.)	47·6	−8·6 ±0·7
		Exp. V‡		
1	5	Casein	81·4	+11·2 ±1·0
2	5	Raw beans	81·4	−14·0 ±1·3
3	5	Germinated beans	81·4	−11·8 ±1·7
4	5	Germinated and cooked beans	81·4	−4·6 ±1·2

*Diet fed *ad libitum*.
†In exp. I the diet consumed and the weights of rats are recorded for a period of 3 weeks, in exp. II, III and V for a period of 2 weeks and in exp. IV for a period of 9 days.
‡Pair feeding.
§Feeding at 18 per cent protein level.

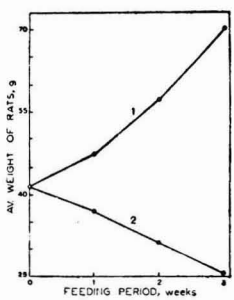

Fig. 1

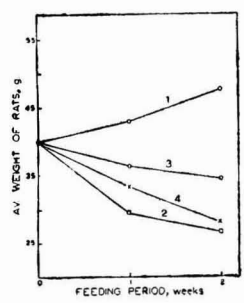

Fig. 2

Fig. 1 — Effect of feeding raw beans *ad libitum* on the growth of rats [(1) Casein and (2) raw beans]

Fig. 2 — Effect of feeding autoclaved beans on the growth of rats [(1) Casein, (2) raw beans, (3) autoclaved (5 min.) beans and (4) autoclaved (4 hr) beans]

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bean meal autoclaved for 4 min. at 15 lb./sq. in. pressure possessed exceptionally high nutritive value for chicks. Beans autoclaved for 4 hr had a low nutritive value.

The heart and kidneys of rats fed on raw or autoclaved bean show significant increase in their weights (Table 2), while the weights of spleen and testes are decreased as compared to the weights of the organs in casein group rats. Scott *et al.*[20-22] have shown that the weights of different organs are affected when the rats are fed on diets deficient in essential amino acids.

The growth inhibition of rats fed field bean at 18 per cent protein level was more pronounced than when fed at 10 per cent protein level (Table 1). The growth depression in rats has been found to be directly proportional to the amount of raw field bean in the diet (Table 3).

Rats fed on germinated beans do not grow properly (Table 1). However, a slight improvement in the loss in weights of rats is observed, perhaps due to the release of amino acids after germination, as reported by Ganguli[23] in the case of *Phaseolus radiatus*. The growth-depressing effect is somewhat lessened by germinating and cooking the beans. This may be attributed to the partial destruction of the possible antigrowth factor by cooking. Similar findings have been reported by Orru and Demel[24] in the case of *Canavalia ensiformis*.

The results of nitrogen balance studies (Tables 4 and 5) indicate that the biological value, true digestibility and the net protein utilization of raw beans by rats is low as compared to casein. The values are increased by autoclaving the beans for 5 min., but the values are still lower than those for casein. Kuppuswami *et al.*[25] have reported the biological value of casein as 72 and that of raw field bean as 57. The values obtained in the present investigation agree with these two values. Jaffe[26] has observed that autoclaved hyacinth beans are 12-15 per cent more digestible than raw beans. In the present investigation, the digestibility of autoclaved field bean has been found to be 18-19 per cent more than that of raw beans. Sohonie and Inamdar[4] have also reported an increase in the digestibility of double bean (*Vicia faba* Moench) by autoclaving.

Rats fed on raw beans excreted larger amounts of essential amino acids, especially methionine and tryptophan, than the rats fed on casein. However,

TABLE 2 — EFFECT OF FEEDING DIFFERENT DIETS ON THE WEIGHT OF DIFFERENT ORGANS*

(*The values represent av. wt of the organs expressed in g./*100 *g. body wt*)

Organ	Diet			
	Casein	Raw bean	Autoclaved bean	
			5 min.	4 hr
Liver	5·15 ±0·46	4·28 ±0·32	5·32 ±0·18	5·55 ±0·32
Heart	0·41 ±0·02	0·59 ±0·03†	0·51 ±0·04†	0·59 ±0·03†
Kidneys	1·08 ±0·02	1·37 ±0·03†	1·30 ±0·06†	1·55 ±0·07†
Spleen	0·50 ±0·08	0·18 ±0·02†	0·21 ±0·01†	0·21 ±0·01†
Pancreas	0·31 ±0·07	0·22 ±0·03	0·30 ±0·02‡	0·21 ±0·03
Adrenals	0·03 ±0·014	0·03 ±0·001	0·04 ±0·001‡	0·04 ±0·002†‡
Testes	0·67 ±0·11	0·26 ±0·01†	0·35 ±0·05	0·32 ±0·02†‡

**t* test was carried out at 5 per cent level of significance.
†*t* value is significant when compared to casein group.
‡*t* value is significant when compared to raw bean group.

TABLE 3 — GROWTH OF RATS FED ON CASEIN AND FIELD BEAN MIXTURES

Group No.	No. of rats	Source of protein in diet		Av. wt of diet consumed per rat in 2 weeks *g.*	Change in wt of rats after 2 weeks *g.*
		Casein *g.*	Bean *g.*		
		SET I (RAW BEAN)			
1	5	10·0	0·0	82·8	+11·2 ±1·0
2	5	9·5	2·5	82·8	+9·4 ±0·7
3	5	9·0	5·0	82·8	+3·4 ±1·5
4	5	8·5	7·5	82·8	+0·8 ±0·1
5	5	8·0	10·0	82·8	−3·0 ±0·9
6	5	0·0	50·0	82·8	−14·0 ±1·3
		SET II (RAW BEAN)			
1	5	10·0	0·0	78·0	+11·2 ±0·8
2	5	7·5	12·5	78·0	−7·1 ±1·3
3	5	5·0	25·0	78·0	−9·1 ±1·0
4	5	2·5	37·5	78·0	−9·8 ±1·4
5	5	0·0	50·0	78·0	−10·3 ±1·5
		SET III (AUTOCLAVED BEAN)			
1	5	10·0	0·0	79·0	+11·2 ±1·0
2	5	7·5	12·5	79·0	+6·6 ±0·2
3	5	5·0	25·0	79·0	+1·6 ±0·2
4	5	2·5	37·5	79·0	−2·8 ±1·5
5	5	0·0	50·0	79·0	−10·4 ±1·4

*In sets I and II raw bean was used.

when autoclaved beans are fed, the excretion of essential amino acids is lower than when the rats are fed raw beans (Table 6). Pearce *et al.*[27], Roth and Allison[28] and Kade and Shepherd[29] have reported that the deficiency of tryptophan and methionine in diets results in the excretion of abnormally high amounts of all other amino acids. Since raw beans are highly deficient in methionine and tryptophan, the excretion of essential amino acids may be more pronounced in the case of rats fed on raw bean diet.

All the above observations appear to indicate that the low nutritive value of field bean might be due to (i) deficiency of essential amino acids in field bean protein, as field bean has been found to be deficient in all essential amino acids except arginine[5], (ii) low availability of essential amino acids from field bean, (iii) presence of trypsin inhibitor in field bean[30], (iv) presence of haemagglutinin in field bean[31] or (v) presence of an antigrowth factor.

Studies on the effect of supplementation of field bean with deficient amino acids on the growth of rats have shown that rats fed on a diet containing raw beans plus amino acids did not grow, while rats receiving a diet of autoclaved beans plus amino acids showed normal growth, indicating thereby that the deficiency of essential amino acids is not the factor causing growth inhibition.

In vitro digestibility studies, reported earlier[5], have shown that the availability of amino acids after tryptic digestion is low in the case of raw beans and is increased by autoclaving the beans for 5 min. Sohonie and Ambe[30] have shown that 40 per cent of the trypsin inhibitor in field bean is destroyed by autoclaving. This may perhaps explain the improvement in the growth inhibition of rats fed on autoclaved beans and the increase in the digestibility and retention of essential amino acids of autoclaved beans. Similar destruction of trypsin inhibitor by autoclaving is reported by Liener *et al.*[32] in the case of soyabean. It has been, however, observed that trypsin inhibitor isolated from raw field bean, when fed to rats receiving casein, does not affect their growth.

TABLE 4 — NITROGEN BALANCE STUDIES AT 10 PER CENT PROTEIN LEVEL

Diet	Biological val.	True digestibility	Net protein utilization
Casein	74·0 ±1·1*	95·7 ±1·7	70·8 ±1·4
Raw bean	57·2 ±1·7	70·7 ±1·6	45·7 ±2·1
Autoclaved bean	64·7 ±1·2	83·6 ±2·6	54·1 ±0·9

*Degree of freedom = 5.

TABLE 5 — STATISTICAL ANALYSIS

Diet	*t* test*		
	Biological val.	True digestibility	Net protein utilization
Casein and raw beans	8·4	10·5	16·8
Casein and autoclaved beans	5·7	6·7	9·9
Raw beans and autoclaved beans	3·6	7·6	9·2

*/ test was carried out at 5 per cent level of significance and the values were significant in all the cases.

TABLE 6 — EXCRETION OF AMINO ACIDS IN URINE AND FAECES OF RATS

Amino acids	Amino acids excreted *mg./100 mg. protein ingested*		
	Casein	Raw bean	Autoclaved bean
Arginine	7·21	4·66	3·79
Threonine	8·79	14·08	9·91
Leucine	6·25	10·35	9·53
Isoleucine	1·12	26·73	12·70
Valine	5·53	14·91	10·38
Tryptophan	15·31	65·77	39·30
Lysine	5·18	15·32	10·38
Methionine	5·14	42·47	23·79
Histidine	4·47	14·00	8·89
Phenylalanine	6·25	15·92	11·04
Cystine	2·65	3·97	3·65
Tyrosine	8·37	15·19	13·27

Acknowledgement

The authors are grateful to the Council of Scientific & Industrial Research, New Delhi, for the award of a junior research fellowship to one of them (K.P.).

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Nutritive Value of Field Bean (*Dolichos lablab*): Part III—Effect of Supplementation of Essential Amino Acids to Raw & Autoclaved Field Bean on the Growth of Albino Rats

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Manuscript received 7 *November* 1961

The effect of feeding raw and autoclaved field bean supplemented with essential amino acids on the growth of rats has been investigated. Raw field bean does not support the growth of rats, even after supplementation with essential amino acids in which the pulse is deficient. The rats fed on diet containing field bean autoclaved for 5 min. and supplemented with essential amino acids show proper growth. The growth inhibition of rats fed on raw field bean does not appear to be due to either amino acid imbalance or antagonism. The presence of some heat labile growth inhibitor is, therefore, postulated.

RAW field bean is deficient in almost all essential amino acids, except arginine, as compared to casein[1]. It has been observed that the rats fed raw or autoclaved field bean do not exhibit proper growth[2]. The present paper reports the effect of supplementation of essential amino acids to the basal raw or autoclaved field bean diet on the growth of rats.

Extensive work has been carried out on the effect of amino acid supplementation to legume proteins[3-8]. Inamdar and Sohonie[9] have also reported that supplementation of essential amino acids to raw double bean (*Vicia faba* Moench) failed to improve the growth of rats. It was, therefore, considered of interest to study the effect of supplementation of amino acids to raw and autoclaved field bean on the growth of rats.

Experimental procedure

Raw and autoclaved (5 min.) field bean diets are deficient in most of the essential amino acids, the major deficiencies being those of methionine, tryptophan and valine (Table 1). The deficiency of each amino acid of the experimental diet was calculated by finding the difference in the contents of that amino acid in casein and experimental diets. In the

first two experiments, the amounts of amino acids corresponding to these differences were added to the experimental diets, so that the final concentrations of those amino acids were the same in all the diets. The supplemented meals were incorporated in the diets at 10 per cent protein level, the composition of the diets being the same as given in the previous paper[2].

Three sets of feeding experiments were carried out (1) in which the experimental diets were supplemented with the major deficiencies of essential amino acids, viz. methionine, tryptophan and valine, (2) in which all the deficient essential amino acids were added and (3) in which all the essential amino acids were added at a level optimum (as recommended by Rose[10]) for the normal growth of rats.

The rats were kept in screen-bottomed cages and were pair fed. Water was available *ad libitum*. The record of daily consumption of food by rats and the weekly records of their weights were kept.

Effect of supplementing the experimental diets with methionine, tryptophan and valine on the growth of rats — The experiment was carried out with 30 young (35-45 g.) male albino rats, divided into five groups, each group receiving its respective diet, viz. (i) casein, (ii) raw field bean, (iii) raw field bean supplemented with methionine, tryptophan and valine, (iv) field bean autoclaved for 5 min. and (v) autoclaved field bean supplemented with methionine, tryptophan and valine (Table 2).

Effect of supplementing the experimental diets with all essential amino acids — A batch of 30 rats was divided into five groups which received the following diets respectively: (i) casein, (ii) raw field bean, (iii) raw field bean supplemented with all essential amino acids in which the diet was deficient, (iv) autoclaved field bean and (v) autoclaved field bean supplemented with all essential amino acids in which the diet was deficient (Table 2).

TABLE 1 — AMINO ACID COMPOSITION OF CASEIN, RAW FIELD BEAN AND AUTOCLAVED FIELD BEAN DIETS AT 10 PER CENT PROTEIN LEVEL

(*Amino acids content expressed in g./100 g. of diet*)

Amino acid	Casein	Raw field bean	Autoclaved field bean
Arginine	0·37	0·87	0·92
Threonine	0·36	0·33	0·47
Leucine	1·11	0·99	1·00
Isoleucine	0·68	0·53	0·49
Valine	0·91	0·55	0·55
Tryptophan	0·13	0·05	0·07
Lysine	0·81	0·74	0·75
Methionine	0·37	0·08	0·12
Histidine	0·35	0·25	0·34
Phenylalanine	0·50	0·39	0·49
Cystine	0·06	0·06	0·06
Tyrosine	0·52	0·50	0·50

TABLE 2 — EFFECT OF SUPPLEMENTATION OF RAW AND AUTOCLAVED FIELD BEAN WITH ESSENTIAL AMINO ACIDS

(*No. of rats in each group, 6; values represent change in wt of the rats after 2 weeks in g.*)

Diet group	Exp. 1	Exp. 2	Exp. 3
Casein	+7·7±1·07	+6·5±0·68	+8·0±0·96
Raw field bean	−13·9±1·36	−12·7±0·30	−12·2±1·02
Raw field bean + amino acids	−12·8±2·54	−11·1±0·42	−11·4±1·00
Autocalved field bean	−7·5±0·84	−8·6±1·39	−6·9±0·81
Autoclaved field bean + amino acids	+4·6±0·61	+6·0±0·82	+8·5±0·32

Exp. 1: Supplementation with methionine, tryptophan and valine; av. wt of diet consumed/rat in 2 weeks, 80 g. Exp. 2: Supplementation with all essential amino acids; av. wt of diet consumed/rat in 2 weeks, 77 g. Exp. 3: Supplementation with all essential amino acids at optimum level; av. wt of diet consumed/rat in 2 weeks, 79·3 g.

The effect of supplementing the experimental diets with all the essential amino acids at optimum levels required for growth was also investigated. A batch of 30 rats was divided into five groups which were fed the following diets respectively: (i) casein, (ii) raw field bean, (iii) raw field bean plus essential amino acids at optimum level, (iv) autoclaved field bean and (v) autoclaved field bean plus essential amino acids at optimum level. In the first two series, the concentrations of all essential amino acids in the experimental diets were raised to the same level as that in the casein diet. In this case, however, all the essential amino acids were added to the experimental diets in order to raise their levels to correspond to the optimum level, as suggested by Rose[10], for the normal growth of rats. Hence, in this series, the concentrations of the essential amino acids were higher than those in the casein diet.

Results and discussion

The rats fed raw field bean showed the same changes in the external appearance as reported earlier[2]. The rats fed raw field bean supplemented either with methionine, tryptophan and valine or with all the deficient essential amino acids or with all essential amino acids at optimum level of requirement did not show any growth (Table 2). However, in the above experiments, when autoclaved (5 min.) field bean was substituted for raw field bean, the rats showed growth. The growth was minimum when autoclaved field bean was supplemented with methionine, tryptophan and valine only. The growth was maximum and of the same order as that obtained with casein when the autoclaved bean diet was supplemented with all essential amino acids.

The possibility of amino acid imbalance, as described by Harper[11,12], or amino acid antagonism as reported by Harper *et al.*[13], does not appear to arise in the case of raw field bean, as the amounts of amino acids in raw field bean diet after supplementation were equal to those in the casein diet. Further, the rats fed raw field bean diet supplemented with amino acids showed growth inhibition, whereas autoclaved and supplemented field bean diets supported normal growth. The results lead to the conclusion that apart from deficiency of essential amino acids, raw beans may contain an antigrowth factor which is destroyed by autoclaving for 5 min.

Acknowledgement

The authors are grateful to the Council of Scientific & Industrial Research, New Delhi, for financial assistance and for a personal grant to one of them (K.P.).

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Pharmacognostic Study of Roots of *Pueraria tuberosa* DC.

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Manuscript received 29 *January* 1962

The macroscopic and microscopic characters and chemical constituents of the roots of *P. tuberosa* (Hindi: *Bidhari kand*) are described. The drug has a slight characteristic odour and a characteristic sweet taste. The roots are fleshy and are swollen at intervals. Secondary cortex cells contain starch grains and stone cells in the form of a continuous band. The stone cells and some of the parenchyma cells contain a single prismatic crystal of calcium oxalate. An uninterrupted band of sclerenchyma in the inner cortex and large pigment cells in the phloem are other distinguishing characters. The roots contain 11-12 per cent protein and free amino acids.

PUERARIA TUBEROSA (*Papilionaceae*; Hindi: *Bidhari kand*) is distributed from the West Himalayas to Sikkim, up to an altitude of 4000 ft, in Kumaon, the lower hills of the Punjab, Mount Abu and the hilly tracts of Bengal and South India[1,2]. In Ayurveda[2,3], the tuber of the plant is used for a wide variety of ailments. It is used as aphrodisiac, tonic, galactagogue, diuretic and alterative. It is prescribed in leprosy, biliousness and in the diseases of the blood. It is considered to cure burning sensation in genito-urinary diseases. The root is given as a demulcent and refrigerant in fevers. It is used in rheumatism and also to reduce swellings of the joints. Our interest in this drug was aroused due to its high place as a tonic in the Unani system of medicine. The present study was necessitated because a number of other tuberous drugs are being sold in the market under the name of *Bidhari kand*.

Morphology of the root

P. tuberosa bears thick fleshy long roots which become swollen at intervals. The tubers thus formed are subspherical or irregularly and pear shaped, varying in diameter from 15 to 60 cm. and in weight from 2·5 to 10 kg. (Fig. 1A). The market

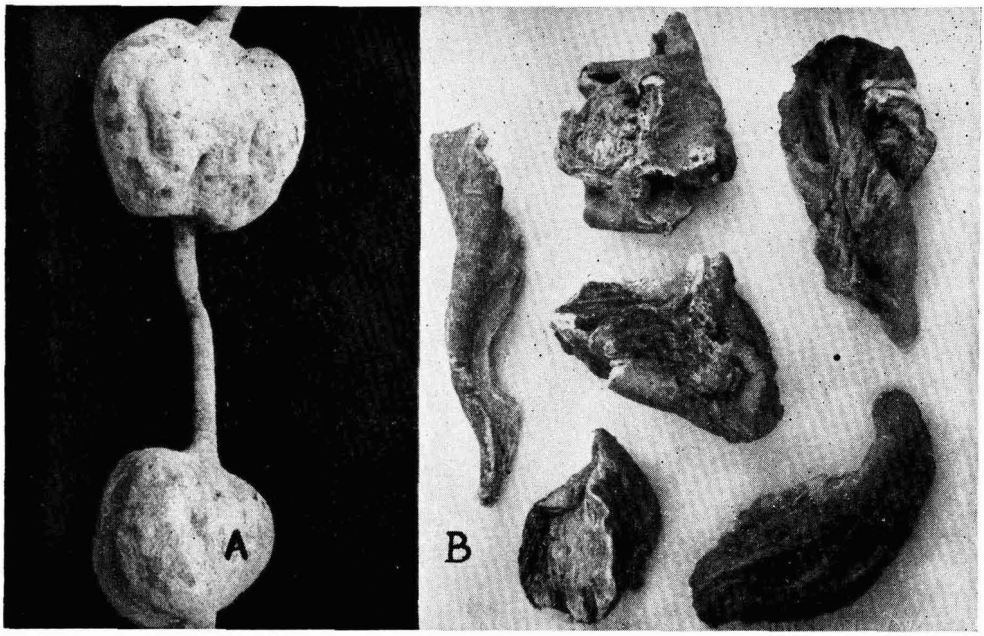

Fig. 1 — Macroscopic characters of the root of *P. tuberosa* showing (A) bulging of the root at intervals (× 15) and (B) the drug as obtained in the market (× 1)

drug (Fig. 1B) consists of dried longitudinally cut, decorticated flat thin slices or longitudinal comparatively thick sectors, the former being of superior quality. The lateral surfaces are white to dirty white and uneven due to longitudinal furrows. External surface in the case of undecorticated drug is yellowish brown, wrinkled and furrowed with a few lenticels. The internal part of the drug loses more water as compared to the peripheral cortical layer with the result that cortical part of the drug pieces converge inwards and partly envelope the internal portion of the drug. The external surface of superior decorticated samples is dirty white and longitudinally striated. Fracture brittle and starchy, and the fractured surface is lamellate. It has a slight characteristic odour and a peculiar sweet taste.

Histology of the roots

The cork (Fig. 2A) consists of several layers of thin-walled suberized rectangular cells measuring 22-**40**-60 μ by 9-**11**-15 μ. In surface view the cork cells appear somewhat squarish to subrectangular or polygonal in outline (Fig. 2, B and C) and measure 22-**52**-100 μ by 17-**35**-44 μ. Beneath the cork are tangentially elongated and somewhat rectangular cells constituting the secondary cortex. These cells contain starch grains. The innermost 2-3 layers of the cortex consist of stone cells in the form of a continuous band (Fig. 2, A and B). In the macerate the stone cells are of various shapes and are highly lignified showing a small lumen and distinct radiating pore canals. They measure up to 130 μ in diameter (Fig. 2, B and C).

One to two layers of the cells of the secondary cortex close to the layers of stone cells are thick walled, slightly lignified and each contains a single prismatic crystal of calcium oxalate. They constitute crystal fibres in longitudinal sections and macerated preparations (Fig. 2C). The crystals measure up to 25 μ.

Phloem consists of radial strands of phloem tissue alternating with phloem rays. Each strand shows a few groups of fibres, several large parenchymatous cells filled with dark brown amorphous pigment, and sieve tube tissue in the form of highly collapsed cells. Each fibre group consists of 15-50 fibres (Fig. 2D). The individual fibres of each group possess thickened lignified walls with a narrow streak-like lumen. They are very long and usually get broken during maceration. They measure up to 20 μ in diameter.

The cambium is composed of 2-3 layers of rectangular or somewhat collapsed cells.

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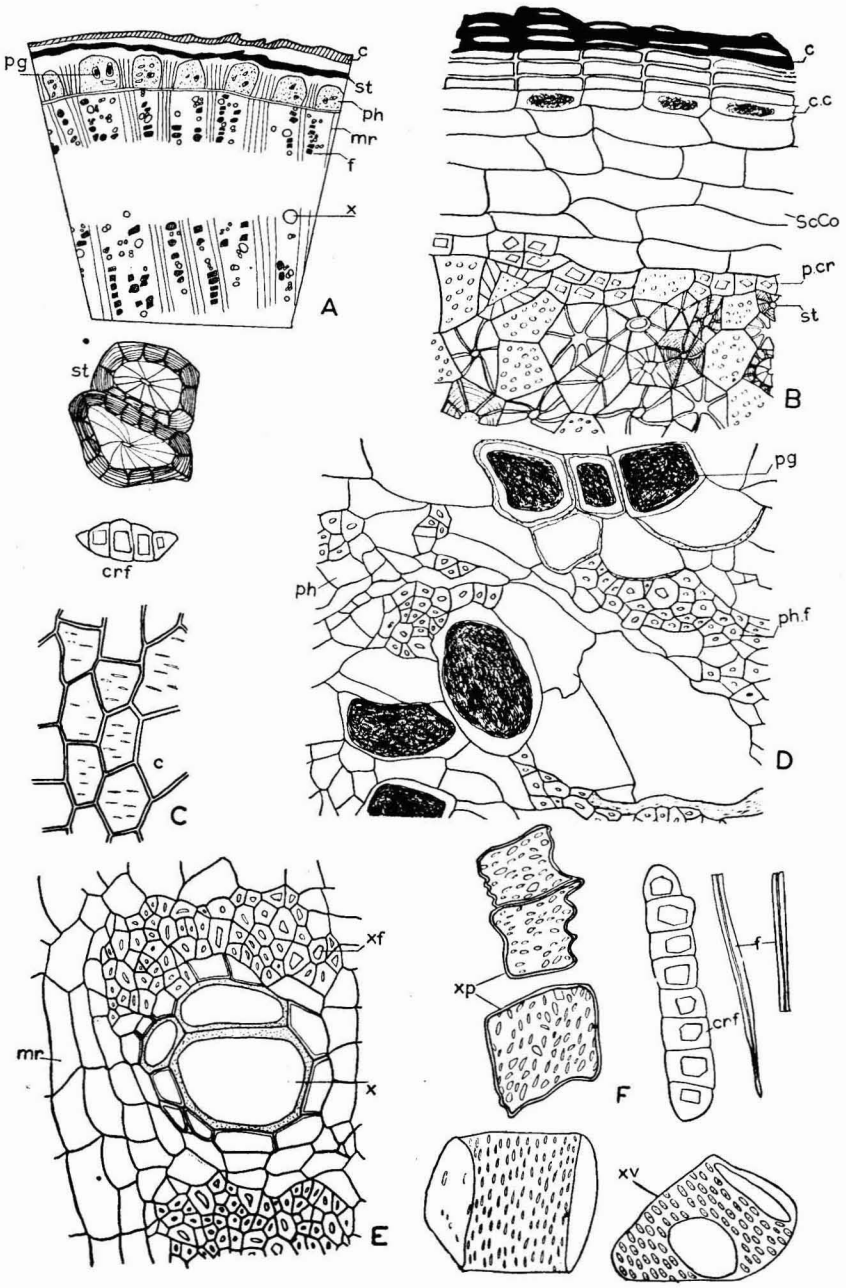

Fig. 2 — Microscopic characters of the dried tubers of *P. tuberosa* [A: Diagrammatic t.s. of drug × 7·2. B: T.s. showing cork, cortex and sclerenchyma band × 212. C: Macerated preparation showing cork and crystal fibres × 212. D: T.s. in phloem region × 212. E: T.s. in xylem region × 212. F: Macerated preparation of xylem × 212. *c*, cork; *c.c*, cork cambium; *crf*, crystal fibre; *f*, fibre; *mr*, medullary rays; *p.cr*, prismatic crystals; *pg*, dark brown amorphous pigment; *ph*, phloem; *ph.f*, phloem fibre; *ScCo*, secondary cortex; *st*, stone cells; *vb*, vascular bundle; *x*, xylem; *xf*, xylem fibre; *xp*, xylem parenchyma; *xv*, xylem vessels]

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Xylem forms the major fleshy portion of the tuber. It is composed of alternating parenchymatous medullary rays and radial strands of xylem. Each xylem strand has a parenchymatous matrix in which the xylem tissue is represented by xylem vessels, xylem parenchyma and groups of xylem fibres (Fig. 2A). The vessels occur singly or in small groups of 2-4, measuring from 25 to 225 μ in diameter (Fig. 2E). The groups of vessels alternate with several bundles of xylem fibres, each composed of 15 to 140 fibres.

In the macerate the vessels are found to consist of elements of various shapes and sizes, the walls of most of which are densely covered with bordered pits having slit-like openings. Some of the vessel elements have simple transversely elliptical pits (Fig. 2F). The xylem fibres resemble the phloem fibres in all respects. Some of the parenchyma cells immediately surrounding the fibre groups of phloem and xylem contain a single prismatic crystal of calcium oxalate. In longitudinal section and maceration such crystal cells are arranged in vertical rows, thus constituting crystal fibres (Fig. 2F).

The overall distribution of xylem vessels and fibre groups is such that concentric rings of xylem tissue alternating with parenchymatous zones are seen, giving the false appearance of annual rings. The cells constituting the parenchymatous zones are irregularly rounded and are filled with compound starch grains measuring 2-9-18 μ.

Chemical examination

Preliminary tests for the usual plant constituents showed the presence of large quantity of protein and free amino acids[4,5]. The protein content of samples obtained from different areas is: Dehra Dun, 11·7; Pathankot, 12·2; Amritsar, 7·8; and Delhi, 6·1 per cent. The first two samples were collected by the authors and the last two were commercial samples.

Acknowledgement

Our thanks are due to Dr K. N. Gaind, Head of the Pharmacy Department, Panjab University, and Hakim Abdul Hamid of the Institute for the History of Medicine, Hamdard Building, Delhi, for their encouragement and interest during the course of this investigation.

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Short Communications

Changes of Thyroid Function in Response to Cadmium Administration in Rats — Studies with I^{131}

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Manuscript received 30 *April* 1962

Administration of cadmium to rats has been shown to depress the thyroidal I^{131} uptake and the relative concentration of thyroxine in the gland. This depression is found to recover slightly after one week though normal values could not be attained even after three weeks of cadmium administration. The possibility of inteference in the pituitary function has been indicated.

CADMIUM, in addition to its effect on the gonadotrophin content of the pituitary[1], has also been shown to produce changes of an unusual nature in the gonads of male and female rats[2,3]. An inhibitory influence of cadmium on the action of sex hormones on their homologous target tissues in gonadectomized rats has also been reported[4]. Sarkar *et al.*[5] showed that cadmium injections in rats caused an increased uptake of phosphorus in the liver and kidney whereas phosphorus incorporation in the muscle was found to be decreased.

The present communication records the changes in thyroid function as studied by I^{131} uptake, after administration of cadmium chloride.

Male albino rats of the Institute colony, fed on normal diet, were injected intramuscularly with cadmium chloride (Judex, 1 mg./100 g. body weight) in the intrascapular region, as a single dose. I^{131} uptake was found at different intervals after cadmium administration for a period of three weeks.

Each rat was injected intraperitoneally with I^{131} in the form of sodium iodide (0·1 μc. with 2 μg. of sodium iodide in 0·2 ml. of saline). The control rats were given saline injection before I^{131} administration.

Four hours after injection of I^{131} the thyroid was dissected out, weighed and digested in 3 ml. of 2 per cent sodium hydroxide according to the method of Rawson *et al.*[6]. The activity was read on a Tracerlab SC-32 Ampliscaler with a P20 AW, well type scintillation counter. This counter gave $11{\cdot}94\times10^5$ counts per minute per μc. of iodine and a background to 772 counts per minute at room temperature.

Chromatographic analysis of thyroid tissue — For the chromatography of the thyroid tissue, four rats were injected with 1·625 μc. of I^{131} 24 hr after cadmium injection. The thyroids were removed after 24 hr, under ether anaesthesia, pooled, weighed and homogenized in conical tissue grinders, in 2 ml. of veronal buffer, *p*H 8·6, with $10\times10^{-4}M$ thiourea to prevent further oxidation of iodine. Trypsin powder (16 mg.) was added to each tube, well dispersed and left for digestion overnight at 37°C. after addition of a drop of toluene. Trypsin (16 mg.) was further added after 16 hr to ensure complete digestion which was allowed to continue for 24 hr. According to Richards and Ingbar[7] this process of digestion hydrolysed 96 per cent of the thyroidal protein. The digested homogenate was well mixed and 0·2 ml. spotted on filter paper (Whatman No. 3 mm.) along with thyroxine, tri-iodothyronine, mono and di-iodotyrosine and sodium iodide as carriers. An aliquot of 0·2 ml. was diluted to 3 ml. and read in the counter for total activity in the thyroids. The ascending chromatograms were run overnight in butanol-acetic acid-water (4: 1: 5) according to Friis and Hall[8]. The chromatograms were dried and placed in casettes with double-coated X-ray films for 48 hr. The films were then developed and the portions of the filter paper corresponding to the active spots were cut out and read directly in vials in the scintillation counter. The fractions were expressed as percentage of the total counts of an individual chromatogram.

The data for the 4 hr I^{131} uptake in normal and cadmium-treated rats (Table 1) show that the percentage uptake for normal rats was 41·76, whereas for the cadmium-treated rats (30 min.) the uptake was 24·20. This depression was found to persist for 24 hr but showed certain recovery after one week, although the normal value could not be attained even after 3 weeks of cadmium administration. Statistical evaluation of the depression at 24 hr period proved it to be highly significant ($p < 0{\cdot}01$). It would, however, be noted that there was no significant difference in the ratio of thyroid to body weight.

I^{131} distribution in different fractions of thyroid hydrolysate (Table 2) indicated that the values for the thyroidal iodo-amino acids in the control animals was similar to that reported by Querido *et al.*[9] and Taurog *et al.*[10]. After cadmium administration the relative percentage of I^{131} in thyroxine, tri-iodothyronine (T_3 and T_4) and di-iodotyrosine (DIT) is decreased, while the relative value for mono-iodotyrosine showed an increase.

The present studies indicate that a single intramuscular injection of cadmium chloride not only

TABLE 1 — UPTAKE OF I^{131} AT DIFFERENT PERIODS AFTER CADMIUM ADMINISTRATION

Interval after Cd administration	No. of animals	Av. body wt *g.*	Thyroid wt *mg.*	Thyroid wt *mg./100 g. body wt*	I^{131} uptake %
0 (normal)	7	51·00	11·36	22·50	41·76
S.E.		±1·23	±0·69	±1·40	±4·44
30 min.	2	62·00	—	—	24·20
24 hr	10	53·90	12·15	22·50	22·68
S.E.		±1·38	±0·77	±1·10	±3·44
1 week	3	125·00	22·00	17·60	34·54
2 weeks	3	122·00	24·10	19·90	34·57
3 weeks	4	142·00	29·60	20·99	30·93

TABLE 2 — EFFECT OF CADMIUM ADMINISTRATION ON THE DISTRIBUTION OF I^{131} IN DIFFERENT FRACTIONS OF THYROID HYDROLYSATE

Fraction	Activity of total I^{131} on chromatogram, %							Uptake %
	At origin	Inorganic I^{131}	Unidentified spot	MIT*	DIT*	T_3T_4*	Solvent front	
Normal	0·018	7·437	21·771	31·83	17·02	16·81	5·33	53·03
Treated†	0·002	4·198	4·454	61·93	14·16	14·00	1·06	29·17

*Nomenclature same as that used by Taurog *et al.*[10].
†I^{131} was injected 24 hr after cadmium administration.

depresses the thyroidal uptake of I^{131} but also decreases the relative percentage of thyroxine in the thyroid. This depression in contrast to the effect of cobalt[11] was seen to recover slightly after one week, although the normal value could not be attained even after 3 weeks. There are several possibilities which could have brought about such a depression. These are the direct effect of cadmium on the thyroidal enzymes, which presume a carry-over and storage of this metal in the gland or the indirect effect via the mediation of the pituitary in which case either the secretion of thyrotropin is reduced or there is a direct inactivation of the circulating thyrotropin. The first explanation may not hold good in view of the observation that oxidative enzymes necessary for the conversion of iodine to organic form are only slightly affected by cadmium[12-16]. The indirect effect via the mediation of the pituitary is supported by the recent studies of Kar *et al.*[1], wherein the effect of cadmium on the pituitary has been shown. This is further supported by the fact that gonadectomy or ovidectomy in rats leads to a decreased uptake of iodine or phosphorus by the thyroid[17,18].

The technical help of Shri R. K. Vaish and Shri L. N. Verma in this work is gratefully acknowledged.

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Inhibition of Vaccinia Virus Pock Formation by 8-Hydroxy-4-quinazolone in Chick Embryo

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Manuscript received 9 *March* 1962

Inhibition of vaccinial pock formation and reduction in mortality of infected chick embryo due to 8-hydroxy-4-quinazolone has been observed. The pock-forming units can be recovered from apparently healthy membranes. The antiviral effect of 8-hydroxy-4-quinazolone seems to be due to changed reaction of the host cells and is not attributable to the virucidal action of the drug.

SUBSTITUTED 8-hydroxy-4-quinazolones, prepared in the Medicinal Chemistry Department[1] of this Institute as potential amoebicides, have been tested against vaccinia virus infection in chick embryo and the results of these studies are reported in this communication.

Egg-adapted vaccinia virus was obtained from the Vaccine Institute, Bangalore. The dilute virus produced regularly discrete plaques on the CAM of 10-12 days old chick embryo in 72 hr. In order to prepare stock virus, the infected chorio-allantoic membranes were suspended in allantoic fluid and crushed in a pestle and mortar with sterile glass powder. The ground material was centrifuged at 2000 r.p.m. for 10 min. to remove tissue debris and supernatant stored at −10°C. when not in use[2]. Five compounds of the quinazolone series, viz. 8-hydroxy-4-quinazolone (I 8), 3-propyl-8-hydroxy-4-quinazolone (I 76), 2-methyl-8-hydroxy-4-quinazolone (I 141), 2-methyl-8-methoxy-4-quinazolone (I 139) and 7-piperidinomethyl-3-propyl-8-hydroxy-4-quinazolone (I 118), were tested. As all these compounds were insoluble in water, they were suspended in 0·1 per cent agar (Difco) and used.

The toxicity of the drugs was assessed by the mortality of embryos caused by 2 mg. of the drugs in 72 hr. The drugs that proved non-toxic were then tested for antiviral activity. Eggs were inoculated first with the appropriate virus dilution that caused discrete pocks, followed by 0·2 ml. of the drug suspension. Antiviral effect of a drug preparation was judged by its ability to cause suppression of pock formation and reduce specific mortality caused by virus multiplication.

Out of the 5 compounds tested, only 8-hydroxy-4-quinazolone and 7-piperidinomethyl-3-propyl-8-hydroxy-4-quinazolone were non-toxic and were, therefore, tested for their antiviral effect. 7-Piperidinomethyl-3-propyl-8-hydroxy-4-quinazolone had no antiviral effect at 2 mg./embryo level, as judged

TABLE 1 — EFFECT OF 8-HYDROXY-4-QUINAZOLONE ON POCK FORMATION

Conc. of drug *mg./embryo*	Av. pock count/embryo	Mortality (dead/total)
2·0	60	1/12
5·0	23	0/12
7·5	5	1/12
10·0	1	1/12
Control	100	1/12

TABLE 2 — EFFECT OF 8-HYDROXY-4-QUINAZOLONE ON POCK COUNT

(*Different dilutions of vaccinia virus inoculum were used to infect the embryos*)

Dilution of virus inoculum*/ embryo	Without drug		With drug (10 *mg./embryo*)	
	Mortality	Mean pock count	Mortality†	Mean pock count
10^{-8}	1/6	50	0/6	1
10^{-9}	0/6	25	0/6	nil

*Different dilutions of virus inoculum were obtained by diluting the stock virus appropriately.
†Mortality/number inoculated.

TABLE 3 — POCK FORMATION BY VACCINIA VIRUS TREATED WITH 8-HYDROXY-4-QUINAZOLONE IN CHICK EMBRYO

Exp. No.	Mean pock count at different dilutions*			
	10^{-0}	10^{-1}	10^{-2}	10^{-3}
1	Confluent plaque	70	30	15
2	do	85	45	20
3	do	80	40	18
4	do	60	25	10

*Mean of six membranes.

by the pock formation and mortality of the embryos. 8-Hydroxy-4-quinazolone more or less completely inhibited pock formation at 10 mg./embryo level (Table 1) and gave nearly 100 per cent protection to the embryos. The effect of 8-hydroxy-4-quinazolone on pock formation, when different concentrations of virus inoculum were used, is evident from the results of tests given in Table 2. The results show that there is a direct relationship between the dose of drug or virus used and the amount of pocks formed.

The chorioallantoic membranes that did not produce pocks in the above experiments were crushed and various dilutions passaged in eggs. The results given in Table 3 show that the potential pock-forming virus units are not destroyed by 8-hydroxy-4-quinazolone.

The authors wish to thank Dr B. Mukerji for his keen interest in the work and Dr B. N. Singh for guidance and helpful suggestions. Thanks are also due to Dr. V. N. Krishnamurthy of the Vaccine Institute, Bangalore, for the supply of egg-adapted Bangalore strain of vaccinia virus.

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Experimental Human Leprosy in Mice & Rats on Pro-oxidant Diet

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Manuscript received 25 *April* 1962

Albino rats, Swiss white mice and black hybrid mice (Chatterji strain), maintained on Bergel's pro-oxidant diet, with daily additions of raw linseed oil (iodine value, 168-80), have been infected (intraperitoneal or intratesticular) with lepromatous material from untreated cases of lepromatous leprosy and examined after 365 days. Examination of smears from lungs, liver, spleen, testis and lymph glands, and of the cells of these tissues, has shown that the animals do not take the infection. M. Bergel's [*Sem. medica*, *B. Aires*, Vol. 111 (1957), 480, 1148; Vol. 113 (1958), 1119] claim of successful transmission of human leprosy in mice maintained on pro-oxidant diet is, therefore, not confirmed.

RESEARCH on leprosy and especially experimental chemotherapy of leprosy has not progressed much because of the difficulty of transmission of leprosy in animals and failure to culture *Mycobacterium leprae in vitro*. Recently, Bergel[1] has claimed that rats kept on a special diet (pro-oxidant diet) can take the infection, specially when the lepromatous material is injected intratesticularly. Kelkar and Ranadive[2] have claimed that mice put on deficient diets take the infection. Still recently, O'Byrne[3] has claimed that hyperthyroidism favours leprosy. All these findings appear to indicate that metabolic and endocrine disorders are potent factors in the causation of leprosy. In view of the significance and importance of Bergel's claims, it was thought desirable to reinvestigate the matter.

Young male albino rats and Swiss mice (three weeks old) and male black hybrid mice as developed by Chatterji (also three weeks old) were put on pro-oxidant diet as advocated by Bergel[1] ten days prior to experiment. Raw linseed oil (iodine value, 165-80) was added to the diet daily.

Material for infecting the animals fed on pro-oxidant diet was obtained aseptically from earlobe biopsies of untreated cases of lepromatous leprosy attending the Faizabad Leprosy House. The earlobe biopsy material was aseptically ground up with sand, suspended in 0·9 per cent NaCl solution and centrifuged at about 1000 r.p.m. for about 5 min. A rough estimate of the number of acid-fast bacteria in the supernatant fluid was made from smears on slide and further diluted with physiological saline

TABLE 1 — MORTALITY IN INOCULATED WHITE RATS AND MICE AND BLACK HYBRID MICE

(Each animal was inoculated with 2000 M. leprae from untreated lepromatous cases)

Patient	Animal	No. of animals and route of inoculation		No. of animals dead	Av. survival period *days*
		IP	IT		
TH	WR	5	5	9	289
	WM	5	5	10	260
	BH	5	5	8	322
LL	WR	5	5	8	313
	WM	5	5	10	274
	BH	5	5	8	316
BR	WR	5	10	10	318
	WM	5	10	13	279
	BH	5	10	11	335
KR	WR	5	10	12	325
	WM	5	10	14	302
	BH	5	10	13	328

WR, white rats; WM, white mice; BH, black hybrid mice; IP, intraperitoneal; and IT, intratesticular.

as required to bring down the count of mycobacteria to 10,000 per ml. Each animal was either inoculated 0·1 ml. in each testes or 0·2 ml. intraperitoneally. The animals were observed for a period of 365 days and surviving animals sacrificed (Table 1).

Post-mortem examination was carried out in each case and smears from lungs, liver, spleen, testes and lymph glands made out on slides, stained Ziehl-Neelsen and examined for acid-fast bacteria. No tubercles were found in any organ. Acid-fast bacteria were not found either in the smears or within tissue cells of any of the above organs. Some of the animals died during summer and in others no specific cause of death could be ascribed.

Bergel's work of producing human leprosy in rats on pro-oxidant diet could not be confirmed in this laboratory. From time to time workers on experimental leprosy have relied on absence of culture of *M. leprae* on artificial culture media and on lepromin test. All that could be said is that *M. leprae murium* also does not grow in artificial culture media and according to Kooij and Gerritsen[4] any particulate matter can give rise to lepromin-like reaction. Mukerjee and Kundu[5] have again shown how in a laboratory rats could be easily infected with *M. leprae murium*. Therefore, some other criteria need to be worked out for evaluating transmission of leprosy in animals and culture of *M. leprae* on artificial media.

The authors' thanks are due to Shri L. N. Sinha for technical assistance and to Dr G. S. Patrick, Superintendent, Faizabad Leprosy Home, for permission to take biopsies.

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Effect of Fermentation on the Nutritive Value of *Idli*

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Manuscript received 9 *April* 1962

The process of fermentation does not increase the growth-promoting value of *idli* flour in rats, over the unfermented flour used as control. The overall growth in either case is fairly high. Thiamine content of the composition is not materially altered by fermentation.

IN continuation of our earlier studies on *idli* fermentation, it was of interest to study whether fermentation, besides imparting a soft and spongy texture to the product, has any effect on the growth-promoting value of the *idli*.

Composite *idli* flour prepared as described earlier[1,2] was allowed to ferment for 20 hr and after steam baking was fed to young weanling rats for a period of 8 weeks. The diet consisted of the cooked *idli* mixed with 1 per cent calcium carbonate, 5 per cent groundnut oil and 1 per cent shark liver oil. The composite mix made into batter with water and steam baked without any fermentation served as a control. Food intake was equalized between the two groups. The results of the growth study carried out for a period of 8 weeks are presented in Table 1.

It will be seen that there is no difference in the growth-promoting value of the *idli* mix as a result of fermentation. The average weekly growth or the increase in weight per gram of protein intake was about the same in both the groups. There was also no significant increase in the thiamine value as a result of fermentation (302, 320 and 281 μg./100 g. of flour, fermented batter and steamed *idli* respectively on a dry basis). It is, however, interesting to note, in this connection, that the *idli* can promote good growth in animals. The average weekly growth is of the order of 13 g. In similar feeding experiments with bread, Morgan[3] obtained an average weekly growth of about 10 g. for equivalent food intake. *Idli* is made from a mixture of cereal and pulse, and is, therefore, better than bread made from a cereal alone. When the *idli* mix is further fortified with Indian multipurpose food, its growth-promoting value is further enhanced[4].

Although the present experiments on rats have shown no increase in nutritive value in the *idli* components as a result of fermentation, it may increase

TABLE 1 — EFFECT OF FERMENTATION ON GROWTH IN RATS

(*Values represent averages of each group*; *No. of animals in each group*, 16)

	Group I: rats fed fermented *idli*	Group II: rats fed unfermented *idli*
Initial body wt, g.	48·40 ±0·27	48·30 ±0·25
Final body wt, g.	151·80 ±2·58	153·80 ±5·09
Increase in body wt, g.	103·40 ±2·39	105·50 ±4·40
Total food intake, g.	530·00	530·00
Total protein intake, g.	57·00	57·00
Increase in wt, g./g. protein intake	1·81	1·84
Av. weekly increase in wt, g.	12·90	13·20

the digestibility or ease of digestion of the *idli* by virtue of its spongy texture and thus facilitating enzyme action during digestion. It is proposed to verify this point by human feeding experiments and by *in vitro* studies.

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Composition & Free Amino Acids of Crocodile Muscle

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Manuscript received 26 *April* 1962

The composition and free amino acids content of the muscles of two specimen crocodiles caught in the Bhavanisagar reservoir have been determined. The muscle of the younger crocodile is richer in protein and phosphorus, and it also contains free proline which is absent in the older crocodile. Iron is present only in the flesh of the older specimen.

CROCODILES are valued mainly for their skin but their flesh is also widely used and is considered to have some medicinal value. There is, however, no reference in literature about the composition of crocodile flesh. It was, therefore, considered of interest to analyse the crocodile muscle for its moisture, protein, fat, ash, mineral and free amino acid contents.

Two crocodiles, one measuring 61 cm. and another 198 cm., were caught in Bhavanisagar reservoir, during 1957 and 1961 respectively, and their muscles analysed according to standard procedures. The free amino acids were detected from the 75 per cent alcoholic extract of the muscle according to the procedure described earlier by the authors[1]. The data presented in Table 1 show that the smaller (younger) crocodile had higher protein and phosphorus content than the larger (older) animal; the latter has a higher fat content. Calcium content does not vary. Iron is present only in the flesh of the older animal. The flesh of the younger crocodile gave a prominent proline band which was not noticed in the case of the flesh of the older animal. The presence of free amino acids in the flesh of the two animals was examined chromatographically. Bands corresponding to leucine, tryptophan, alanine, glycine-serine-aspartic acid, and an unidentified band below that of cystine, were obtained with the flesh of both the animals. The flesh of the younger animal gave in addition a prominent band corresponding to proline and a band for histidine. These were absent in the chromatogram of the flesh of the older animal. On the other hand, the flesh of the older animal gave bands corresponding to glutamic acid — threonine, taurine and lysine, and an unidentified band below that for alanine and cystine. A band corresponding to an organic acid was also observed in the chromatograms of the flesh of both the animals. Bands for free sugars and polyphenols were not present.

TABLE 1 — CHEMICAL COMPOSITION OF CROCODILE MUSCLE

	Smaller crocodile (*length*, 61 *cm.*)	Larger crocodile (*length*, 198 *cm.*)
Moisture, %	77·39	77·59
Protein (M×6·25), %	20·97	16·20
Fat, %	0·74	4·33
Ash, %	1·37	0·99
Phosphorus (as P_2O_5), mg. %	680·00	358·00
Calcium, mg. %	130·00	127·90
Iron, mg. %	—	11·00

Whether the differences observed in the composition of the flesh of the two specimens are due to age or due to different environmental and feeding conditions prevailing during 1957 and 1961 cannot be definitely stated. In view of the scanty knowledge about crocodiles, the present data would be useful.

Our thanks are due to Shri R. Soundara Raj for his assistance. The paper is published with the permission of the Director of Fisheries, Madras.

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Printed and published by Shri B. N. Sastri, Council of Scientific & Industrial Research, New Delhi, at the Catholic Press, Ranchi, India

Regd. No. P-842