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CONTENTS

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Amino Acid Composition of Indian Silk Fibroins & Sericins: Part I—Fibroins*

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The amino acid composition of the fibroins of four indigenous silks, viz. mulberry, tussah, muga and eri, has been determined by ion-exchange chromatography. The fibroins show significant variations in amino acid composition, particularly with respect to glycine, alanine, aspartic acid, serine and arginine. The mulberry fibroin is uniquely characterized by a higher content of glycine than alanine, while the other three types have a higher alanine than glycine content. The classification of the fibroins based on their chemical composition is discussed.

FOUR different types of silks are produced in India, viz. mulberry, tussah, muga and eri[1]. The insects which secrete these silks are *Bombyx mori*, *Antheraea mylitta*, *Antheraea assama* and *Attacus ricini* respectively. *Bombyx mori* larvae feed almost exclusively on mulberry leaves, while the larvae of the other three species are less specific in their nutritional requirements and feed on leaves of *sal* (*Shorea robusta*), *arjun* (*Terminalia arjuna*), *sum* (*Machilus odoratissima*) and castor oil plant. The four varieties of silks differ markedly in their colour, lustre and other physical properties[2]. The amino acid composition of the fibroin of mulberry silk has been extensively investigated[3] and a few studies have been reported on the composition of tussah silk fibroins[4,5], whereas the fibroins of the other two silks have not so far been studied. In the present investigation the amino acid composition of the fibroins and sericins from the four different types of Indian silks has been determined. This communication deals with the results of studies on the fibroins of Indian silks.

Experimental procedure

Methods — The fibroins were isolated from the raw silks by degumming and defatting according to the method of Lucas *et al.*[6]. Moisture was determined by drying to constant weight over P_2O_5 at 110°C. at a pressure of 2 mm. of mercury. Total nitrogen was determined by the micro-Kjeldahl method and the ash content of the samples according to the standard A.O.A.C. method[7].

Degummed and defatted samples (50-150 mg.) of the fibroins were hydrolysed with 6*N* hydrochloric acid in sealed tubes according to the method of Hirs *et al.*[8]. Excess hydrochloric acid was removed by repeated evaporation under reduced pressure and finally by leaving over KOH pellets in a vacuum desiccator. The residue was dissolved in 0·5*N* hydrochloric acid and made up to volume with water. The hydrolysates were then analysed for amino acids by chromatography on Dowex-50 (200-400 mesh, 8 per cent cross-linked) as described by Moore and Stein[9]. Amino acids were estimated by the ninhydrin method of Moore and Stein[9] using a Klett-Summerson photoelectric colorimeter with a 560 mμ green filter. A 0·9 × 15 cm. column was used for the analysis of basic amino acids with a load of about 5 mg. of protein hydrolysate. A 0·9 × 100 cm. column was used with a load of about 3-4 mg. of protein hydrolysate for the separation of all the amino acids except glycine and alanine which were estimated separately on the 100 cm. column with a load of only 0·20-0·25 mg. of the hydrolysate, on account of the very high content of these two amino acids in fibroin. Tryptophan was determined separately after alkaline hydrolysis

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according to the method of Horn and Jones[10]. Proline was estimated separately in the protein hydrolysates by the method described by Chinard[11] as the ninhydrin reaction was not adequately sensitive for the low levels of the amino acid present in the fibroins.

Results and discussion

The moisture, ash and nitrogen contents of the different silks are given in Table 1 and the amino acid composition of the four fibroins (on moisture-free basis) is recorded in Table 2.

It will be seen from the results presented in Table 1 that the nitrogen contents of the various fibroins vary only within the narrow limits of 18·60 to 18·90 per cent, while the ash contents show an appreciable difference, with mulberry and muga fibroins having 0·13 and 0·14 per cent ash respectively, and tussah and eri having 0·05 per cent each. Since only individual samples representative of each species were analysed, it would be difficult to decide whether the differences in ash contents are significant or not. Also, all the four fibroins contain high proportions of glycine, alanine, serine and tyrosine with smaller amounts of the other common amino acids. The glycine and alanine residues together constitute 72, 63, 67 and 61 per cent of the total nitrogen in the mulberry, tussah, muga and eri fibroins respectively. In this respect none of the fibroins analysed belongs to the type of *Anaphe* fibroins which probably constitute the simplest class of fibroins consisting almost exclusively of alanine and glycine residues, in some cases these amino acids making up 90 per cent or more of the weight of the protein[5]. The mulberry fibroin is unique from the analytical point of view, being characterized by a higher content of glycine than alanine. The ratio of glycine to alanine obtained in the present investigation is 1 : 0·66 and is in good agreement with that reported by other workers[4,12].

A common feature of the tussah, muga and eri fibroins is the higher content of alanine than glycine, the ratio of glycine to alanine being 0·6 : 1. Similar results have been reported for tussah fibroins by earlier workers[4,12,13]. In fact, the tussah type has been found to be the commonest and includes a large number of the species of Saturniinae sub-family.

Eri silk is characterized by a low serine content (4·96 per cent) compared to mulberry, tussah and muga (10·0, 8·62 and 7·88 per cent respectively).

The low content of proline and cystine in all the four fibroins follows the general pattern characteristic

TABLE 1 — MOISTURE, ASH AND NITROGEN CONTENT OF FIBROINS

(Ash and nitrogen expressed on moisture-free basis)

Silk	Moisture %	Ash %	Nitrogen %
Mulberry	7·70	0·13	18·90
Tussah	9·50	0·05	18·87
Muga	9·00	0·14	18·61
Eri	8·80	0·05	18·60

TABLE 2 — AMINO ACID COMPOSITION OF SILK FIBROINS

(Values expressed as g. amino acid/100 g. protein and amino acid N as % of total N on moisture-free basis)

Amino acid	Mulberry		Tussah		Muga		Eri	
	g./100 g. protein	Amino acid N/ total N	g./100 g. protein	Amino acid N/ total N	g./100 g. protein	Amino acid N/ total N	g./100 g. protein	Amino acid N/ total N
Glycine	44·29	43·75	24·49	24·24	25·47	25·55	26·32	26·42
Alanine	32·50	29·05	44·44	39·00	40·64	34·34	41·80	35·35
Valine	2·92	1·85	1·05	0·67	0·82	0·53	0·84	0·54
Leucine	0·75	0·42	0·62	0·35	0·70	0·40	0·67	0·38
Isoleucine	0·94	0·53	0·64	0·36	0·57	0·39	0·91	0·52
Serine	14·16	10·00	12·20	8·62	11·00	7·88	6·92	4·96
Threonine	1·46	0·90	0·51	0·32	1·27	0·80	0·56	0·36
Aspartic acid	2·70	1·51	9·90	5·53	8·52	4·82	5·74	3·25
Glutamic acid	2·14	1·07	1·54	0·78	2·28	1·17	1·29	0·66
Phenylalanine	1·13	0·50	0·96	0·43	1·23	0·56	1·40	0·64
Tyrosine	13·23	5·42	11·49	4·71	11·10	4·61	12·91	5·37
Lysine	0·59	0·60	0·05	0·05	0·28	0·29	0·29	0·30
Histidine	0·25	0·36	1·17	1·68	0·73	1·06	1·05	1·53
Arginine	1·10	1·90	6·95	11·84	7·07	12·25	4·01	6·95
Proline	0·78	0·50	1·10	0·70	0·72	0·47	0·80	0·52
Tryptophan	0·55	0·39	2·83	2·04	3·79	2·80	0·54	0·39
Methionine	—	—	—	—	—	—	—	—
Cystine	0·13	0·08	0·46	0·29	—	—	—	—
	119·62	98·83	120·40	101·61	116·19	97·92	106·05	88·14

of these fibres. In these features the fibroins are distinct from the collagens[14] which are rich in proline and hydroxyproline and the keratins[15] which have high levels of cystine.

Shaw and Smith[5] have suggested that *Bombyx mori* fibroin, which is deficient in basic and dicarboxylic amino acids, does not contain cross-bonding of the salt-linkage type. The high content of these amino acids in the non-mulberry fibroins suggests that salt linkages may exist in these proteins. Further investigation, however, would be necessary to establish the presence of such linkages.

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The Effect of Trace Elements, Other Nutritional Factors & *p*H on the Growth & Sporulation of *Penicillium* Species

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In experiments with different strains of two perithecial and twenty-two non-perithecial species of *Penicillium*, sporulation has been found to be most often stimulated by manganese and iron as trace elements. Vegetative growth has been found to be stimulated in the perithecial species most clearly by manganese, and in the non-perithecial species most often by zinc. Cobalt, copper and molybdenum are generally either without effect or inhibitory. Stimulation of growth and sporulation frequently do not occur together. Changes are often induced in mycelial colour and texture, and in the colour of the medium. For the fruiting of the non-perithecial species, the best nitrogen source among amino acids is tryptophane, methionine being the least suitable. An initial *p*H below 5·4 is favourable.

THE effect of carbon and nitrogen sources and vitamins on the growth and sporulation of two perithecial and several non-perithecial species of *Penicillium* has been reported in a previous communication[1]. In this study, the influence of trace elements, and of amino acids and *p*H on a limited scale, on growth, conidia formation and perithecia formation is investigated. The perithecial species comprised seven strains of *Penicillium vermiculatum* and six of *P. wortmanni*, which

belong to the *P. luteum* series. They were homothallic, since single ascospores, single conidia or hyphal tips developed fully perithecial colonies in which the complete cycle of the fungus was reproduced. The non-perithecial organisms studied comprised 29 strains, representing 22 species belonging to the main divisions of the genus.

Materials and methods

At the beginning of this study, three strains (77C, 77.10 and 77.32) of *P. vermiculatum* had lost the capacity of producing mature perithecia in subculture, and did not do so on any of the experiments here reported; they are not mentioned in the experimental results. Strains 77.29, 77.62 and 77.81 of this species usually produced perithecia, while 77.80B did so regularly on malt agar, but only under certain circumstances on potato-glucose agar. The six strains of *P. wortmanni* fruited normally. These fell into two rather distinct morphological groups[2], distinguished by the integral code numbers 126 and 130. The non-perithecial species tested included, apart from those mentioned in Table 4, *P. citrinum* 37, *P. citrinum* 37.1, *P. citrinum* 37.2, *P. cyaneum* 124, *P. frequentans* series 135, *P. implicatum* series 101 and *P. purpurogenum* 134.

The organisms were isolated mostly from rotten jute material over the last few years. In the identification of species the classification of Raper and Thom was followed. Fungi were subcultured since isolation alternately on malt agar and potato-glucose agar at intervals of 6 months. The basal medium (pH 7) contained $NaNO_3$, 2 g.; K_2HPO_4, 0·75 g.; KH_2PO_4, 0·25 g.; KCl, 0·5 g.; $MgSO_4.7H_2O$, 0·5 g.; and water, 1 litre. The carbon source was glucose — 1 per cent for perithecial and 3 per cent for non-perithecial species. All chemicals were of the highest available purity, and the water used was distilled twice in an all-glass Pyrex apparatus. Only Pyrex glassware, cleaned before each test with hot chromic acid, was employed. The media were taken in 10 ml. quantities in 50 ml. conical flasks, which were closed with aluminium caps. Sterilization was at 10 lb./sq. in. for 15 min.; incubation was at 27°C. The term 'fruiting' indicates perithecia formation only. Perithecia not showing ascospores after 14 days have been referred to as immature. The intensity of perithecia or conidia formation has been denoted arbitrarily in an increasing order with the numbers 0, 1, 2, 3 and 4; np denotes no perithecia. Mycelial dry weight was taken as a measure of growth. Mycelia were harvested on previously weighed filter paper circles (Whatman No. 1), and these were dried at 105°C. for 4 hr after excess water had been removed by placing them on blotting paper.

Results and discussion

Trace elements: (i) Perithecia formation and growth — Six mineral elements, namely Fe, Zn, Mn, Cu, Co and Mo, were tested over a range of concentration. The salts used were $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $MnSO_4.4H_2O$, $CuSO_4.5H_2O$, $Co(NO_3)_2.6H_2O$ and $Na_3PO_4.12MoO_2$; the concentrations of the salts are expressed as p.p.m. metal. Stimulation of the perithecial species was largely confined to the first three elements, and selected data are given in Tables 1-3. It is seen from the results given in Table 1 that the favourable effect of Fe was general, all strains of both species being benefited; the effect on perithecial crop was maximum on strain 77 29 of *P. vermiculatum* and the 130 strains of *P. wortmanni*. Fruiting was sometimes stimulated even at 1 p.p.m., but on the whole the optimum range was 100-200 p.p.m., beyond which signs of inhibition became increasingly apparent. Inhibition of vegetative growth was also seen at the higher concentrations, though not in strains 77 80B and 77.81 of *P. vermiculatum*; the latter strain actually showed a two-fold increase in mycelial weight at 1000 p.p.m., the maximum concentration tried. Except in this organism, clear stimulation of vegetative growth was not seen with Fe. The yellow mat of *P. wortmanni* changed to white submerged growth at the high Fe levels, and in the 130 group the medium turned from yellow to colourless.

It is seen from the results given in Table 2 that only strain 77.80B of *P. vermiculatum* remained unaffected by Zn. The optimum concentration was around 10 p.p.m.; at higher Zn levels its toxicity became apparent and there was complete inhibition of fruiting at 200 p.p.m. in all strains except one. The 126 group of *P. wortmanni* was distinctly less sensitive to Zn inhibition than the 130 group; the yellow colour of the mycelial mat of the latter strains turned nearly white at the higher Zn levels and the surface appeared wrinkled. Inhibition of vegetative growth occurred side by side with that of fruiting in all the organisms.

Table 3 shows that the effect of Mn, like that of Fe, was general and was felt even at 1 p.p.m. Again, the optimum concentration for both species was between 100 and 250 p.p.m. *P. wortmanni* tolerated much higher concentrations of Mn than *P. vermiculatum*. Stimulation of vegetative growth was much clearer with Mn than with Fe, and as before, strains 77.80B and 77.81 gave increasing mycelial weights up to the highest metal concentration

TABLE 1 — EFFECT OF DIFFERENT CONCENTRATIONS OF Fe ON FRUITING OF *P. VERMICULATUM* AND *P. WORTMANNI*

(*Glucose* 1 *per cent. Increasing intensity of fruiting denoted by numbers* 0-4)

Strain No.	Day of appearance of perithecia								Intensity of fruiting at 14 days							
	Control	1	10	100	150	200	250	500	Control	1	10	100	150	200	250	500
							P. vermiculatum									
77.29	np	np	np	6	8	5	6	10	0	0	0	3	3	3	3	2
77.62	9	8	6	6	6	5	6	10	1*	1*	2	3	3	3	3	2
77.80B	np	np	np	12	9	6	8	np	0	0	0	1*	1*	2	2	0
77.81	np	np	np	np	8	6	6	np	0	0	0	0	1	2	2	0
							P. wortmanni									
126.24	10	10	10	8	6	6	9	np	2	2	2	2	2	3	3	0
126.31	10	8	8	8	6	6	9	np	2	2	2	2	2	3	2	0
126.78	10	11	8	7	6	6	10	np	2	2	2	3	2	2	2	0
130.30	np	9	6	5	5	5	10	np	0	2	2	3	3	3	2	0
130.63	np	9	6	6	5	5	9	np	0	2	2	3	3	3	2	0
130.64	np	10	6	5	5	5	10	np	0	2	2	3	3	3	2	0

np = no perithecia. *Immature perithecia.

TABLE 2 — EFFECT OF DIFFERENT CONCENTRATIONS OF Zn ON FRUITING OF *P. VERMICULATUM* AND *P. WORTMANNI*

(*Glucose* 1 *per cent. Increasing intensity of fruiting denoted by numbers* 0-4)

Strain No.	Day of appearance of perithecia					Intensity of fruiting at 14 days				
	Control	1	10	100	200	Control	1	10	100	200
				P. vermiculatum						
77.29	np	8	8	10	np	0	2	2	1*	0
77.62	9	8	8	11	np	1	2	2	1*	0
77.80B	np	np	np	np	np	0	0	0	0	0
77.81	np	np	10	10	np	0	0	1*	1*	0
				P. wortmanni						
126.24	6	6	6	6	np	2	3	3	3	0
126.31	6	6	6	7	np	2	2	2	1	0
126.78	5	5	4	4	4	2	2	2	2	1
130.30	np	np	9	np	np	0	0	1	0	0
130.63	np	np	9	np	np	0	0	1*	0	0
130.64	np	np	9	np	np	0	0	1*	0	0

np = no perithecia. *Immature perithecia.

TABLE 3 — EFFECT OF DIFFERENT CONCENTRATIONS OF Mn ON FRUITING OF *P. VERMICULATUM* AND *P. WORTMANNI*

(*Glucose* 1 *per cent. Increasing intensity of fruiting denoted by numbers* 0-4)

Strain No.	Day of appearance of perithecia								Intensity of fruiting at 14 days							
	Control	1	10	100	250	500	2000	4000	Control	1	10	100	250	500	2000	4000
							P. vermiculatum									
77.29	np	8	8	8	8	8	np	—	0	1*	1	2	2	2	0	—
77.62	9	9	7	6	8	8	np	—	1*	1*	1	2	3	3	0	—
77.80B	np	np	np	9	7	8	13	—	0	0	0	1	2	1	1	—
77.81	np	np	np	8	8	8	12	—	0	0	0	1	2	2	1	—
							P. wortmanni									
126.24	10	7	7	8	9	9	9	9	2	2	2	3	2	2	1	1
126.31	9	7	7	7	7	8	9	9	2	2	3	3	2	2	2	2
126.78	10	5	5	5	5	8	8	9	2	2	2	3	2	2	2	2
130.30	np	6	5	4	5	5	9	9	0	2	2	3	3	3	2	1
130.63	np	5	5	5	5	8	9	9	0	2	2	3	3	3	3	1
130.64	np	9	7	5	5	5	8	10	0	2	2	3	3	3	2	1

np = no perithecia. *Immature perithecia.

TABLE 4 — EFFECT OF TRACE ELEMENTS ON GROWTH AND SPORULATION OF *PENICILLIUM* SPECIES

(*Glucose 3 per cent. Figures in parentheses represent p.p.m. metal*)

Fungus and ref. No.	Sporulation markedly stimulated by	Maximum increase in mycelial wt (%)					
		Fe	Zn	Mn	Cu	Co	Mo
P. adametzi 128	Fe (500)	92 (100)					56 (1)
P. canescens series 136	Fe (10-100)		34 (100)				
P. fellutanum 7		56 (100)				26 (10)	
P. fellutanum 125					31 (1)		
P. fellutanum series 135			31 (100)				
P. funiculosum series 150	Mn (5-500)		32 (100)				
P. implicatum 53		19 (100)					
P. luteum 28	Mn (10)						
P. notatum 138	Zn (10)						
P. ochro-chloron 153.1		23 (10)			23 (500)	17 (10)	
P. oxalicum 96	Fe (100)						
P. roseo-purpureum 148						30 (1)	30 (10)
P. rubrum 127	Fe (100) Zn (10) Mn (10-100)						
P. simplicissimum 4	Mn (10-100)						
P. steckii 137	Mn (500)		27 (100)	27 (100)			
P. tardum 132	Mn (5-50)						
P. variabile 34				24 (10)			
P. variabile 121	Fe (100) Mn (100)					20 (1)	27 (1)
P. variabile 121.1		48 (10)	21 (100)		32 (1)		
P. variabile 121.2	Fe (10-100)		18 (10)		24 (1)		
P. verruculosum 158			69 (100)				
Penicillium sp. 40		69 (10)	44 (10)	33 (100)	54 (1)		
Penicillium sp. 98			64 (100)				

tried. In the presence of Mn the yellow mat of *P. wortmanni* turned greenish yellow, with orange exudate.

The effect of Cu, Co and Mo was mainly inhibitory on both growth and fruiting. Mycelial growth was completely suppressed by Cu even at 10 p.p.m. in all strains except 126.78 of *P. wortmanni*. With Co, however, the 126 strains of this species showed hardly any difference in growth and fruiting up to 250 p.p.m., although no perithecia were formed and growth nearly stopped in the other organisms at 10 p.p.m.; some sign of stimulation was seen with strain 77.29 of *P. vermiculatum* and the 130 group of *P. wortmanni* at 1 p.p.m., in the formation of immature perithecia. Tolerance to Mo was least among the 126 strains of *P. wortmanni* and greatest in *P. vermiculatum*, of which some strains showed no difference in vegetative growth up to 200 p.p.m. Mo, however, engendered a few fertile perithecia in the 130 strains of *P. wortmanni* at 10 p.p.m., and in strains 77.29 and 77.62 of *P. vermiculatum* between 50 and 100 p.p.m.

(ii) Conidia formation and growth — All instances of definite stimulation of conidia formation or increase in mycelial weight by more than 15 per cent in the non-perithecial species are recorded in Table 4. The time required for conidia to appear could always be correlated with the intensity of sporulation after 14 days.

Cu, Co and Mo never caused marked stimulation of sporulation at 1 or 10 p.p.m.; sometimes there was inhibition at 10 p.p.m.

It is seen that Mn stimulated the maximum number of species, closely followed by Fe, and that *P. rubrum* was sensitive to all three elements. Considerable variation was observed between strains in *P. variabile*. Organisms which did not sporulate on the control medium but showed slight conidia formation with a trace element were: *P. cyaneum* type 124 (10-100 p.p.m. Zn), *P. implicatum* series 101 (10-100 p.p.m. Zn) and *P. simplicissimum* 4 (100 p.p.m. Fe and 10-100 p.p.m. Zn).

Fe, Zn and Mn sometimes delayed conidial germination (as judged from the first appearance of visible mycelia) and sporulation slightly, though having a favourable ultimate effect on sporulation. With Fe, where the action on sporulation was most marked, there was a relatively striking change in the appearance of the mycelium and often in the colour of the culture solution; for example, between 10-100 p.p.m. Fe, the white mat of *P. simplicissimum* 4 changed to grey-white and the medium turned yellow; the mycelia of *P. rubrum* 127 and *P. adametzi* 128 changed to orange-green and bluish white respectively. The *P. variabile* strains, which ordinarily produced pinkish white mycelia, gave blue to bluish green mats with 10 p.p.m. Zn, but reverted to the original colour at 100 p.p.m., where the solution turned brown.

Stimulation of vegetative growth was seen more or less clearly in 17 organisms, and these were very often different from those showing increased sporulation. Most of the organisms responded to more than one trace element, the most sensitive being *Penicillium* sp. 40, in which, however, conidial growth could never be induced. Zn was the metal most often effective, followed by Fe. Instances of inhibition were seen with all the metals, particularly at the higher concentrations, and with Cu, Co and Mo. However, some selected organisms, when tested with higher metal concentrations, showed growth stimulation up to 500 p.p.m. metal. Maximum growth was obtained at 500 p.p.m. Cu with *P. ochro-chloron* 153.1, which has previously been shown to be extremely copper-tolerant[3].

Mixtures of trace elements registering at least a 10 per cent increase in mycelial weight were next tried, at optimum concentrations, on *P. funiculosum* series 150 (Fe + Zn + Mn), *P. implicatum* 53 (Fe + Cu), *P. ochro-chloron* 153 1 (Fe + Cu + Co), *P. variabile* 121.1 (Fe + Zn + Cu) and *Penicillium* sp. 98 (Fe + Zn). Definite stimulation, either in growth or sporulation, could always be explained by the effect of one of the metals alone. On the other hand, with *P. variabile* 121.1, sporulation was clearly less intense with Fe, Zn and Cu than with Fe alone. This kind of metal antagonism was found by Mann[4] with *Aspergillus niger*; he showed that although Zn stimulated growth, and Ca had no effect, a combination of the two was harmful.

B vitamins plus trace elements — Thiamin, which in the previous study[1] had shown marked stimulation of perithecial fruiting in some instances, was tested in mixtures with Fe, Mn, Zn, Co and Mo at their optimum concentrations for the perithecial strains, at an initial *p*H 5·0 and 1 per cent glucose concentration. Only in *P. vermiculatum* was the total effect of the mixture distinctly superior to that of any single component, as seen in quickness and intensity of fruiting; strains 77.29 and 77.62 also showed increased perithecial size, and strain 77·80B formed large pinkish perithecia. Similar mixtures particularly enhanced conidial formation in the three non-fruiting strains of this species. In *P. wortmanni*, the mixture showed no improvement over single components and sometimes there were signs of inhibition.

Thirteen selected non-perithecial organisms were also tested against mixtures of trace elements and vitamins of the B group which had previously shown a favourable effect on growth or sporulation. In no instance did the improvement exceed that due to a single component.

That the trace elements tested often showed only an enhancing effect does not preclude the possibility of absolute requirement for growth or normal spore formation; such requirements are usually very small and are supposedly met from impurities in the medium constituents, glassware and inoculum. Stimulation was, however, seen occasionally at relatively high metal concentration, and this suggests roles as structural nutrients; in smaller amounts, these metals probably acted as components of enzymes or as coenzymes, such as in the stimulation of fruiting of one group of *P. wortmanni* at 1 p.p.m. Fe.

Work based mainly on the single organism *Aspergillus niger* has led to the conclusion that sporulation and growth in fungi have qualitatively the same mineral requirements[5]. This is not supported by the present data, nor by our previous findings with *Chaetomium* species[6].

Amino acids — Six amino acids were tried as N sources for the perithecial species on the 1 per cent glucose medium, the N content being the same as in the basal medium. It is seen from the results given in Table 5 that all the organisms fruited on tryptophane and none did so on methionine. The former induced even better fruiting in *P. vermiculatum* than peptone[1]. Next to tryptophane, leucine and histidine were favourable for perithecial formation. With the strains of *P. wortmanni*, fruiting

TABLE 5 — EFFECT OF DIFFERENT AMINO ACIDS ON FRUITING OF *P. VERMICULATUM* AND *P. WORTMANNI*

(*Glucose* 1*per cent. Increasing intensity of fruiting denoted by numbers* 0-4)

Strain No.	Day of appearance of perithecia						Intensity of fruiting at 14 days					
	Glycine	Alanine	Leucine	Histidine	Tryptophane	Methionine	Glycine	Alanine	Leucine	Histidine	Tryptophane	Methionine
P. vermiculatum												
77.29	np	np	12	7	10	np	0	0	1	2	2	0
77.62	np	np	10	7	10	np	0	0	1	2*	2	0
77.80B	np	np	np	np	11	np	0	0	0	0	1	0
77.81	np	np	10	np	10	np	0	0	2	0	2	0
P. wortmanni												
126.24	5	5	7	5	9	np	3	2	2	3	2	0
126.31	6	6	8	8	7	np	2	2	2	2	2	0
126.78	6	6	10	6	8	np	2	2	2	2	2	0
130.30	5	5	7	7	6	np	1	1	2	2	2	0
130.63	5	5	8	10	8	np	1	1	2	2	2	0
130.64	5	5	8	10	8	np	1	1	2	2*	2*	0

np = no perithecia. *Immature perithecia.

occurred on all the amino acids except methionine. Mycelial growth of all the fungi tested was lowest on methionine and higher on the amino acids supporting better fruiting. Methionine often retarded the initial vegetative growth of the *P. vermiculatum* strains. Steinberg[7] has observed S-containing amino acids to be poor N sources.

Effect of pH — Following up previous indications that the pH of the medium could have considerable influence on the fruiting of the perithecial species, tests were carried out at different initial pH values, the pH of the medium being adjusted with dilute HCl or NaOH. Buffered media could not be used since the chemicals introduced proved to be inhibitory. Growth was obtained over the entire range, there being only slight inhibition of vegetative growth in the alkaline range occasionally. Growth and fruiting were most favoured below pH 5·4, *P. vermiculatum* fruiting only within that range and usually best at pH 4·6 (strain 77.80B again did not fruit throughout). In *P. wortmanni*, although the optimum pH lay in general in this region, the strains showed greater tolerance of a higher initial pH, particularly the 126 group. They showed wide powers of adjusting the pH of the medium.

P. luteum 28, *P. rubrum* 127, *P. simplicissimum* 4 and *Penicillium* sp. 98, which showed no conidial formation on the 3 per cent glucose medium at pH 7, did so at pH 5.

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Studies on the Metabolic Effects of Chloramphenicol

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The effects of prolonged administration (7-14 days) of chloramphenicol on carbohydrate, lipid and protein metabolism in rabbits have been investigated. Administration of the drug has been found to increase the fasting blood sugar and the plasma beta lipoprotein levels and to decrease serum proteins, serum albumin and plasma alpha lipoproteins. The administration of the drug also increases the cholesterol content of liver and skin, and the phospholipid content of skin. Carbohydrate utilization is not hampered after the administration of chloramphenicol. The metabolism of lipids and of protein, in particular, is altered by the drug.

ANTIBIOTICS are widely used by the physicians in the treatment of infectious diseases. There are many reports of the alterations of carbohydrate and lipid metabolism after treatment with antibiotics. In studies with healthy adult men and women it was observed by Befani and Angioletti[1] that administration of 2 g. chloramphenicol daily for 10 days led to hypoglycaemia. Pacheco[2] also reported hypoglycaemia in patients treated with chloramphenicol. Roccuzzo and Sciacca[3] showed that the blood cholesterol of children with typhoid was lowered by treatment with chloramphenicol. Banerjee *et al.*[4] reported that glucose tolerance diminished and liver glycogen decreased in rabbits and rats fed terramycin. No such effects were observed after similar treatment with achromycin. Total cholesterol content of whole body of rats increased after they were fed terramycin or achromycin. Chatterjee *et al.*[5,6] showed that prolonged administration of penicillin and dihydrostreptomycin increased blood sugar and lowered glucose tolerance of rabbits. The metabolic effects of the various antibiotics should, therefore, be known before they are used in the treatment of diseases.

The effects of administration of chloramphenicol on blood sugar, glucose tolerance, plasma lipids, serum proteins and tissue lipids of rabbits have been investigated and the results are presented in this communication.

Materials and methods

Healthy male albino rabbits, weighing 1·5-2·0 kg., were fed *ad libitum* germinated Bengal gram (*Cicer arietinum*) and green grass. After overnight fasting, they were fed glucose, 4 ml. of a 50 per cent solution per kg. body weight, and samples of blood were withdrawn from the ear vein before and at intervals after glucose for the estimation of blood sugar. A fasting blood sample was withdrawn next morning. Plasma and serum of these blood samples were used for the estimation of lipids and proteins. After the above studies were completed, the animals were given a daily intramuscular injection of 125 mg. chloramphenicol per animal for 7 days. On the 8th day the glucose tolerance test was repeated and fasting blood samples used for the determination of lipids and proteins.

Chloramphenicol was injected, 125 mg./animal/day, for 14 days in 4 rabbits. The rabbits which did not receive the injection served as normal controls. All the animals were sacrificed on the 15th day by stunning and decapitation. Heart, liver, kidney, adrenals, intestine, brain and skin were removed, weighed, dried at 85°C., soxhleted for 18 hr with petroleum ether and aliquots of the measured extract used for the estimation of total cholesterol and phospholipids.

Blood sugar was estimated by the method of Hagedorn and Jensen as described by Hawk *et al.*[7]. Plasma and tissue cholesterol were determined by the method of Abell *et al.*[8]. Plasma and tissue phospholipids were measured by determining the phosphorus content of the ether extract, multiplying the phosphorus value by 25 and expressing the result in terms of lecithin[7]. Non-esterified fatty acids were estimated by the method of Dole *et al.*[9]. Lipoproteins were separated by paper electrophoresis[10]. Electrophoresis of unstained plasma was also performed along with electrophoresis of plasma stained with acetylated Sudan Black B. Paper strips with

TABLE 1 — EFFECT OF CHLORAMPHENICOL ADMINISTRATION ON PLASMA LIPIDS OF RABBITS

(*No. of animals employed*, 10; *the animals were given* 125 *mg. of the drug daily for* 7 *days*)

	Total plasma cholesterol *mg./*100 *ml.*	β-Lipoprotein cholesterol %	Plasma phospholipid as lecithin *mg./*100 *ml.*	Non-ester fatty acid *m.eq./litre*	Lipoproteins, %	
					α	β+O
Before chloramphenicol	41±2·6	94±4·2	92±11	593±105	25±0·6	75±1·5
After chloramphenicol	48±4·0	72±4·3	148±27	666±63	15±2·0	85±2·1
t	1·46	4·7*	1·92	0·60	4·78*	3·81*

*Significant at 5 per cent level.

TABLE 2 — EFFECT OF CHLORAMPHENICOL ADMINISTRATION ON SERUM PROTEINS OF RABBITS

(*No. of animals employed*, 10; *the animals were given* 125 *mg. of the drug daily for* 7 *days*)

	Total serum protein %	Serum proteins, % of total protein			
		Albumin	α-Globulin	β-Globulin	Υ-Globulin
Before chloramphenicol	6·95±0·49	48·7±3·13	22·4±2·75	14·4±2·02	14·4±2·89
After chloramphenicol	5·58±0·22	38·4±1·66	25·9±3·19	18·3±1·84	17·4±2·20
t	2·34*	2·9*	0·83	1·42	0·82

*Significant at 5 per cent level.

TABLE 3 — ORAL GLUCOSE TOLERANCE TEST

[*The rabbits were administered* 2 *g./kg. glucose before and after injection of chloramphenicol; the animals were given an injection of chloramphenicol* (125 *mg./animal*) *daily for* 7 *days*]

	Fasting blood sugar *mg./*100 *ml.*	Rise in blood sugar (*mg./*100 *ml.*) from fasting level after feeding glucose				
		0·75 hr	1·5 hr	2·25 hr	3·0 hr	4·0 hr
Before chloramphenicol	117±5·7	84±12	106±11	85±14	49±8	3±3
After chloramphenicol	134±2·3	101±9	127±9	92±16	39±15	1±9
t	2·7*	1·12	1·46	0·33	0·57	0·19

*Significant at 5 per cent level.

TABLE 4 — CHOLESTEROL CONTENTS OF TISSUES

(*Values represent mg. of cholesterol/*100 *g. wet wt of tissue*)

Animals	Heart	Kidney	Liver	Intestine	Skin	Adrenals	Brain
Normal	195±4	428±8	336±4	314±41	51±22	6510±755	3425±111
Chloramphenicol treated*	194±21	446±11	372±9	271±21	110±13	7430±1370	3450±157
STATISTICAL ANALYSIS							
Diff. of means	1	18	36	43	59	920	25
Standard error of diff.	21·63	13·91	10·18	46·54	25·95	2089	192·93
t	0·04	1·29	3·5†	0·92	2·27†	0·44	0·12

*These rabbits were injected with chloramphenicol (125 mg./animal/day) for 14 days and killed on the 15th day. There were 4 animals in each group. The values are mean ± standard error of mean.

†Significant at 5 per cent level. Other values of *t* were not significant.

TABLE 5 — PHOSPHOLIPID CONTENTS OF TISSUES

(*Values represent mg. of phospholipid/*100 *g. wet wt of tissue*)

Animals	Heart	Kidney	Liver	Intestine	Skin	Adrenals	Brain
Normal	487±34	317±15	933±29	527±33	38±14	2647±238	5124±97
Chloramphenicol treated	491±63	425±60	991±77	432±65	85±17	2806±180	4977±293
STATISTICAL ANALYSIS							
Diff. of means	4	108	58	95	47	159	147
Standard error of diff.	71·72	61·91	82·58	72·7	22·07	299	308·6
	0·05	1·74	0·702	1·30	2·1*	0·53	0·47

*Only this value of *t* was significant at 5 per cent level.

the unstained plasma corresponding to beta lipoproteins of the stained plasma were cut, dried, extracted with alcohol-acetone mixture (1 : 1), the extract evaporated to dryness, cholesterol estimated[8] and the results expressed as beta lipoprotein cholesterol. Different fractions of serum proteins were determined by paper electrophoresis and of total protein by micro-Kjeldahl procedure[11].

Results

Cholesterol, phospholipid, non-esterified fatty acids and lipoprotein values of plasma obtained from rabbits, with or without chloramphenicol treatment, are given in Table 1. Total cholesterol, phospholipids, non-esterified fatty acids and beta lipoproteins of plasma increased after treatment of the animals with chloramphenicol but the increase was statistically significant only in the case of plasma beta lipoproteins. Beta lipoprotein cholesterol expressed as per cent of total cholesterol and the alpha lipoprotein of plasma significantly decreased after chloramphenicol treatment. There was increase in the beta-alpha lipoprotein ratio in the plasma of rabbits treated with chloramphenicol.

Values for the total proteins and different fractions of serum proteins are given in Table 2. There was significant diminution in the total serum proteins and of albumin and an increase in serum globulins without any change in the relative proportions of its different fractions after treatment of animals with chloramphenicol.

There was a significant increase in the fasting blood sugar level after the rabbits received chloramphenicol for 7 days (Table 3). Although the blood sugar values in all the samples of blood, collected at different times after the feeding of glucose, were at higher levels in the animals receiving chloramphenicol as compared to the values in the untreated animals, they were not statistically significant. This indicated that utilization of glucose was not affected by chloramphenicol administration.

The cholesterol contents of tissues are given in Table 4 and of phospholipids in Table 5. Cholesterol content significantly increased in liver and skin and phospholipids increased significantly only in skin, after the animals were treated with chloramphenicol. Other tissues studied did not show any significant change in the distribution of cholesterol and phospholipid after chloramphenicol administration.

Discussion

Administration of chloramphenicol increased the fasting blood sugar values. This is in contradiction to earlier reports[1,2] where hypoglycaemia was observed under similar conditions. In spite of the higher fasting blood sugar values of the treated animals, administered glucose disappeared from the blood at the same rate as the untreated controls. This indicated that the metabolism of carbohydrates was not affected by chloramphenicol administration.

A decrease in the alpha lipoprotein with increase in the beta fraction observed in the plasma of chloramphenicol-treated rabbits was similar to the observations of Jencks *et al.*[12] in patients with myocardial infarctions. A significant increase in the cholesterol content of liver and skin and of phospholipids of skin along with the change in the lipoprotein patterns of plasma in animals receiving chloramphenicol treatment indicated derangement of lipid metabolism. Prolonged use of the drug, therefore, might lead to atherosclerotic changes. Diminution of total serum proteins and of serum albumin after chloramphenicol indicated disturbed protein metabolism. The drug, therefore, should be used very carefully bearing in mind the metabolic derangement likely to be produced after its prolonged use.

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Nutritive Value of Field Bean (*Dolichos lablab*): Part V—Nature of Possible Antigrowth Factor

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It has been shown that the trypsin inhibitor present in the field bean does not inhibit the growth of rats. The antigrowth factor is not extracted by methanol, ethanol (70 per cent) and chloroform. The protein fraction precipitated at *p*H 4·5 from water extract of raw field bean as well as the protein isolated by aqueous sodium chloride (3 per cent) extraction of raw field bean retard the growth of rats; acid and tryptic hydrolysates of protein from the latter source retard the growth of rats even when supplemented with deficient essential amino acids. It is concluded that the antigrowth factor is neither a protein nor a peptide but is associated with the protein component of the field bean.

EARLIER communications from this laboratory[1] have indicated that the low nutritive value of field bean might be due to the presence of an antigrowth factor. It was, therefore, considered of interest to study the nature of this factor in detail.

Dihydroxyphenylalanine[2], β-nitropropionic acid[3,4], γ-aminobutyric acid[5,6], β-aminopropionitrile[7] and canavanine[8-10] found in certain legumes and vegetables have been reported to be toxic to and retard the growth of animals. These substances, with the exception of dihydroxyphenylalanine, have been shown earlier to be absent in the field bean[11], and, therefore, the antigrowth factor must be some other substance. It has been reported by some workers that the low nutritive value of legumes as well as the growth retardation of animals[12] are due to the presence of trypsin inhibitor. Trypsin inhibitor is present in the field bean[13] and studies have, therefore, been carried out to find out if this is responsible for the growth inhibition in rats. Also, the effect of adding trypsin to field bean diet has been investigated as it has been reported that addition of trypsin to soyabean counteracts growth retardation in rats[14,15]. The nutritive value of soyabean has been reported to improve after extracting it with methanol[16], and, therefore, studies have been carried out to find out whether by extracting the field bean with different solvents, the antigrowth factor can be removed. The effects of feeding field bean flour after extraction with water and of feeding protein precipitated from the water extract has also been investigated. The protein isolated from raw double bean (*Vicia faba* Moench) has been shown to inhibit the growth of rats and this property of the protein is lost by hydrolysing it with acid[17]. Therefore, the effects of feeding the protein isolated from the field bean by sodium chloride (3 per cent) extraction and its hydrolysates (acid and enzymic) have been investigated. The results of these studies are reported in this paper.

Experimental procedure

Young male albino rats (35-45 g.) were employed in these studies and pair feeding method was resorted to in all the experiments. The composition of the diet employed was: protein, 10; sucrose, 31·2; salt mixture[18], 4; and groundnut oil, 6 per cent; the total was made up to 100 per cent by the addition of corn starch. Before feeding the diets, 0·06 g. of a vitamin mixture[19] 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). Casein was used as the standard reference protein. The rats were placed in separate cages and water was supplied *ad libitum*. The protein in all the diets was maintained at 10 per cent level. A daily record of the amount of diet consumed and a weekly record of the weights of the rats were maintained.

Results and discussion

Raw field bean flour was tested for the presence of dihydroxyphenylalanine*. However, it was found

*Raw field bean flour (0·2-0·3 mg.) was extracted with 2 ml. of 0·1*N* HCl for 1 hr and the extract chromatographed using *n*-butanol-acetic acid-water (4:1:5) mixture as the solvent system. Dihydroxyphenylalanine was identified according to Block *et al.*[20].

to be absent in field bean. It could, therefore, be concluded that the antigrowth factor present in field bean is different from the toxic substances mentioned earlier.

Effect of trypsin inhibitor present in the field bean on the growth of rats — A batch of 15 rats was divided into three groups receiving the following diets: (i) casein; (ii) raw field bean; and (iii) raw field bean plus crude trypsin at 2 per cent level. The results presented in Table 1 show that addition of trypsin to raw field bean diet does not overcome growth retardation in rats. This indicates that the trypsin inhibitor in field bean has no effect on the growth of rats.

The above finding was confirmed by the following experiment in which trypsin inhibitor isolated from field bean was added to the diet of rats. A batch of 20 rats were divided into four groups receiving the following diets: (i) casein; (ii) casein plus trypsin inhibitor (obtained from 50 g. of raw field bean per 100 g. of diet); (iii) field bean autoclaved for 5 min. at 15 lb./sq. in. pressure; and (iv) autoclaved field bean plus trypsin inhibitor (the amount added was equal to that destroyed when the beans are autoclaved, i.e. 40 per cent[21]). The results recorded in Table 2 show that addition of trypsin inhibitor to the diets does not bring about any retardation in the growth of rats. Similar observations have been made in the case of double bean trypsin inhibitor[22].

Effect of solvent extracted field bean on the growth of rats — Raw field bean flour was extracted separately with methanol, 70 per cent ethanol and chloroform in a continuous soxhlet extractor. The residual flours were dried in air, freed from the solvents and the extracts were concentrated *in vacuo*. The nitrogen content of the extracted flours and the concentrated extracts was determined.

A batch of 40 rats was divided in eight groups receiving the following diets: (i) casein; (ii) raw field bean; (iii) methanol extracted field bean; (iv) ethanol extracted field bean; (v) chloroform extracted field bean; (vi) casein plus methanol extract; (vii) casein plus ethanol extract; and (viii) casein plus chloroform extract. The amount of the extracts added to 100 g. casein diet corresponded to that extracted from 50 g. of bean flour. The results of these studies recorded in Table 3 show that solvent extracted flours failed to improve the growth of rats and that the antigrowth factor was still retained in the extracted flours.

Effect of feeding water extracted bean flour, protein precipitated from water extract and residual extract — Raw bean flour was extracted with water for 4-5 hr at room temperature and the residual flour dried at 37°C. The *p*H of the water extract was adjusted to 4·5 and the protein was precipitated out, separated and dried at 37°C. The clear filtrate was concentrated *in vacuo*. The nitrogen content of all the three was determined.

A batch of 30 rats was divided into six groups receiving the following diets: (i) casein; (ii) raw bean flour; (iii) water extracted field bean flour; (iv) water extracted field bean flour supplemented with deficient amino acids; (v) protein precipitated from water extract at *p*H 4·5; and (vi) water extract after removal of this protein.

TABLE 1 — EFFECT OF ADDITION OF TRYPSIN TO RAW FIELD BEAN DIET AT 2 PER CENT LEVEL

(*No. of rats in each group*, 5; *av. wt of diet consumed per rat in 2 weeks*, 80 *g.*)

Group No.	Source of protein in diet	Change in wt of rats after 2 weeks *g.*
1	Casein	+9·4±0·8
2	Raw field bean	−12·0±0·7
3	Raw field bean + trypsin	−11·8±0·7

TABLE 2 — EFFECT OF FEEDING TRYPSIN INHIBITOR ISOLATED FROM RAW FIELD BEAN

(*No. of rats in each group*, 5; *av. wt of diet consumed per rat in 2 weeks*, 81·4 *g.*)

Group No.	Source of protein in diet	Change in wt of rats after 2 weeks *g.*
1	Casein	+6·6±0·3
2	Casein + trypsin inhibitor	+6·2±0·6
3	Autoclaved field bean	−7·8±0·9
4	Autoclaved field bean + trypsin inhibitor	−8·0±1·0

TABLE 3 — EFFECT OF EXTRACTION OF RAW FIELD BEAN WITH DIFFERENT SOLVENTS

(*No. of rats in each group*, 5; *av. wt of diet consumed per rat in 2 weeks*, 76·4 *g.*)

Group No.	Source of protein in diet	Change in wt of rats after 2 weeks *g.*
1	Casein	+6·4±0·9
2	Raw field bean	−13·2±2·9
3	Methanol extracted raw field bean	−12·6±1·3
4	Ethanol (70%) extracted raw field bean	−11·6±1·7
5	Chloroform extracted raw field bean	−13·1±2·6
6	Casein + methanol extract	+6·2±2·0
7	Casein + ethanol (70%) extract	+6·2±1·0
8	Casein + chloroform extract	+6·3±1·2

TABLE 4 — EFFECT OF EXTRACTION OF RAW FIELD BEAN WITH WATER

(*No. of rats in each group*, 5; *av. wt of diet consumed per rat in* 2 *weeks*, 80 *g.*)

Group No.	Source of protein in diet	Change in wt of rats after 2 weeks *g.*
1	Casein	+8·4±0·7
2	Raw field bean	−12·0±0·5
3	Water extracted raw field bean	−11·8±1·2
4	Water extracted raw field bean + amino acids	−11·6±0·7
5	Protein ppted from water extract	−11·8±0·7
6	Casein + remaining water extract	+8·6±1·1

TABLE 5 — EFFECT OF FEEDING PROTEIN ISOLATED FROM RAW FIELD BEAN

(*No. of rats in each group*, 5; *av. wt of diet consumed per rat in* 2 *weeks*, 81·2 *g.*)

Group No.	Source of protein in diet	Change in wt of rats after 2 weeks *g.*
1	Casein	+11·2±1·0
2	Raw field bean	−13·6±1·3
3	Protein isolated from raw field bean	−13·0±0·8
4	Protein isolated from raw field bean + amino acids	−13·0±1·4

The results given in Table 4 show that the antigrowth factor in the field bean is partially extracted by water and is associated with the protein precipitated from the water extract.

Effect of feeding protein isolated from field bean by extraction with sodium chloride solution and its hydrolysates — Raw field bean flour was extracted with 3 per cent sodium chloride solution and the proteins were isolated[23]. The major protein fraction was precipitated by dialysing the extract against water, separated and dried at 37°C.

A batch of 20 rats divided into four groups received the following diets: (i) casein; (ii) raw field bean; (iii) isolated protein; and (iv) isolated protein supplemented with deficient amino acids. The results recorded in Table 5 show that protein isolated by extraction of field bean with sodium chloride solution does not support the growth of rats even after supplementation with the deficient essential amino acids.

Effect of feeding field bean protein hydrolysate — Acid hydrolysate of the protein was prepared as follows. The protein isolated by extraction of the flour with sodium chloride and casein were hydrolysed by autoclaving for 8-10 hr at 15 lb./sq. in. pressure, the hydrolysates adjusted to *p*H 5·0 with barium hydroxide, filtered and the precipitates washed with water till free from amino acids. The filtrates, after adjusting their *p*H to 7·0, were concentrated *in vacuo*. The amounts of tryptophan destroyed during hydrolysis were added to the hydrolysates.

Tryptic hydrolysate of the protein was prepared as follows: Suspensions of casein and the field bean protein in distilled water were adjusted to *p*H 8·0 and hydrolysed with trypsin at 37°C. for 8 days. The rate of hydrolysis was studied by determining the rate of release of amino-nitrogen after a definite interval of time. After completion of hydrolysis, the hydrolysates were heated in a boiling water bath for 30 min., filtered, *p*H adjusted to 7·0 and concentrated *in vacuo*.

A batch of 30 rats was divided into six groups receiving the following diets: (i) casein acid hydrolysate; (ii) casein enzyme hydrolysate; (iii) field bean protein acid hydrolysate; (iv) field bean protein enzyme hydrolysate; (v) field bean protein acid hydrolysate plus deficient essential amino acids; and (vi) field bean protein enzyme hydrolysate plus deficient essential amino acids. The results recorded in Table 6 show that the protein hydrolysates even after supplementation with essential amino acids show growth inhibition in rats. The results presented in Tables 5 and 6 indicate that the antigrowth factor in field bean is not a protein or a peptide.

From the results of the present and earlier investigations it can be concluded that the low nutritive value of raw field bean might be due to the presence of some antigrowth factor which is destroyed by autoclaving the beans for 5 min. The antigrowth factor is not a peptide or a protein,

TABLE 6 — EFFECT OF FEEDING ACID AND ENZYME HYDROLYSATES OF PROTEIN ISOLATED FROM RAW FIELD BEAN

(*No. of rats in each group*, 5; *av. wt of diet consumed per rat in* 2 *weeks*, 77·0 *g.*)

Group No.	Source of protein in diet	Change in wt of rats after 2 weeks *g.*
1	Casein acid hydrolysate	+5·4±1·1
2	Casein enzyme hydrolysate	+5·3±0·9
3	Raw field bean protein acid hydrolysate	−13·2±1·4
4	Raw field bean protein acid hydrolysate + amino acids	−11·4±0·7
5	Raw field bean protein enzyme hydrolysate	−13·5±2·5
6	Raw field bean protein enzyme hydrolysate + amino acids	−13·2±0·6

but it appears to be associated with the protein component of the beans. Attempts are being made to isolate this factor.

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Studies in Fermented Foods: Part I—Nutritive Value of *Idli*

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A comparative study of the nutritive value of proteins of *idli* from the fermented and unfermented *idli* mix of rice and black gram *dhal* by the growth and nitrogen balance experiments on albino rats has been made. Experiments for evaluating the relative value of casein and the proteins of fermented and unfermented *idli* mix for the regeneration of haemoglobin and red blood cells have also been carried out. The results have shown that (i) the nutritive value and digestibility of protein of *idli* mix is not improved as a result of fermentation and (ii) *idli* from the fermented and unfermented batter is as efficient as casein in the regeneration of red blood cells and haemoglobin.

FERMENTED foods are being used by the people in their daily diets in many countries. They are generally considered by the people as highly nourishing foods. But very little information is available on the nutritive value of fermented foods[1-3]. Recently, a study on the nutritive value of *idli* prepared from a fermented batter of rice and black gram *dhal* fortified with Indian multipurpose food or Bengal gram flour by the growth method on albino rats has been reported[4]. It is reported that during the process of fermentation, vitamins and proteins are formed and supplements of these nutrients of microbial origin enhance the nutritive value of restricted vegetarian diets[5-7].

In view of this, it was considered of interest to study systematically the nutritive value of *idli*, a fermented food, consumed widely in South India.

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In the present communication are reported the results of a comparative study on the nutritive value of *idli* prepared from the fermented and unfermented batter of rice and black gram *dhal* by the growth and nitrogen balance experiments on albino rats, and by the regeneration of haemoglobin and red blood cells.

Experimental procedure

Preparation of idli — *Idli* was prepared according to the procedure described by the previous workers in this laboratory[8,9]. The blend of four parts of coarsely powdered black gram (*Phaseolus mungo*) *dhal* and one part of coarsely powdered rice were mixed with about two times the weight of hot water at 80°C. and made into a batter which was allowed to ferment at room temperature for about 20-24 hr. The fermented batter was then cooked in an ordinary cooker. The corresponding unfermented preparation was prepared by mixing the above blend with cold water, making into a batter and cooking the batter immediately without allowing it to ferment.

Experimental diets — The composition of experimental diets is given in Table 1. Diets 1 and 2 contained 10 per cent protein and diets 3 and 4 contained 18 per cent protein (on moisture-free basis) from *idli* prepared from the unfermented and fermented *idli* mix respectively. Besides *idli*, the diets contained starch, cane sugar, refined groundnut oil, vitamins A, D and E in oil, and Wesson's salt mixture[10]. Diets 1 and 2 were used for investigations on protein efficiency ratio and biological value, whereas diets 3 and 4 were used for studies on regeneration of red blood cells and haemoglobin. In addition to the diet, each rat received the following daily supplements of water-soluble vitamins: B_1, 25 μg.; B_2, 25 μg.; B_6, 20 μg.; calcium pantothenate, 100 μg.; niacin, 100 μg.; folic acid, 5 μg. and choline chloride, 5 mg.

Experimental animals — (a) Protein efficiency ratio: Twenty male albino rats, about 5 weeks old and weighing about 40-45 g., were evenly distributed into two groups and housed in the individual cages. They were fed *ad lib.* on experimental diets 1 and 2 and the growth was observed for a period of six weeks. A record of food intake was also maintained.

(b) Nitrogen metabolism: Six adult male rats, weighing about 170 g. and about 5 months old, were kept first on a protein-free diet (Table 1) for 7 days, followed by a period of 7 days for rehabilitation on casein diet (Table 1). Then they were fed on diets 1 and 2 by rotation. The duration of feeding each diet was one week. The collection of urine and faeces were made on the last four days, the first three days being the adjustment period. Urine was collected over phenol solution containing thymol. Faeces were collected and dried in an air-oven at 100°C., after moistening with oxalic acid. Nitrogen was determined by micro-Kjeldahl procedure.

(c) Regeneration of haemoglobin and red blood cells: Twenty-four adult male rats were evenly distributed into three groups. After determining the initial levels of haemoglobin and red blood cell count, they were rendered anaemic by administration of phenylhydrazine according to the technique described by Yeshoda and Damodaran[11] and fed on casein diet for three days. The animals in the two groups were given experimental diets 3 and 4 from the 4th day of injection, when the peak of anaemia was reached. Haemoglobin and red blood cell count were determined every 3-4 days till the animals recovered from anaemia, according to the methods adopted by Ambegaokar and Chandran[12].

Results

Growth experiments — The results of growth experiments given in Table 2 show that the average gain in body weight of animals in the two groups is almost equal. Also, there was no significant difference in the mean protein efficiency ratios of *idli* prepared from the fermented and unfermented *idli* mix. The results further show that the nutritive value of *idli* mix of rice and black gram determined by the growth experiment is not improved as a result of fermentation.

Nitrogen metabolism — The results of nitrogen metabolism experiment on two groups of adult rats fed on two different diets are given in Table 2. The table shows that the mean biological value of protein of the unfermented *idli* mix was higher than that of protein of the fermented *idli* mix. On the other hand, the digestibility of *idli* prepared from the fermented *idli* mix was comparatively higher. However, the differences in the mean biological values and digestibility coefficients of proteins of the fermented and unfermented *idli* mix were not statistically significant. The results obtained have shown that the biological value and digestibility of proteins of the blend of rice and black gram *dhal* are not affected by fermentation.

Regeneration of haemoglobin and red blood cells — The results of experiments on the regeneration of red blood cells and haemoglobin of blood of rats fed on the three different diets are given in Table 3. The average haemoglobin level and red blood cell count of animals in the three groups decreased by about 27 and 33 per cent respectively, when the peak of anaemia was reached on the 4th day

TABLE 1 — COMPOSITION OF DIETS

Diet	Starch	Sugar	Salt mixture[10]	Refined groundnut oil	Groundnut oil fortified with vitamins* A, D and E	Source of protein
Casein diet	57·0	10·0	4	10	1	18 g. casein
Protein-free diet	75·0	10·0	4	10	1	Nil
Diet 1	19·5	19·5	4	10	1	85 g. of fresh *idli* prepared from the unfermented *idli* mix corresponding to 46 g. of dry material containing 10 g. protein
Diet 2	20·5	20·5	4	10	1	79 g. of fresh *idli* prepared from the fermented *idli* mix corresponding to 44 g. of dry material containing 10 g. protein
Diet 3	1·0	1·0	4	10	1	153 g. of fresh *idli* prepared from the unfermented *idli* mix corresponding to 83 g. of dry material containing 18 g. protein
Diet 4	3·0	3·0	4	10	1	141 g. of fresh *idli* prepared from the fermented *idli* mix corresponding to 79 g. of dry material containing 18 g. protein

*2000 I.U. of vitamin A, 200 I.U. of vitamin D and 5 mg. of alpha-tocopherol acetate/g. of oil.

TABLE 2 — PROTEIN EFFICIENCY RATIO AND BIOLOGICAL VALUE

	Growth (av. in respect of 10 animals)				Nitrogen metabolism (exp. period 4 days; av. in respect of 6 animals)						
	Initial wt *g.*	Increase in wt (6 weeks) *g.*	Protein intake *g.*	P.E.R.	N intake *mg.*	Food faecal N *mg.*	Food urinary N *mg.*	N absorbed *mg.*	N retained *mg.*	Digestibility coeff.*	B.V.†
Diet 1	43·6	39·2	16·65	2·28 ±0·16	693·5	146·8	112·1	546·7	434·6	78·98 ±1·55	79·25 ±2·74
Diet 2	40·1	38·5	14·68	2·55 ±0·16	858·9	151·2	214·5	707·7	493·2	82·47 ±0·87	69·59 ±2·70

*t test not significant.
†t test significant at 5 per cent level but not significant at 1 per cent level.

TABLE 3 — RECOVERY OF RATS FROM EXPERIMENTALLY PRODUCED ANAEMIA

(*Red blood cells in millions/cu. mm.; haemoglobin in g./100 ml. blood*)

Diets	No. of animals		Initial levels	Interval after administration of phenylhydrazine (*days*)			
				4 (peak of anaemia)	11	14	18
Casein	8	RBC	8·65	5·33	6·93	7·56	8·49
		Haemoglobin	15·66	10·71	14·93	16·75	17·31
Diet 3	9	RBC	8·01	5·88	7·18	7·57	8·83
		Haemoglobin	15·09	11·93	16·94	16·84	16·56
Diet 4	8	RBC	8·73	5·80	7·44	8·12	8·62
		Haemoglobin	15·78	11·41	15·75	16·44	16·78
				Av. red blood cell counts based on 100% level at the peak of anaemia			
Casein				100	131·6	144·9 ±5·9	162·6
Diet 3				100	122·8	130·6 ±9·1	148·0
Diet 4				100	132·6	146·0 ±11·9	156·7

of administration of phenylhydrazine. Yeshoda and Damodran[11], whose technique was followed for the production of anaemia in the present investigation, had obtained about 50 per cent reduction in haemoglobin and red blood cell content. Repeated doses of phenylhydrazine on 2-3 consecutive days[13] might produce a severe anaemia in the strain of rats used in the present experiment.

Table 3 indicates that the rate of formation of red blood cells in rats fed on fermented *idli* mix was apparently higher than that in animals fed on other two diets. The average red blood cell count in rats fed on fermented *idli* mix attained the initial level on the 14th day, whereas the counts in rats in other two groups were below the initial level during the same time interval. In the latter two groups, viz. those on unfermented *idli* mix and casein, red blood cell count touched the initial level on the 18th day.

But, when the results are analysed statistically on the basis of standardized level at the peak of anaemia on the 4th day, the differences in the rate of regeneration of red blood cells in the three groups are not significant (Table 3).

As far as the regeneration of haemoglobin was concerned, there was no significant difference in the mean haemoglobin content of blood of rats fed on the fermented and unfermented *idli* mix, and casein.

The results have shown that the nutritive value of protein of *idli* mix of rice and black gram *dhal* for the regeneration of red blood cells and haemoglobin is not increased by the fermentation and that *idli* from the fermented and unfermented batter is as efficient as casein in the regeneration of red blood cells and haemoglobin.

Discussion

The results obtained in the present investigation have shown that processing of *idli* mix of rice and black gram *dhal* by fermentation for the preparation of *idli* is not of any special advantage as far as the nutritive value of protein is concerned. The digestibility of the protein of *idli* is also not affected by fermentation. It is shown[1-3] that the nutritive value of protein of milk is not improved by making curds from it by fermentation. But the significance of fermentation of foodstuffs in nutrition is not restricted only to the nutritive value of protein, because during the process of fermentation other essential nutrients or vitamins are produced[14-16] and feeding of such fermented food (curds) to children caused increase in their body weight, faecal and urinary thiamine excretions and blood thiamine levels[17]. Recent work[16] carried out in this laboratory indicated that feeding of *idli* prepared from the fermented batter brought about a significant reduction in the fat content of livers of rats fed on high fat low protein diet.

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Pharmacognostic Study of *Dodonaea viscosa* Linn.

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The macroscopic and microscopic characters of the different parts of the plant *D. viscosa* are described. The following are the distinguishing characters of the different parts. The leaves are isobilateral with palisade cells under both the surfaces. Epidermal cells show prominent cuticular striations. Both the surfaces of the leaves are covered by multicellular peltate scales. Sclereids protect vein terminations. The stem shows rhytidoma which consists of 2-3 alternate layers of cork, crushed phelloderm and sclerenchyma of phloem origin. Vessels and tracheids bear bordered pits, and wide zones of fibre occur in the wood. In the root, the growth of the xylem is eccentric, and pericyclic fibres and sclereids are present. Calcium oxalate rosettes, cubes and diamond-shaped crystals are present in leaf, petiole and stem. Tannin, mucilage and resin occur in the leaf. The roots possess high haemolytic activity (haemolytic index 4000), whereas the leaf has no activity.

DODONAEA VISCOSA Linn. (*Sapindaceae*), a shrub or a small tree growing in warm countries, is reported to grow throughout India, Ceylon and Malacca[1]. Leaves of *D. viscosa* are used as alterative, laxative and in rheumatism[1]. They are used as febrifuge in Madagascar and chewed as a stimulant in Peru. Their infusion is considered as sudorific in La Reunion. Decoction of the wood is used for astringent bath in Madagascar, while the entire plant is employed in South Africa for stomach disorders[2]. Powdered leaves are applied over wounds, burns and scalds. The juice of the leaves is used as a wash on swellings and also used as a poultice[2].

The leaves contain gums, albumen and tannin besides two acrid resins[1]; an alkaloid saponin has also been reported from the leaves. Physiological investigations (Sukkawala, V. M., unpublished data) have revealed that the leaves possess cardioinhibitory, spasmolytic, antihistaminic, antibarium and antiacetylcholine effects; they also possess anthelmintic and antibacterial property.

As no previous work has been recorded on the pharmacognosy of *D. viscosa*, the present investigation was undertaken.

Materials and methods

Microtome and hand sections of the leaf, petiole, stem and root from different levels were made permanent after staining with safranin and light green. Stem and root pieces were macerated as per Schultze's maceration process.

Description of the plant (Plate I, Fig. 1)

D. viscosa has twiggy branches, mostly angled and softly pubescent. Leaves are simple, alternate and tristichous. They contain a yellow resinous exudation. Small greenish yellow, unisexual flowers occur in short few-flowered cymes. Each of the 8 stamens bears an oblong-linear anther as long as the sepal. Ovary is 2-3 angled, hairy externally and possesses a long style. Membranous capsule, notched at the base and the apex has 2-3 wide marginal wings.

Macroscopic characters

Leaf (Plate I, Fig. 1) — The leaf is simple, entire, subsessile, oblanceolate with a subacute or shortly apiculate apex and a much tapering base. Its viscid, glabrous, shining surface possesses dark green colour. It measures 4-10 $\times$ 0·5-1·5 cm. and has reticulate venation. Marginal veins anastomose. Taste is mucilaginous and slightly bitter.

Stem — The stem and branches are woody. Each is many-angled or cylindrical. Nodes are prominent and internodes are 2-3 cm. long. Uppermost part of a young branch is greenish and prominently angled. This part develops thin, brown bark as it grows old. An old branch shows longitudinal striations, some of which are so prominent that

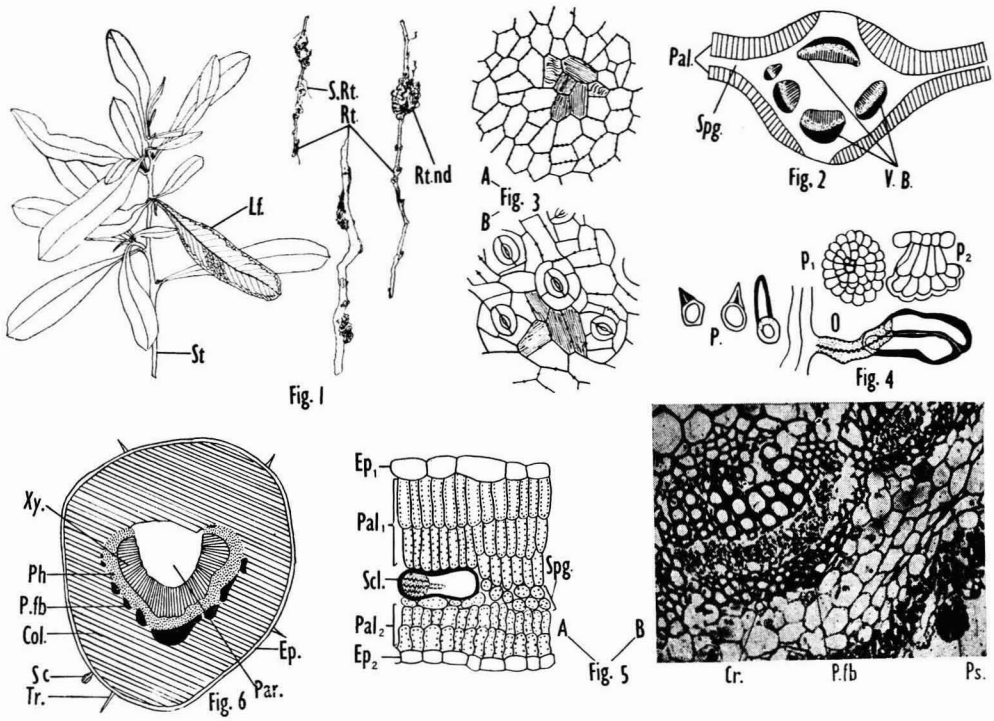

Plate I — Macroscopic characters of the twig and root and microscopic characters of the leaf of *D. viscosa* [Fig. 1: Twig × 0·33. Fig. 2: T.s. of leaf passing through midrib × 23. Fig. 3: A, upper epidermis with prominent cuticular striations × 83; and B, lower epidermis showing stomata and cuticular striations × 146. Fig. 4: P, trichome × 146; P_1, peltate scale in surface view × 146; P_2, l.s. of peltate scale × 260; and O, vein ending surrounded by sclereids × 146. Fig. 5: A, t.s. of lamina × 146; and B, midrib showing vascular bundle × 80. Fig. 6: T.s. of petiole × 83; O, vein termination protected by sclereid; P, P_1 and P_2, multicellular peltate scales. *Cr*, crystal; *Ep*, epidermis; Ep_1 and Ep_2, upper and lower epidermis; *Lf*, leaf; *Ph*, phloem, *Rt*, root; *Ps*, peltate scale; *St*, stem; *Xy*, xylem; *V.B*, vascular bundle; *Col*, collenchyma; *Pal*, palisade; Pal_1, palisade below upper epidermis; Pal_2, palisade below lower epidermis; *Par*, parenchyma; *P.f.b*, pericyclic fibre; *Spg*, spongy tissue; *Rt.nd*, root nodule]

the branch appears a little angled. Besides, it bears numerous, small, amorphous yellowish masses which can be removed easily. The brownish outer part of a branch usually splits longitudinally into lenticular areas exposing the inner cortex. Wood is dark brown, hard and heavy.

Root (Plate I, Fig. 1) — Roots are long spreading and dark brown in colour. Young root shows minute tubercle-like swellings which commonly develop into big knot-like structures at a later stage. Occasionally, rootlets may emerge from the tubercles in addition to the non-tubercular parts. They give a clay-like consistency to the root powder.

Microscopic characters

Leaf (Plate I) — Transverse section of the leaf shows isobilateral lamina, as palisade is present under both the upper and the lower epidermis (Figs. 2 and 5A). The enlarged cells of the upper epidermis are tubular in shape; they possess straight anticlinal walls (Fig. 5). Thick layer of cuticle protects both the dermal surfaces. In surface view, the polygonal cells of both the epidermises show beads on their walls and exhibit straight, wavy or interruptedly wavy cuticular striations (Figs. 3A and 3B). Cells of the upper epidermis are, however, relatively straight walled and show more beads; cuticular striations are also more prominent here. Cells of the lower epidermis and the cells around the veins on the upper epidermis are interrupted with numerous small rubiaceous stomata. Unicellular trichomes as well as multicellular peltate scales are present on both the surfaces; the former mostly appear on the veins or on the margin while the latter are scattered throughout the lamina (Fig. 4, P, P_1 and P_2). Trichomes around the veins are longer, measure 70·5-188 μ in length, and are abundant on the upper surface; those of the margin are shorter

and measure 66-90 μ in length. Peltate scales measure 88-120 μ in diameter and are found scattered on both the surfaces. A vein termination is well protected by a group of sclereids (Fig. 4).

Two to three layers of elongated cells of the palisade are present below the upper epidermis. The two layers of palisade cells below the lower epidermis are relatively shorter and possess more wavy anticlinal walls. Two to three layers of rounded or oval cells in the centre and in between the upper and lower palisade layers constitute the spongy tissue (Fig. 5).

Veins and veinlets are cut transversely or commonly obliquely in the laminar region. They are surrounded by a bundle sheath which can be observed only after clearing the sections with chloral hydrate as the cells of the mesophyll are studded with chloroplasts. Midrib slightly projects above but more so on the abaxial side (Fig. 2). In the transverse section, palisade is discontinuous over and under the vascular ring. It reaches halfway on both sides in the midrib (Fig. 2). The vascular ring is dissected into 4-6 conjoint, collateral and open vascular bundles; 3-5 of these form an arch on the abaxial side while the remaining one occupies the adaxial side. A group of pericyclic fibres form a protective arch towards the outer side of each vascular bundle (Figs. 2 and 5B). Xylem of the bundle faces the inner side and consists of radiating rows of vessels and xylem parenchyma in between. Phloem faces the outer side and consists of sieve tubes, companion cells and phloem parenchyma. Cortex and pith of midrib are parenchymatous. However, cells in 2-3 layers below the upper and the lower epidermises in the midrib may turn collenchymatous. Leaf constants are recorded in Table 1.

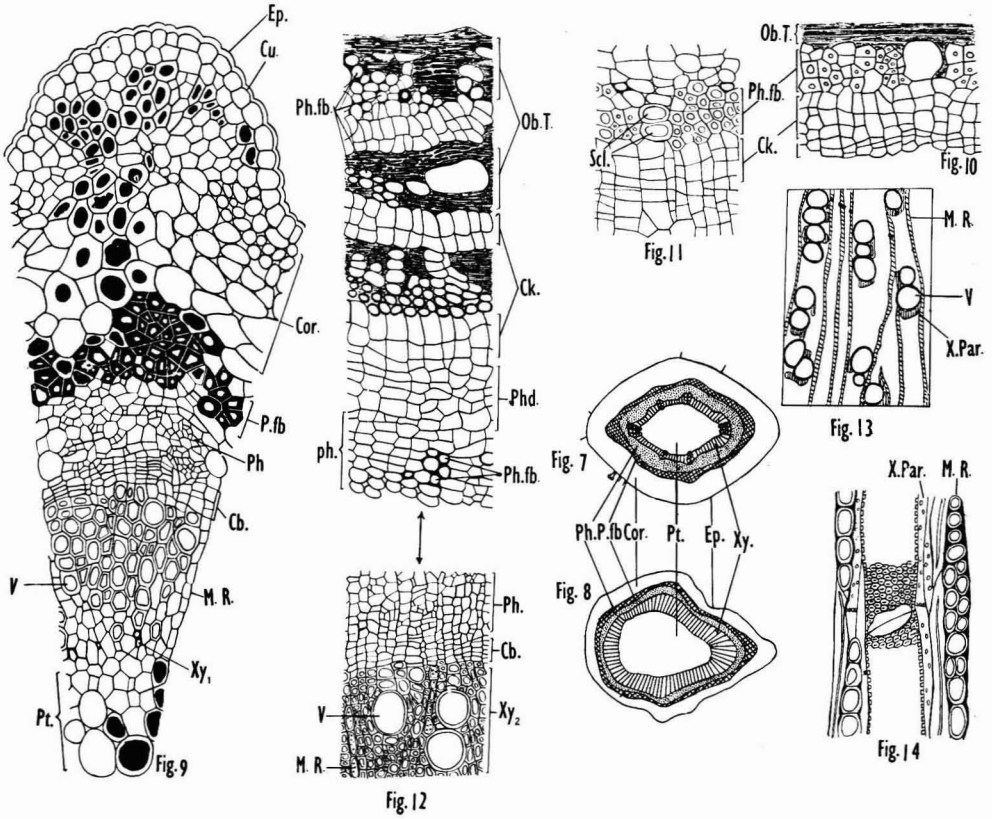

Plate II — Microscopic characters of the stem of *D. viscosa* [Figs. 7 and 8: T.s. of young stem × 23. Fig. 9: T.s. of young stem showing the cell contents × 260. Fig. 10: T.s. of stem showing phellogen development in the phloem region × 146. Fig. 11: T.s. of old stem showing development of sclereids among phloem fibres × 146. Fig. 12: T.s. of outer part of an old stem showing three layers of rhytidoma × 146. Fig. 13: T.s. of old stem showing the central woody region × 83. Fig. 14: T.l.s. of old stem showing the wood × 146. *V*, vessel; *Cb*, cambium; *Ck*, cork; *Cu*, cuticle; *Ep*, epidermis; *M.R*, medullary ray; *Ph*, phloem; *Pt*, pith; *Xy*, xylem; Xy_1, primary xylem; Xy_2, secondary xylem; *Cor*, cortex; *Ob.T*, obliterated tissue; *P.fb*, pericyclic fibre; *Phd*, phelloderm; *Scl*, sclereid; *Ph.fb*, phloem fibre; *X.Par*, xylem parenchyma]

TABLE 1 — LEAF CONSTANTS

Leaf surface	Location*			Range of variation	Mean val.
	Base	Middle	Apex		
		STOMATAL INDEX			
Upper	0	0·95	0	0-1·29	0·32
Lower	6·7	7·2	5·6	5·3-8·2	6·5
		PALISADE RATIO			
Upper	8·8	8·75	7·2	5-12·5	8·25
Lower	3·7	4·5	4·65	2·25-7	4·28
		VEIN ISLET NUMBER			
	14	9·8	12·5	8-18	15·4
		PELTATE SCALES/SQ. MM.			
	9	6·4	14·6	3-19	10

*Values are mean of 12 readings for each of the leaf parts.

Petiole (Plate I, Fig. 6) — Transverse section of petiole is subspherical in shape. Epidermis bears peltate scales as well as trichomes, as in the leaf. Cortex is collenchymatous. Xylem of the stele takes the form of a strongly developed arch. Xylem and phloem of the stele as usual face adaxial and abaxial sides respectively. Groups of lignified fibres arrange into a discontinuous arch on the abaxial side of the stele. The channel on the adaxial side, formed by the ends of the xylem, is parenchymatous.

Stem (Plate II) — Transverse section of young stem appears irregularly angled (Figs. 7 and 8). Secondary growth starts early, as a young stem of 968 × 660 μ or 935 × 715 μ shows secondary growth. Initially, six groups of primary xylem are visible. The young stem is protected from outside by a thick cuticle. Its epidermis bears trichomes and multicellular peltate scales. Cortex is parenchymatous; occasionally, two hypodermal layers of small spherical cells may develop cellulose thickening at the corners. Inner cortex is composed of large parenchyma cells with distinct spaces (Fig. 9). Pericyclic fibres form a complete ring around the vascular tissues. Secondary phloem zone is as large as xylem. Cambium is 2-6 layered. Pith is parenchymatous.

Trichomes and peltate scales are also present in the portion of the stem very close to the apex. The primary xylem groups become obscure (Fig. 8).

Two layers of phellogen originate from the inner side of the pericyclic fibres (Figs. 10 and 11). The first phellogen remains active for a short time, during which it gives out 3-5 layers of cork cells towards its outer side and 2-3 layers of phelloderm towards the inner side. Cork cells are thin-walled, lignified and suberised and almost isodiametric. Tissues external to the cork usually get crumpled to form an obliterated mass. This mass is rhytidoma, as it consists of the cork, phloem, phloem fibres and phelloderm (Fig. 12). While the activity of the first phellogen is going on, isolated cells of phloem starts depositing lignin (Fig. 12). Thus, groups of phloem cells modify into fibres which later on form a ring. The cells of the phloem rays here occasionally transform to form sclereids (Fig. 11). When the activity of the first phellogen ceases, a new one starts its origin within the ring of phloem fibres and sclereids. Thus, in an old stem rhytidoma consists of not more than three alternate layers of cork, and obliterated mass (Fig. 12).

Phloem is a wide zone which is composed of all the usual elements. Cambium is distinct and 2-5 layered (Figs. 9 and 12). Xylem forms the wood of the stem. It consists of vessels, tracheids, xylem parenchyma, secondary medullary rays and fibres. Vessels mostly occur in radial groups of 2-5 (Plate II, Figs. 9 and 13). Often a large vessel may show tylosis. Their lignified walls may show hexagonal bordered pits with slit-like openings or more commonly scattered bordered pits (Fig. 14). A few tracheids present are mostly fibro-tracheids. Fibres are numerous and appear in masses. They are mostly septate and hardly show a few pits. Some of the fibres also show branched ends. Xylem parenchyma are mostly of vesicentric type and possess many big oval pits. Secondary medullary rays are generally uniseriate, though biseriate ones are not uncommon (Fig. 14). A ray is 2-44 cells long or occasionally even more so. Cells of the ray are

TABLE 2 — LENGTH AND BREADTH OF STEM ELEMENTS

Elements	Length μ	Breadth μ
Vessel	183-230-**303**-5-381-426	34-**54**-5-80
Tracheid	188-250-**324**-357-428	14-**21**-5-28
Sclereid	42-68·6-**80**-141-164·5	20-**31**-42
Fibre	320-496-**592**-742·5-880	9·5-**14**-21
Pitted parenchyma	34-**59**-76	16·5-**25**-34
Cork	17·8-26·8	17-28

TABLE 3 — LENGTH AND BREADTH OF ROOT ELEMENTS

Elements	Length μ	Breadth μ
Vessel	181-229-**285**-343-398	35-**64**-89
Tracheid	195·5-274-**329**-376-470	15·5-**23**·5-33
Fibre	464-554-**683**-810-944	11-**20**·5-33
Sclereid	70-**136**-169	28-**35**-42
Pitted parenchyma	47-**94**-128	12-**22**-28

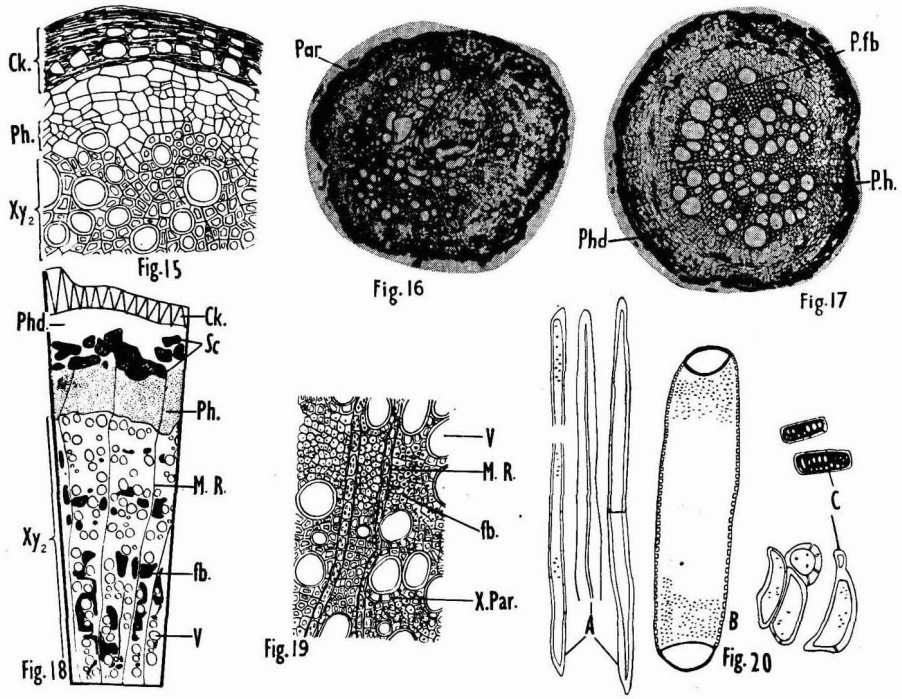

Plate III — Microscopic characters of the root of *D. viscosa* [Fig. 15: T.s. of young root × 260. Fig. 16: T.s. of root from root nodule region showing parenchyma invading xylem portion × 33. Fig. 17: T.s. of root showing eccentric growth × 57. Fig. 18: T.s. of old root × 83. Fig. 19: A portion of vascular tissue of root magnified × 146. Fig. 20: Maceration of root; A, tracheid and septate and unseptate fibre; B, a vessel element; and C, sclereids and pitted parenchyma (all × 146). *V*, vessel; *Ck*, cork; *Fb*, fibre; *M.R*, medullary ray; *Ph*, phloem; *Sc*, sclereid; Xy_2, secondary xylem; *Par*, parenchyma; *P.fb*, pericyclic fibre; *Phd*, phelloderm; *X.Par*, xylem parenchyma]

radially elongated, and show spherical pits on their wall. In an old stem, these cells become much lignified to form sclereids. The length and breadth of stem elements are recorded in Table 2.

Root (Plate III) — A young root (diam. 167 μ) exhibits secondary growth (Fig. 15). Phellogen throws off the primary cortex and develops 5-6 layers of thin-walled radially elongated cork cells on its outer side and 2-3 layers of phelloderm on the inner one. Transverse section of a root at a nodule shows development and multiplication of parenchyma towards the infected side; this parenchyma may invade xylem. Small groups of pericyclic fibres are also present (Fig. 16).

Secondary growth in an old root may be eccentric (Figs. 16 and 17). In an old root (diam. 825 μ), only 3-4 layers of cork cells are observed (Figs. 16-18). Cells of the cork are thin-walled, lignified and are arranged radially; usually, they are filled with blackish to brownish cell contents. Phelloderm is 2-3 layered. Groups of fibres associated at times with a few sclereids, delimit the phloem from the phelloderm region (Plate III, Fig. 18). At a later stage a few cells of the phelloderm also get modified into sclereids. The fibre groups alone or in association with sclereids are surrounded by small parenchyma cells each containing a crystal of calcium oxalate. Cambium is distinct and is 2-4 cell layered. Phloem consists of the usual elements. Xylem forms the central solid core which is composed of vessels, tracheids, fibres, xylem parenchyma and secondary medullary rays. Vessels mostly occur in irregular groups of 2-4 (Figs. 16-19). They show slit-like bordered pits on their wall. Majority as in the stem show pointed ends. Big vessels may show tylosis. Tracheids are comparatively few and exhibit a few bordered pits. Fibres are numerous, mostly septate and show a few pits. As in stem, fibres form wide zones in root too (Figs. 16-19). Secondary medullary rays are mostly uniseriate. The dimensions of root elements are recorded in Table 3.

Microchemical tests

Calcium oxalate crystals — Parenchyma and phloem of the midrib cortex and midrib vascular bundles respectively, parenchyma around the laminar vascular bundles, cortex of petiole and phloem of stem contain rosettes, cubes and diamond-shaped crystals of calcium oxalate. Rosettes of calcium oxalate in the midrib cortex and around the laminar vascular bundles are bigger in diameter (10·4-15·6-18 μ) than those present in the phloem of midrib vascular bundles, cortex of petiole and phloem of stem (5·2-7·8-10 μ).

Tannin — The cell contents of cortex, phloem parenchyma and phloem ray, and peripheral cells of pith in the case of stem (Plate II, Fig. 9) and a few outermost cells of petiole as well as those around the pericyclic fibres when treated with ferric chloride turn blue-black, indicating the presence of tannin.

Mucilage — The leaf was tested for the presence of mucilage according to Youngken[3]. The cells of layers of epidermis and of the bundle sheath were found to contain mucilage.

Resin — The leaves were tested for the presence of resin according to Youngken[3]. The innermost palisade layers of both the sides were found to contain resin which was indicated by the emerald green colour taken up by the cell contents.

Evaluation of saponin content by froth number and haemolytic index — Froth numbers of the leaf and root of *D. viscosa*, determined at *p*H 7·8 according to Kofler's method[4] were 333 and 666 respectively (froth number of both *Polygala senega* root and quillaia bark[5] is 3000). Haemolytic indices[4] of the leaf and root of *D. viscosa*, determined using ox blood with isotonic buffer solution at *p*H 7·38, were 0 and 4000 respectively (haemolytic indices for *P. senega* root and quillaia bark are 2500-6000 and 4000-6000 respectively[6]).

Acknowledgement

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Short Communications

The Isolation of a Toxic Principle from *Lathyrus sativus* Seeds

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The insect larva *Corcyra cephalonica* St. has been found to respond to the toxic principles present in *L. sativus* seeds. A new ninhydrin-positive compound has been isolated from the seeds of *L. sativus* and some of the properties of the compound are reported. This compound is highly toxic to several microorganisms in minute amounts.

THE consumption of *L. sativus* seeds as the principal dietary constituent causes a neuropathological disease called 'lathyrism' among the rural population in certain parts of Central India[1]. Difficulty has been experienced in producing pathological symptoms characteristic of lathyrism in laboratory animals[2-4]. Uncertainty exists regarding the exact nature of the causative factors for the disease, though high contents of Se[5] and Mn[6] as well as low contents of methionine[7] and tryptophan[8] in the seeds have been suggested as possible etiological factors. The absence of alkaloids[9] and poisonous aminonitriles[10,11] in the seeds has also been reported. It is, therefore, of interest to examine other chemical constituents of the seeds for their biological activity. As a first step, the effect of *L. sativus* seeds on the insect larva *Corcyra cephalonica* St., an organism with nutritional requirements resembling those of higher organisms[12], has been investigated. In the present communication, the response of *Corcyra* to feeding of *L. sativus* seeds, and the isolation of a new ninhydrin-positive compound toxic to several microorganisms are reported.

Incorporation of *L. sativus* seed meal at 30 per cent level in the basal wheat flour diet[13] led to a marked growth inhibition to the extent of 60 per cent. A gross fractionation of the seed meal with different organic solvents was carried out to isolate the inhibitory factors. Ether and chloroform extracts failed to affect the growth of the organism while the seed residues still retained inhibitory properties. However, aqueous ethanol (75 per cent) extract proved deleterious to larval development, indicating the extractability of the toxic principles by aqueous ethanol. The seed meal residue allowed normal growth of the insect at comparable dietary levels.

In view of the reported presence of a pharmacologically active amine in *L. sativus* seeds[14], the alcohol extract was examined for the possible presence of unusual nitrogenous constituents such as rare amino acids. Paper chromatography on Whatman No. 1 filter paper using *n*-butanol-pyridine-water-acetic acid (4: 1: 1: 2; vol./vol.) revealed two prominent ninhydrin-positive components (R_f values 0·09 and 0·24) not corresponding to the usual amino acids found in proteins. The bulk isolation of these two constituents was, therefore, attempted and the isolation of one of them is described below. The isolation and characterization of the other compound will be reported elsewhere.

The seed meal (400 g.) was refluxed with 600 ml. of aqueous ethanol (75 per cent) for 90 min. and the extraction repeated thrice with fresh portions of the solvent. The pooled extract was filtered, concentrated *in vacuo* (40-45°C.) to one-sixth the volume and shaken with an equal volume of chloroform to remove lipids and pigments. The aqueous layer (*p*H 5-6) was passed through a column of Dowex-50×8 (200-400 mesh; resin volume 117 ml.) in the H^+ form. The break-through fluid containing starch and sugars was rejected and the column washed with 1 litre of distilled water. The effluent contained only the ninhydrin-positive component (R_f value 0·09) and the washing continued to secure its complete elution. The eluate was concentrated by freeze-drying to one-fourth the volume in a 'Virtis' freeze mobile and the compound precipitated by addition of acetone and purified by repeated acetone precipitation from aqueous solutions; yield 0·8 g.

The acidic character of the isolated product was suspected by its behaviour on the Dowex-50 column and an aqueous solution (2 mg./ml.) had a *p*H of 2·5. The compound reduced the Folin-Ciacalteu reagent[15] and could be quantitatively estimated by the method of Lowry *et al.*[16], the colour intensity per mg. being equivalent to that due to 0·04 μmole of tyrosine. When treated with ninhydrin according to Rosen[17] the colour yield obtained per mg. was equivalent to that due to 5·35 μmoles of leucine.

TABLE 1 — R_f VALUES OF ACIDIC COMPOUND

Solvent system	Chromatographic technique	R_f value
n-Butanol-acetic acid-water (4: 1: 1)	Circular	0·15
Pyridine-water (80: 20)	do	0·45
Phenol-water (80: 20)	Ascending	0·14
Aqueous ethanol (75%)	Circular	0·50

The homogeneity of the isolated compound was established by paper chromatography in 4 different solvent systems and the R_f values obtained are given in Table 1. Further, when adsorbed on a column of Dowex-1×8 (200-400 mesh) in CH_2ClCOO^- form and eluted with 0·1*M* $CH_2ClCOONa$, the elution pattern also showed a single symmetrical peak in conformity with the paper chromatographic data.

On treatment with 6*N* HCl or a saturated solution of $Ba(OH)_2$ at 105°C. for 24 hr, the compound yielded another ninhydrin-positive substance (R_f value 0·28) on circular paper chromatograms with *n*-butanol-acetic acid-water (4: 1: 1) as the solvent. The latter, unlike the parent substance, could be adsorbed on Dowex-50 and eluted with 2*N* HCl. When treated with periodate reagent[18], ammonia could be detected on spraying with Nessler's reagent. It also fluoresced under ultraviolet light on treatment with acetylacetone[19] unlike the original compound. These reactions indicate the presence of an amino and a hydroxyl group in juxtapositions in this product. On continuing the acid or alkali treatment for 72 hr two additional ninhydrin-positive bands (R_f values 0·38 and 0·46) were obtained on paper chromatograms with the above solvent system.

The isolated compound inhibited the growth of *Neurospora crassa* (wild, Em 5297a) by 50 per cent at a concentration of 4 μg./ml. and completely at 8 μg./ml. of the basal medium when the mold was grown on the minimal medium[20] for 72 hr at 30°±1°C. with shaking. Preliminary data indicated that casein hydrolysate at 250 μg./ml. level counteracted the toxicity to a marked extent. The growth of *Staphylococcus aureus* N-15, *Escherichia coli* N-52 and *Candida albicans* Z-247 was also inhibited (about 50 per cent) by the compound at 7 μg./ml. and completely at 15 μg./ml. of the culture medium[21] when the organisms were grown for 24 hr.

The data obtained rule out the possibility of this compound being identical with those recently isolated and characterized as lathyrin and homoarginine from the seeds of several *Lathyrus* species[11,22] (also Rao, S. L. N., Ramachandran, L. K. & Adiga, P. R., unpublished data). Further work is in progress

to characterize this compound and to study its effects on other organisms.

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The Infrared & Ultraviolet Absorption Spectra of Phenylthiohydantoin of L-Cystine

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The phenylthiohydantoin of L-cystine has been prepared by coupling phenylisothiocyanate with L-cystine, and the infrared and the ultraviolet absorption spectra of the compound have been measured. The physicochemical data obtained reveal that this compound can be synthesized and identified by paper chromatography.

THE phenylthiohydantoin procedure for the stepwise degradation of simple peptides was first described by Edman[1]. This method involves the characterization of the amino acid residues through the 3-phenyl-2-thiohydantoins. Most of the phenylthiohydantoins of amino acids, excepting for a few amino acids like serine, threonine and cystine, were synthesized by Edman[1]. Those of hydroxyamino acids, serine and threonine, have recently been prepared by Ingram[2] and Levy[3], and were found to undergo decomposition readily. However, there seems to exist some doubt regarding the formation of phenylthiohydantoin derivatives of cysteine and cystine. Levy[3,4] has reported the formation of phenylthiohydantoin cystine and observed that on hydrolysis with 20 per cent HCl at 150°C. the phenylthiohydantoins of cystine and cysteine yielded free cystine and alanine. Sjöquist[5] suggested recently that cystine or cysteine should be oxidized to cysteic acid before coupling with phenylisothiocyanate since neither of them forms phenylthiohydantoin derivatives. We have re-examined the claims of both Levy[3] and Sjöquist[5] and have been able to obtain the phenylthiohydantoins of cystine and cysteic acid. That of L-cystine was prepared according to the method described by Levy[3]. It was a lemon-yellow crystalline compound and melted with decomposition around 120°C. (Found: C, 50·05; H, 3·67; N, 11·58. $C_{20}H_{18}O_2N_4S_4$ requires C, 50·60; H, 3·82; N, 11·81%.)

Ramachandran *et al.*[6] have reported the application of infrared absorption spectra of the phenylthiohydantoins of amino acids (excluding those of cystine and cysteic acid) for the identification of N-terminal groups in peptides.

The characteristic infrared absorption spectrum of L-cystine phenylthiohydantoin recorded with a Perkin-Elmer infracord is presented in Fig. 1. The pure crystalline sample (10 mg.) was dissolved in 1 ml. of redistilled chloroform and was taken in a cell of 1 mm. thickness. The spectrum exhibited characteristic bands assigned to

$$\underset{\|}{\overset{O}{}}\!\!-C-,\quad C_6H_5-,\quad \underset{\|}{\overset{S}{}}\!\!-C-\quad \text{and}\quad -\underset{|}{N}H$$

vibrations at 1770-1740 cm.$^{-1}$, 1600 cm.$^{-1}$, 1425-1400 cm.$^{-1}$ and 3300-3150 cm.$^{-1}$ respectively of the phenylthiohydantoin of L-cystine. The ultraviolet absorption spectrum measured with a Beckman DU spectrophotometer in the region of 250-330 mμ is shown in Fig. 2. The sample exhibited a high absorption peak at 272 mμ [ϵ_{max} 18,000 (in ethanol)] and another small peak at 315-320 mμ; the ϵ_{max} value in diisopropylether reported by Levy and Chung[3] was higher. However, the absorption band at 320 mμ was found to increase slowly with time. Levy[7] noted that L-cystine phenylthiohydantoin was unstable even during storage in the crystalline form and its absorption maximum gradually shifted to 315-320 mμ to

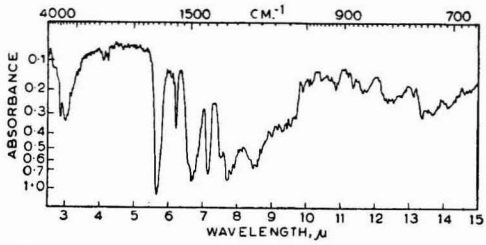

Fig. 1 — The infrared absorption spectrum of phenylthiohydantoin of L-cystine

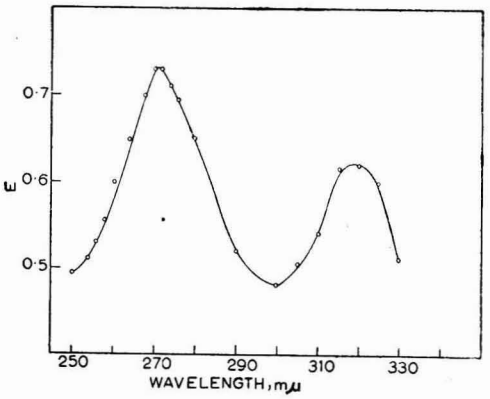

Fig. 2 — The ultraviolet absorption spectrum of phenylthiohydantoin of L-cystine

form an insoluble polymer. Our sample had an unchanged infrared absorption spectrum with the characteristic bands pertaining to the phenylthiohydantoin ring, when stored in cold in a perfectly dry condition. Further, the phenyl thiohydantoin of L-cystine was separated by the paper chromatographic procedure given by Sjöquist[8] using heptane-*n*-butanol-formic acid (2: 2: 1) as the solvent system. The compound ($R_f = 0{\cdot}9$) was identified on paper chromatograms by spraying the iodine-azide reagent (1: 1) and by directly observing under an ultraviolet lamp ('Mineralight' short wave UV model SL 2537, UV Products Inc., San Gabriel, Calif.). Hydrolysis of phenylthiohydantoin of L-cystine was also carried out with 5·7*N* HCl in an evacuated sealed tube at 150°C. for 20 hr and the products were identified by paper chromatography after evaporation of HCl using the upper phase of *n*-butanol-acetic acid-water (4: 1: 5) as the solvent system. Alanine, cystine and traces of two other unidentified ninhydrin-positive spots were noted on the chromatograms. This is in accord with the observations made by Levy[4].

The present study reveals that phenylthiohydantoin of L-cystine can be obtained and identified as reported by Levy[3]. However, the phenylthiohydantoin method can be applied without any difficulty for the identification of L-cystine only after oxidation. This is illustrated in the case of oxytocin which has cystine in its N-terminal position[9]. No phenylthiohydantoin amino acid could be extracted in the first step when the stepwise degradation technique was applied to oxytocin, since the N-terminal cystine remains attached to the peptide through its disulphide linkage[10]. As suggested by Edman[11], the difficulties that arise when the phenylthiohydantoin method is applied for the stepwise degradation of proteins can be obviated only when the disulphide bonds are first oxidized by performic acid.

The authors thank Prof. D. K. Banerjee and Dr B. H. Iyer of the Organic Chemistry Department of this Institute for the microanalysis of the sample, and for providing facilities for taking the infrared absorption spectrum. The financial assistance from the Rockefeller Foundation is gratefully acknowledged.

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Heat Resistance & Destruction Rates of *Saccharomyces cerevisiae* in Mandarin Orange (*Citrus reticulata* Blanco) Juice

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The heat resistance and destruction rates of *S. cerevisiae* in Mandarin orange juice (*p*H 3·6) and *M*/20 phosphate buffer (*p*H 7·0) have been determined at 45°, 50° and 55°C., and the heat destruction curves have been plotted. The rate of destruction of yeast cells has been found to be rapid during the initial stages of heating and the rate slows down considerably on subsequent heating. *S. cerevisiae* shows greater resistance to destruction by heat in orange juice than in phosphate buffer.

A PORTION of the Mandarin orange (*Citrus reticulata* Blanco) crop produced in the country is utilized in the preparation of orange juice. Unless

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properly preserved, the juice is susceptible to spoilage as a result of contamination with molds and yeasts. On account of the low *p*H of the juice, bacterial contamination in it is rare[1]. The predominating types of spoilage organisms associated with spoilage in orange juice include yeasts which cause gaseous fermentation during storage.

Unlike spore-forming bacteria, yeasts and molds are heat labile and get killed in citrus juice within a few minutes at 70-75°C. Pederson and Beavens[2] have shown that fruit juices contaminated with fermenting yeast can be successfully preserved by heating the juice for several hours at 57°C., for 30 min. at 65°C., or for 1 min. at 75°C. Similar studies on the heat resistance of bakers' yeast by White[3] in sodium phosphate-citric acid buffers of varying *p*H revealed that variations in *p*H had little effect on the destruction of yeast cells. Further, according to White, heating for 4 min. at 60°C. was sufficient to destroy all the yeast cells in a buffer of *p*H 7·0. These studies were carried out in order to find out the end point of destruction rather than the rates of destruction of yeast cells. The latter aspect of the problem has now been investigated using *Saccharomyces* sp. as the test strain and Mandarin orange juice and phosphate buffer as substrates.

Mandarin orange juice used in the present investigations was extracted with a mixmaster from locally purchased fresh and sound oranges from Nagpur. The juice was quickly strained through a 35 mesh stainless sieve under aseptic conditions and stored at −18° to −20°C. in sterile 4 oz. medicinal bottles. The juice on analysis gave: total soluble solids, 10 per cent; total sugars (as glucose), 8·4 per cent; reducing sugars, 3·9 per cent; acidity (as citric acid), 0·78 per cent; and vitamin C, 32·2 mg./100 ml.; the *p*H of the juice was 3·6.

Yeast strain — Two strains of yeasts were isolated from the orange juice. One of these was identified as *S. cerevisiae*[4] and the second was a pink yeast which was not identified. As the former possessed greater heat resistance, it was used as the test organism in the present studies.

The suspension of yeast cells was prepared from a 24 hr culture grown on potato-sucrose-agar[4]. The cells were washed in glass distilled water on a centrifuge and finally prepared into a suitable suspension which on 50-fold dilution gave an approximate viable count of 30×10^5 cells per ml.

Heat resistance of yeast cells — Six conical flasks (capacity, 250 ml.), each containing 50 ml. of sterile phosphate buffer (*p*H 7·0; prepared by mixing 7 volumes of *M*/20 Na_2HPO_4 and 3 volumes of *M*/20 K_2HPO_4) or thawed orange juice (*p*H 3·6), were heated in a water bath to 45°, 50° and 55°C. (±1°C.). One ml. of yeast culture suspension (viable count 15×10^7 yeast cells) was added to the flasks and the contents mixed. After suitable intervals, 1 ml. of the heated buffer or juice samples was withdrawn and added to 9 ml. of precooled phosphate buffer in dilution tubes. Suitable dilutions of these suspensions were plated in duplicate on potato-sucrose-agar. Plates showing 30-300 colonies only were counted. Log number of survivors were plotted against time.

Results and discussion — It is seen from the results presented in Fig. 1 that the rate of destruction curves for *S. cerevisiae* at all the three temperatures could not be represented by a single straight line. The rate of fall of viable counts was highest at 55°C. and lowest at 45°C. Further, there is a steep fall in viable counts in the initial stages of heating. On further heating, the rate of fall in viable counts is considerably slowed down and the destruction curves become less inclined to the time axis. Further, the yeast strain was found to survive for longer time in heated orange juice than in phosphate buffer at the same temperature.

The present investigation reveals that the rate of destruction curves for *S. cerevisiae* when heated in orange juice or phosphate buffer are non-logarithmic

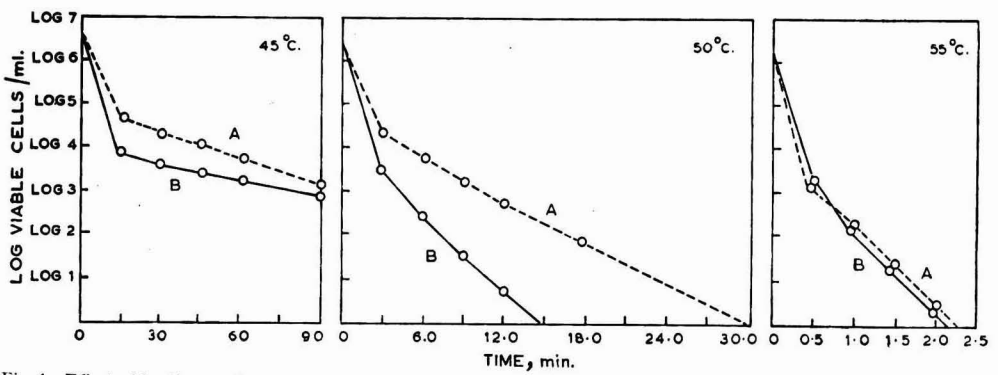

Fig. 1—Effect of heating at different temperatures on the viability of yeast cells in (A) orange juice and (B) phosphate buffer

in nature, i.e. equal percentages of cells are not destroyed in unit interval of time. The rapid rate of death in the initial stages of the heating process indicates that the bulk of the population comprised cells of low heat resistance. Slower death rates in the later stages may be attributed to the protective influences of the large number of dead cells in the suspension. Heating at 45°C. has a low lethal value and is insufficient to bring about total destruction of yeast cell within a short interval. At 50° and 55°C., the destruction of yeast cells occurs in 30 and 2·2 min. respectively. Yeast cells show greater resistance to heat in orange juice (*p*H 3·6) than in phosphate buffer (*p*H 7·0) at all the temperatures investigated. The difference in the resistance of the yeast cells to heat in the two substrates was more marked at 50°C. than at 45° or 55°C.

The ability of the yeast cells to resist the effects of heating for longer periods in media of low *p*H value than in neutral media is rather contrary to general expectations. However, these results are in agreement with those obtained by White[3].

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Some Aspects of Carbohydrate Metabolism of Brain in Experimentally Induced Convulsive Seizures

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The carbohydrate metabolism in rat's brain has been studied after injection of caffeine sodi benzoate. Caffeine sodi benzoate in a dose of 195 mg./rat has proved to be an efficient convulsive agent. The glycogen content of the brain has been found to diminish during convulsion after the injection of caffeine sodi benzoate. The hexosamine content of brain of normal rat, which is 18·3±1·64 mg./100 g. tissue, increases significantly during convulsion to 38·8±3·3 mg./100 g. tissue; the blood sugar level increases at the peak of convulsion. The possible causes of increased hexosamine content and diminished glycogen content of brain have been discussed, with special reference to hyperglycaemia during convulsive seizures.

EXPERIMENTAL convulsion in lower animals can be induced either by electric shock or by injecting convulsive drugs. In the lower mammals, injection of large quantities of caffeine is followed by symptoms closely resembling those induced by strychnine. Klein and Olsen[1] have observed the generalized convulsive properties of caffeine and other drugs. Kerr and Ghantus[2] have observed that the brain glycogen, unlike liver glycogen, is rather more stable. They have also noted that in fasting, pancreatectomy, phloridzine poisoning or adrenaline injection does not cause significant change in brain glycogen. Kerr and Antak[3] have noted slight increase of free sugar content of the brain after the convulsion induced by strychnine and cardiazole in anaesthetized (amytal) animals. Klein and Olsen[1] have noted an increased metabolism of glucose during induced convulsion in animals. They have also observed a decreased concentration of brain glycogen, glucose and phosphocreatine and increased concentration of lactate and inorganic phosphorus. Richter and Dawson[4] have reported that the direct stimulation of the brain or the administration of convulsive agent increased the ammonia level of rat's brain. Ghosh *et al.*[5] have studied the mechanism of biosynthesis of glucosamine with labelled glucose and noted that glucosamine formation takes place from fructose-6-phosphate and ammonia in the presence of glucosamine-6-phosphate-deaminase. Glucosamine-6-phosphate can also be formed from fructose-6-phosphate and glutamine in the presence of enzyme L-glutamine-D-fructose-6-phosphate-transamidase.

All the above observations do not conclusively prove that significant changes occur in carbohydrate metabolism during convulsive seizures. As electric convulsive therapy is one of the commonest methods used in psychiatric practice, it was considered of interest to study the changes in carbohydrate metabolism during experimentally induced convulsions. Studies have, therefore, been carried out with albino rats to note any possible change in the carbohydrate metabolism in the brain and in the blood during convulsions, and the results of these studies are reported in this paper.

Twenty-four healthy albino rats of both sexes weighing 140 ± 10 g. were selected for the experiment. The animals were divided into two groups and each pair of animals was selected in such a way that the weight of the pair of the animals was the same and both were of the same sex. The animals were placed in individual metabolic cages and fed *ad libitum*.

The blood samples for the estimation of glucose were collected from all animals of both groups. In

one animal of an individual pair 1 ml. caffeine sodi benzoate solution containing 195 mg. of the chemical was injected and in the other animal of the pair, 1 ml. of pyrogen-free distilled water was injected. The dose of caffeine sodi benzoate was determined previously by trial and error method, and it was noted that 1 ml. of caffeine sodi benzoate solution containing 195 mg. of the chemical was most satisfactory for the induction of convulsive seizures. The convulsion in rat started usually 15-20 min. after injection. During pre-convulsive state the increased rate of respiration and walking backward reaction were noted. During convulsive state the second blood samples for glucose estimation were collected by cardiac puncture. The blood was withdrawn from the other animal of the pair injected with distilled water at the same time for the estimation of sugar.

During convulsion the animals were sacrificed by decapitation and the brain of each rat was removed as quickly as possible to estimate its glycogen and hexosamine content. The animals serving as controls for each pair were also sacrificed at the same time and the brains were removed for the estimation of glycogen and hexosamine.

Blood glucose was estimated by Hagedorn and Jensen[6] method. Glycogen was estimated by the method of Pluger as modified by Kerr[7]. Hexosamine was estimated by the Elson and Morgan procedure[8,9] as modified by Rimington[10] and Boas[11].

The results of experiments on the effect of intramuscular injection of caffeine sodi benzoate on blood sugar level, and glycogen and hexosamine content of brain of the experimental animals are recorded in Table 1.

From the results given in Table 1 it is evident that the glycogen content of brain decreases significantly during the peak of convulsive seizures. The hexosamine content of the whole brain increases significantly during convulsion induced by caffeine sodi benzoate. The blood sugar level also shows significant rise during the peak of convulsion. The rise of blood sugar during convulsion is to be normally expected. This may be either due to sympathetic overaction or excess secretion of adrenaline-like substances. Although it has been suggested by Kerr and Ghantus[2] that the brain glycogen remains in a stable condition, from the result of the present study it is evident that there is some amount of glycolysis. The present study also confirms the results obtained by Klein and Olsen[1] who suggested that during seizure there may be increased oxygen utilization and that a great degree of hypoxia may result in glycolysis and increased lactic acid content of brain. Kerr and Antak[3] have suggested that convulsion is not regularly associated with any significant change in the carbohydrate fractions studied. Decreased glycogen content of the brain noted by us at the peak of the convulsion suggested the possibility of glycolysis which is not common in ordinary stress conditions in fasting and pancreatectomized animals. The results of the present study suggest a mode of carbohydrate metabolism involving glycolysis during convulsion. The increased content of hexosamine in the brain during convulsion may be a detoxicating mechanism due to excess accumulation of ammonia in the brain as noted by Richter and Dawson[4]. Excess formation of hexosamine may also be due to increased activity of deaminase and transamidase enzymes during convulsion. The increased blood sugar during the convulsive phase also may be a remote causative factor in the increased hexosamine content of brain. But one should be guarded in attributing excess hexosamine formation to hyperglycaemia during convulsive state. Hexosamine content of brain has been estimated at the peak of convulsion and the time factor in the formation of hexosamine in the brain due to hyperglycaemic condition of blood has to be considered. The short experimental period may not be favourable to rapid formation of hexosamine in the brain from the excess blood glucose. Moreover, the rise of blood sugar may not cause a rise in the glucose content of the brain due to the existence of a blood brain barrier. It may be assumed that the barrier mechanism of glucose is not very marked but again the short time interval may not be favourable for the synthesis of hexosamine in the brain.

From the results of the present studies it may be surmised that the diminished glycogen content and increased hexosamine content of brain during convulsion reflects the possibility of changes in carbohydrate metabolism during convulsion.

TABLE 1 — EFFECT OF CAFFEINE SODI BENZOATE ON BLOOD SUGAR LEVEL AND GLYCOGEN AND HEXOSAMINE CONTENTS OF BRAIN OF RATS

[*No. of observations made in each case*, 12; *dose of caffeine sodi benzoate solution injected intramuscularly*, 1 *ml.* (=195 *mg.*) *per animal*; *blood sugar values expressed in mg.*/100 *ml. of blood*; *glycogen and hexosamine values expressed in mg.*/100 *g. brain tissue*]

	Before convulsion	During convulsion	*t* value
Blood glucose level	108·3±2·2	155·8±5·2	27·1
Glycogen content of brain	69·5±2·1	50·1±5·3	11·8
Hexosamine content of brain	18·3±1·6	38·8±3·3	19·2

*t value significant at 1 per cent level.

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Biochemical Studies on Indian Camel (*Camelus dromedarius*): Part III — Plasma Insulin-like Activity & Glucose Tolerance

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Normal blood glucose, plasma insulin-like activity and intravenous glucose tolerance of Indian camel have been reported.

IN earlier publications from this laboratory, blood proteins and lipids[1] as well as inorganic constituents[2] of the serum of *C. dromedarius* were reported. In the present communication, the plasma insulin-like activity (ILA), normal blood glucose and intravenous glucose tolerance of the Indian camel are reported.

Materials and methods — The camels used in this study were males, aged 4-7 years, and were in the rut period. Food was withdrawn 15-20 hr before the blood was collected. Blood was collected by jugular vein puncture, in heparinized tubes containing potassium fluoride for the estimation of blood glucose.

When present in low concentrations, insulin gets adsorbed on inert surfaces like glass[3,4]. Therefore, for plasma ILA, blood was collected in siliconized glass tubes. Siliconization was done with Rhodrosil, kindly presented by Voltas Ltd, Bombay. Plasma was separated in the usual way. Glucose was estimated by the method of Hagedorn and Jensen[5].

For glucose tolerance test, 500 ml. of 25 per cent sterile glucose solution was injected in the left jugular vein of a camel and blood was withdrawn from the right jugular vein at 30 min. intervals for 2 hr. Glucose injection was given by gravity method and took about 5 min.

Plasma ILA was estimated by a method essentially similar to that of Vallance-Owen and Hurlock[6]. Male rats of 120-150 g. body weight were used. The animals were stunned, decapitated, abdomen opened up and diaphragm excised. Hemidiaphragms from each animal were washed in cold Krebs-Ringer bicarbonate and kept in separate beakers containing buffer with a trace of glucose at 4°C. for 15-20 min. Glucose was dissolved in Krebs-Ringer to give a final concentration of 300 mg./100 ml. Two ml. of glucose in buffer were pipetted out into a 25 ml. siliconized flask with a standard B19 stopper. One of the hemidiaphragms from an animal was gently blotted and transferred to glucose buffer, while the other was transferred to the flask containing plasma-glucose mixture (1 ml. plasma plus 1 ml. glucose). As controls, flasks containing glucose and plasma-glucose mixture were used. All the vessels were gassed with carbogen (95 per cent O_2 and 5 per cent CO_2) for 5 min., stoppered and incubated in a shaker at 37°C. for 1 hr. Each plasma sample was assayed in duplicate. At the end of the incubation period, the residual glucose concentration was determined. Hemidiaphragms were washed and dried at 110°C. overnight. The plasma ILA values have been expressed as mg. of glucose used up per hour per g. dry weight of diaphragm over the basal glucose uptake. For comparison, standard insulin was used after appropriate dilution in Krebs-Ringer bicarbonate solution.

Results and discussion — Normal blood glucose values of the camel are given in Table 1 and plasma ILA values in Table 2. The results of intravenous glucose tolerance tests are given in Table 3.

The results given in Table 1 show that blood glucose values of camel are comparable to those of guinea-pigs. Camel is a pseudo-ruminant while cattle are ruminants. The disparity among the

TABLE 1 — BLOOD GLUCOSE VALUES OF INDIAN CAMEL AND OTHER ANIMALS

(*Values expressed in mg./100 ml. whole blood*)

Species	Blood glucose	Range	Ref.
Camel (10)*	110±11·4	98-137	Present work
Guinea-pig (11)	111±4·6	—	7
Horse	73±9	—	8
Sheep	45±6	—	8
Cattle	50	—	8
Dog	77	—	8

*Figures in parentheses indicate the number of animals.

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TABLE 2 — PLASMA ILA OF CAMEL AND CAT EXPRESSED AS GLUCOSE UPTAKE/ML./HR/G. DRY WEIGHT DIAPHRAGM

	Glucose uptake *mg.*	Range *mg.*	Ref.
Camel (8)*	5·87±0·67	3·2-8·1	Present work
Cat (7)	6·60±0·73	—	9
200 micro U standard insulin	6·77	—	Present work

*Figures in parentheses indicate the number of animals.

TABLE 3 — GLUCOSE TOLERANCE IN INDIAN CAMEL

[*The values represent the rise in blood glucose above fasting level (expressed in mg. glucose/*100 *ml. blood) at intervals of* 30 *min. after glucose administration*]

No. of animals	30 min.	60 min.	90 min.	120 min.
4	+63±8·2	+42±7·3	+39±10·7	+21±10·3

blood glucose values of camel, cattle and sheep is noteworthy.

It has been recently shown that plasma insulin is present in ' free ' and ' bound ' forms, and that the biologically active form of insulin present in most of the tissues is the ' free ' form[10,11]. By the rat diaphragm method, only the ' free ' form is determined[12-14]. It is evident from the results given in Table 2 that plasma ILA values of camel compare well with those of the cat. Recent studies have shown that plasma contains factors other than insulin, which can affect the glucose uptake by the diaphragm[15]. In view of these observations plasma ILA values have been expressed in terms of glucose uptake rather than in absolute units.

Camel is a polygastric animal and as such *per os* glucose tolerance is not feasible. In fact, it was observed in a pilot experiment that there was no change in blood glucose values for 3 hr after feeding glucose. The results given in Table 3 show that an intravenous load of glucose gives a peak value in blood 30 min. after glucose injection. These values suggest that the pancreas of camel respond to glucose load in the usual way and the utilization of glucose in camel under normal conditions is influenced by the pancreatic activity.

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Papers Accepted for Publication

The following papers have been accepted for publication in Section C of the *Journal of Scientific & Industrial Research* and will be published in subsequent issues:

Influence of α-Chymotrypsin & Trypsin on Beef Liver Catalase & Horse Radish Peroxidase — T. M. Radhakrishnan, E. Raghupathy & P. S. Sarma, Department of Biochemistry, Indian Institute of Science, Bangalore

Mode of Action of Psoralen in Pigment Production: Part II — Inactivation of —SH Groups by Irradiated Psoralen — Rashid Ali & S. C. Agarwala, Central Drug Research Institute, Lucknow

Long-acting Pharmaceutical Preparations : Part I — Ephedrine & Chlorpheniramine Resin Complexes — E. D. Bharucha & Y. K. Hamied, Cipla Research Laboratories, Bombay

The Pharmacology of Certain Terpene Alcohols & Oxides — B. J. Northover & J. Verghese, Christian Medical College, Vellore

Studies on Some Umbelliferous Fruits: Part I — Pharmacognosy of the Fruits of Trachyspermum roxburghianum Benth. & Hook. — S. M. J. G. Qadry & C. K. Atal, Institute of History of Medicine & Medical Research, Delhi & Pharmacy Department, Panjab University, Chandigarh

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Abstract — The abstract should indicate the scope of the work and the principal findings of the paper. It should not exceed 3 per cent of the length of the paper, and, except in exceptional cases, it should not exceed 200 words. The abstract prepared should be in such a form that abstracting periodicals can use it without modification.

Tables — Tables should be typed on separate sheets of paper without any text matter on the page. They should be numbered consecutively in Arabic numerals and should bear brief titles. Column headings should be brief. Units of measurement should be abbreviated, typed in small letters (underlined) and placed below the headings. Nil results should be indicated and distinguished clearly from absence of data. Graphs as well as tables, both representing the same set of data, must be strictly avoided. Inclusion of structural formulae inside the tables should be avoided as far as possible.

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