3. Group 2. After 2 weeks on radioactive diet. Exposure time 144 h. The rectangle indicates the region enlarged in Pl. 2.
4. Group 3. Ten weeks after returning to non-radioactive diet. The interstitial activity is still comparable with that in 3.

PLATE 2

Photomicrographs of cortical regions of the autoradiographs.

1. Group 1. Uniform activity due to full incorporation of the radioactive diet during growth.
2. Group 2. The photomicrograph shows the uniformity of the interstitial activity throughout the thickness of the pre-radioactive cortical bone. The outline of the photomultiplier slit is superposed on the photograph.

PLATE 3

Autoradiographs of upper incisors.

1. Group 2. After 4 weeks on radioactive diet. Exposure time 4 h. Only the uniformly radioactive growth zone in the dentine, and new growth of enamel is revealed.
2. Same as 1, exposure time 8 days. Even with a very long exposure no interstitial activity in the pre-radioactive dentine is detectable.
3. Group 3. Ten weeks after return to non-radioactive diet. In this interval nearly the whole tooth has been renewed by growth. The variation with time of the serum-Ca activity has been recorded in the deposited dentine which shows the persisting activity due to the return of resorbed radioactive bone mineral to the circulation.

Growth and Amino-acid Intakes of Children on a Cereal-Legume-Vegetable Diet

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Opinion is as yet divided on the adequacy of vegetable protein as the sole protein in the diet of growing children. On the one hand, Woolley (1945) has demonstrated the existence of a growth factor associated with certain proteins. On the other hand, vitamin B_{12}, which is present in plants in scarcely detectable amounts (Smith, 1950–1), has been claimed to be necessary for optimum growth (Zucker & Zucker, 1948; Maddy & Swift, 1952). In recent papers (Chick, 1951; Nicol, 1952) the low biological value of vegetable proteins has been ascribed to deficiency in certain of the essential amino-acids, especially lysine and tryptophan. Clinical findings in infants and children fed on predominantly vegetable diets have shown such varied features as low serum albumin, oedema, fatty liver, anaemia and symptoms resembling those arising from B-vitamin deficiencies (Brock & Autret, 1952) all of which occur in the condition called kwashiorkor or malignant malnutrition. It is now recognized that kwashiorkor is liable to occur in all races (Williams, 1953) and is amenable to treatment with skim milk, the curative factor being either the essential amino-acids of the milk proteins or a growth factor associated with the animal protein and designated animal protein factor (APF).

Preliminary reports by Dean (1949, 1951) indicated that a mixture of cereals with soya or sunflower seed could produce excellent growth in rats, and the trials have been extended to growing children of 1–2 years of age. A further report (Dean, 1952) suggests that vegetable proteins in sufficient variety would suffice to prevent kwashiorkor. In his latest report on the subject, Dean (1953) concludes that 'nothing in the composition of human or cow's milk reveals any reason why plant sources should not be able to provide a satisfactory alternative.... Adequate substitutes for milk could probably be provided from plant sources'.

Studies on the amino-acid composition of staple foodstuffs consumed in Ceylon (Baptist, 1952, 1954*a*, *b*) and on the growth of rats on vegetable diets (Baptist, 1955) have shown that though lysine is the essential amino-acid most seriously lacking in rice diets, this deficiency can be largely remedied by the inclusion of the lysine-rich proteins of legumes and leafy vegetables in as large quantities as practicable. On the basis of these studies the construction of a diet devoid of animal protein but containing a well-balanced mixture of essential amino-acids from vegetable proteins was considered feasible. Such a diet would be presumably deficient or entirely lacking in the APF.

The essential amino-acid requirements for maintenance of nitrogen equilibrium have been established by Rose (1949) on the basis of feeding experiments with young male adults. Rose has arbitrarily recommended twice these levels as a safe intake to guard against all normal variables. The human maintenance requirements have also been calculated from published data on human feeding experiments by Harte & Travers (1947), by Mitchell (1950) and by Baptist (1953) (these figures are included in Table 8 for ease of reference). The figures of Rose and of Mitchell, unlike those of the others, do not take account of the sparing effect of cystine on methionine or of tyrosine on phenylalanine.

The requirements for human infants and for young children have been only partially established, and the data available have been summarized by Albanese (1950). Dean (1953) has used the data of Czerny & Keller (1925) to calculate the infant's requirements.

The purpose of this paper is to report the results of feeding children aged 1–6 years on an entirely vegetable diet containing a varied mixture of plant proteins from three cereals (rice, finger millet and wheat flour), four legumes (lentil, green gram, black gram and pigeon pea), numerous locally available vegetables (see Table 1) and coconut. The adequacy of this diet in APF was judged by feeding a control group with the test diet plus a daily ration of skim milk. The diet also furnished a means of evaluating the requirements of essential amino-acids for growth by children in this age group.

EXPERIMENTAL

Subjects and methods

Selection of children. The children used in the study were selected from about fifty children attending the Maligakanda* Crèche. They came from poor homes of working-class families with an income of Rs. 50 (£3. 15*s*. 0*d*.) or less per month and

* One of the municipal wards of Colombo.

lived within walking distance from the crèche. A survey of the diets of the families in their homes revealed that very few of them consumed any beef, fish or milk and those who partook of such animal foods did so in extremely small quantities. The children were usually brought by their parents early in the morning and removed to their homes in the evening after their final meal.

The children were examined clinically, and a Mantoux test and X-ray of the chest were done in each case. Total plasma protein and plasma albumin (Members of the Department of Experimental Medicine, Cambridge, and Associated Workers, 1951) were determined on fifteen children, and erythrocyte count, leucocyte count and haemoglobin (Sahli) determinations were carried out on twenty-eight children selected at random. The following values were obtained (the mean with its standard error is given): total plasma protein, $6{\cdot}99 \pm 0{\cdot}15$ g/100 ml.; plasma albumin, $4{\cdot}30 \pm 21$ g/100 ml.; erythrocytes, $4{\cdot}79 \pm 0{\cdot}11$ millions/mm^3; leucocytes, $11{,}810 \pm 690$/mm^3; haemoglobin, $11{\cdot}20 \pm 0{\cdot}19$ g/100 ml. The total protein and albumin determinations only were repeated on seven of the children at the end of the experiment and were found to be not significantly different. The values given above were considered to be normal judging from previous work by one of the authors (de Mel, unpublished data) on middle-class schoolchildren in this age group.

Faecal examination revealed a large incidence of infestation with *Ascaris lumbricoides*; all the children were therefore dewormed with diethylcarbamazine (Hetrazan syrup, Lederle Laboratories Inc.). This treatment was repeated after 10 weeks in a few children in whose faeces *Ascaris* ova were still present. Owing to the incidence of mild signs of vitamin deficiency such as angular stomatitis, phrynoderma and xerosis of the skin, all the children were given one multivitamin tablet and one teaspoonful of shark-liver oil once a day at a meal. In view of the low haemoglobin level, an iron preparation was also given for a short period. This treatment produced a noticeable improvement in the general condition of the children. Thirty children free from diseases likely to interfere with the experiment were ultimately selected.

Experimental procedure. During a preliminary 15-week (pre-experimental) period, the children were fed on the usual diet given at the crèche (Table 1). The experimental period itself lasted 17 weeks. The heights (to the nearest 0·25 cm) and weights (to the nearest oz.) of the children were recorded weekly, immediately before the midday meal. By the time the experiment was due to begin both the crèche authorities and the parents of the children were very willing to co-operate with us as our intervention had resulted in a considerable improvement in the health of the children. The crèche was, however, unable to house the children at night or attend to them on Sundays, even though the parents were willing to bring them. The purpose of our experiment was therefore explained to the parents, who readily agreed to the dietary plan suggested and the rigid omission of any animal foods from the diets of the children on the days when they did not attend the crèche.

The thirty children were divided into the age subgroups 1–2, $2\frac{1}{2}$–3, $3\frac{1}{2}$–4, $4\frac{1}{2}$–5, $5\frac{1}{2}$–6 years, and each subgroup was divided at random into two by drawing lots. The group to be fed on the experimental 'test' diet was called the 'test' group and the other group receiving the test diet and skim milk was called the 'control' group. For various reasons a few children, four in the control group and three in the test group ceased to attend at the crèche shortly after the experimental period began, and the experiment was completed on eleven children in the control group and twelve in the test group.

Table 1. *Plan of the diets used in the experiment*

Meal	Crèche diet	Experimental diet	Control diet
Morning meal	Bread, rusk and condensed milk	Selected from the following: bread, rusk, green gram, finger millet, milk rice, jack seeds, jam, coconut and brown sugar	As experimental diet with 0·5 oz. skim milk/child/day
Mid-morning drink	Barley water and sugar	Orange juice and sugar	
Midday meal	Rice, legume* curry and *mellun*‡	Rice, legume† curry, vegetable§ curry and *mellun*‖	
Afternoon tea	Plain tea and sugar	Plain tea and sugar	
Evening meal	Rice and vegetable¶ curry with beef once a week	Rice, legume† curry, vegetable§ curry and *mellun*‖	

* Lentil (*Lens esculenta*).

† One of the following: lentil, green gram (*Phaseolus aureus* Roxburgh), black gram (*Phaseolus mungo* Linn.), pigeon pea (*Cajanus cajanus*).

‡ Kankun leaf (*Ipomoea aquatica*).

§ One of the following: yellow pumpkin (*Cucurbita maxima*), ash plantain (*Musa sapientum*), brinjal (*Solanum melongena*), drumstick bean (*Moringa pterygosperma*), snake-gourd (*Trichosanthes anguina*), jack fruit (*Artocarpus integrifolia*), jack-fruit seeds.

‖ One of the following: kankun leaf, murunga leaf (*Moringa pterygosperma*), agati leaf (*Sesban a grandiflora*), mukunuwenna leaf (*Alternanthera triandra*), tampala leaf (*Amarantus* var.), spinach (*Basella alba*).

¶ One of the following: yellow pumpkin, ash plantain, okra (*Hibiscus esculentus*), potato.

Diets of children. During the experimental period (17 weeks), all the children were given three meals a day at 7–8 a.m., 11–12 a.m. and 4–5 p.m., with a drink of orange juice at 10 a.m. The rice used was a parboiled undermilled variety known locally as 'country rice' which was cooked in the manner customary in Ceylon. No cooking water was discarded in this process. The finger-millet seed was ground to a coarse flour, made into *pittoo* with grated coconut and served with jaggery*, or brown sugar, at the morning meal. Wheat flour (70% extraction) was used only in the form of bread. Four legumes, lentil, green gram, black gram and pigeon pea, and several green leafy and non-leafy vegetables were prepared as vegetable curries and *melluns* (chopped leafy vegetables and grated coconut suitably spiced and lightly cooked without addition of water). The only condiments and seasoning agents used consisted of turmeric, ginger, coriander, cumin seed, pepper, salt, red chillies and lime (fruit) juice in small quantities. The diet followed a fixed plan which was repeated weekly. The food was prepared using standardized recipes, and portions weighed to the nearest ¼ oz. were given to each child. In addition, each child was given every day half a compound tablet of calcium, vitamin D and ascorbic acid (Calcium-D-Redoxon,

* Crude sugar obtained by evaporating the sap of the young inflorescence of the toddy (kitul) palm (*Caryota urens*).

Roche Products Ltd)*, and five drops of a liquid multivitamin preparation (Protovit, Roche Products Ltd)†. The skim-milk powder, 0·5 oz./child/day, was dissolved in water and given in the forenoon. The health of the children as a whole remained good throughout the experimental period, but some children (seven in the control and six in the test group) suffered from temporary ailments such as colds and sore eyes.

The nutrients in the diets were calculated from the figures provided by Platt (1945), McCance & Widdowson (1946), Joachim & Pandittesekera (1938), Kandiah & Koch (1938), Nicholls (1945), Hodges, Fysh & Rienits (1949), Chatfield (1949) and unpublished data from this laboratory. Where appreciable differences were observed in the respective tables, determinations were carried out on the foodstuffs themselves, especially for protein. The amount of nutrients in any one portion was calculated as that present in the raw equivalent of the cooked food.

RESULTS

Heights and weights. Tables 2 and 3 give the weights and heights of each child in each group at the beginning of the pre-experimental period, at the beginning of the experimental (the end of the pre-experimental) period and at the end of the experimental period for the test and control groups respectively. The results for the twenty-three children who completed the experiment (see Table 4) were subjected to statistical analysis with the following conclusions.

(1) In the experimental period there was no significant difference between control and test groups in percentage increase in weight when all age groups were taken together ($0·3 < P < 0·4$). However, when the age groups $3\frac{1}{2}$–6 years were considered

Table 2. *Heights and weights of the children in the test group*

Subject	Age group (years)	Weight (oz.): At beginning of pre-experimental period	Weight (oz.): At end of pre-experimental period (beginning of experimental period)	Weight (oz.): At end of experimental period	Height (cm): At beginning of pre-experimental period	Height (cm): At end of pre-experimental period (beginning of experimental period)	Height (cm): At end of experimental period
Yw	<2	208	210	232	68	$68\frac{1}{2}$	$70\frac{1}{2}$
Hr	<2	270	300	336	$70\frac{1}{2}$	$74\frac{1}{2}$	$77\frac{1}{2}$
Cl	$2\frac{1}{2}$–3	340	352	386	78	80	$83\frac{1}{2}$
Sl	$2\frac{1}{2}$–3	336	370	432	$81\frac{1}{2}$	83	$85\frac{1}{2}$
Gw	$3\frac{1}{2}$–4	328	313	348	79	79	$80\frac{1}{2}$
Pd	$3\frac{1}{2}$–4	316	328	366	82	83	85
Kl	$4\frac{1}{2}$–5	458	462	506	109	$109\frac{1}{4}$	110
Sp	$4\frac{1}{2}$–5	476	492	528	$99\frac{1}{2}$	101	102
Ws	$4\frac{1}{2}$–5	—	496	532	—	108	109
Dd	$5\frac{1}{2}$–6	466	482	522	108	$109\frac{1}{2}$	110
Rt	$5\frac{1}{2}$–6	592	600	644	110	111	$114\frac{1}{2}$
Mr	$5\frac{1}{2}$–6	608	617	660	116	117	$117\frac{1}{4}$

* According to the manufacturer's statement, $\frac{1}{2}$ tablet contained calcium triphosphate 0·42 g; ascorbic acid 8·5 mg; vitamin D 150 i.u.

† According to the manufacturer's statement, five drops contained vitamin A 833 i.u.; thiamine 0·41 mg; riboflavin 0·20 mg; nicotinic acid 2·10 mg; vitamin B_6 0·20 mg; 'panthenol' 2·0 mg; 'vitamin H' 0·02 mg; ascorbic acid 10·5 mg; vitamin D 208 i.u.; vitamin E 0·62 mg.

separately, the percentage increase in weight in the test group was almost significantly greater than in the control group ($0{\cdot}5 < P < 0{\cdot}1$). The increase in height was not significantly different between control and test groups for all age groups considered together ($0{\cdot}6 < P < 0{\cdot}7$), or for the age groups 3½–6 years considered separately ($0{\cdot}1 < P < 0{\cdot}2$).

Table 3. *Heights and weights of the children in the control group*

Subject and age group (years)	Weight (oz.): At beginning of pre-experimental period	Weight (oz.): At end of pre-experimental period (beginning of experimental period)	Weight (oz.): At end of experimental period	Height (cm): At beginning of pre-experimental period	Height (cm): At end of pre-experimental period (beginning of experimental period)	Height (cm): At end of experimental period
Tr < 2	308	311	340	75½	77½	78½
Sw 2½–3	288	308	370	80	81¾	85
Js 2½–3	350	382	426	81	84½	87½
Sd 3½–4	416	432	461	96	98½	101
Cr 3½–4	412	428	458	93	94	95½
Hp 3½–4	456	450	486	91½	93½	96
Rw 4½–5	432	450	474	94½	96	99
Lw 4½–5	428	456	462	98	99	102
Bd 4½–5	512	520	552	102	103	105
Vt 5½–6	544	553	594	112	112¾	113½
Gw 5½–6	544	548	594	107	108	109

Table 4. *Statistical analysis of weights and heights of the eleven children in the control group and the twelve children in the test group who completed the test*

(*a*) Comparison of mean percentage increases in weight and height for the test and control groups during the experimental period of 17 weeks

Age group	Weight: Control group	Weight: Test group	Weight: Difference	Weight: S.E. of difference	Height: Control group	Height: Test group	Height: Difference	Height: S.E. of difference
All ages	8·40	9·87	1·47	1·59	2·31	2·09	0·22	0·53
3½–6 years	6·43	8·68	2·25	1·08	2·06	1·34	0·72	0·49

(*b*) Comparison of mean weekly percentage increases in weight and height during the pre-experimental (15 weeks) and the experimental periods for each group

Group	Weight: Pre-experimental	Weight: Experimental	Weight: Difference	Weight: S.E. of difference	Height: Pre-experimental	Height: Experimental	Height: Difference	Height: S.E. of difference
Control	0·23	0·49	0·26*	0·10	0·14	0·15	0·01	0·34
Test	0·27	0·59	0·32**	0·09	0·10	0·13	0·03	0·40

(*c*) Comparison of mean changes in rates of percentage weekly increases in weight and height in experimental over those in pre-experimental period for each group

Weight: Control group	Weight: Test group	Weight: Difference	Weight: S.E. of difference	Height: Control group	Height: Test group	Height: Difference	Height: S.E. of difference
0·26	0·32	0·06	0·10	0·01	0·03	0·02	0·03

* Significant at $P < 0{\cdot}02$. ** Significant at $P < 0{\cdot}01$.

(2) When the pre-experimental and the experimental periods were compared for each group (test and control), the rate of percentage increase in weight (i.e the percentage increase in weight per week) was greater in the experimental period than in the pre-experimental period. The difference between the means for the two periods was statistically significant (control group: $0.01 < P < 0.02$; test group: $P < 0.01$). When the rate of percentage increase in height for the two periods was calculated similarly, the difference was not significant (control group: $0.6 < P < 0.7$; test group: $0.4 < P < 0.5$).

(3) When the two groups (test and control) were compared, the mean changes in rates of percentage increase in weight and height from one period to the other were not significantly different (weight: $0.5 < P < 0.6$; height: $0.6 < P < 0.7$).

Table 5. *Mean daily food consumption per head of the children, on the crèche and the entirely vegetable test diet, together with the moisture and protein contents of the chief foods consumed*

Food item	Moisture (%)	Protein (%)	Crèche diet, all ages (g)	Test diet 1–3 years (g)	Test diet 3½–6 years (g)
Rice (parboiled)	10·7	8·7	125·3	118·0	156·0
Bread (white)	37·0	8·1	77·0	11·7	11·7
Finger millet	13·7	7·1	—	4·0	4·0
Lentil	13·0	25·0	11·8	18·0	18·0
Green gram	13·7	23·9	—	17·7	17·7
Black gram	12·3	26·6	—	7·0	7·0
Pigeon pea	11·4	24·0	—	7·2	7·2
Leafy vegetables	77–90	3·0–8·0	3·8	19·1	19·1
Non-leafy vegetables	68–95	0·7–2·2	41·7	21·2	21·2
Coconut (kernel, mature fresh)	41·0	4·4	—	7·2	7·2
Coconut milk*	81·5	1·6	30·8	61·4	61·4
Jack seeds	52·0	4·6	—	2·8	2·8
Condensed milk	28·0	8·2	22·8	—	—
Boiled beef	46·0	28·0	1·2	—	—

* Prepared as follows: 200 g of finely grated coconut kernel were mixed with an equal weight of warm water (60°) and expressed by hand. The process was repeated with the residue. The yield of milk was 450 g, and its composition was water 81·5, protein 1·6, carbohydrate 1·8, fat 15·1 %.

Food consumption and intake of nutrients on the diets. Table 5 gives the details of food consumption on the crèche and experimental diets.

Table 6 gives the nutrient intake of the children of all ages during the pre-experimental period. Table 7 gives the nutrient intake of the children under 3 years of age during the experimental period. The others received also an additional 4 oz. cooked rice/child/day.

Essential amino-acid content of the diets. The essential amino-acids per 100 g food consumed in these experiments have been calculated from the figures given by Block & Bolling (1951) and Baptist (1954*a*, *b*) on the basis of the moisture and protein contents quoted in Table 5. For the vegetables, small differences that may exist between the amino-acid composition of the whole vegetables and that of the 'crude proteins' prepared from them have been disregarded and the former have been calculated, using the published figures for the latter (Baptist, 1954*a*, *b*). The values for rice have been calculated from the figures of Baptist (1953) and for condensed milk from the figures for evaporated milk given by Block & Bolling (1951). In estimating the tyrosine intake, only the chief contributing foods were taken into account, as information about many of the others was lacking; the tyrosine intakes therefore represent minimal values. As no reliable values were available for pigeon pea, its amino-acid distribution was taken as identical with that of lentil; the minor contributions of orange juice, jam and broth have been omitted as negligibly small. The amino-acid intakes on the crèche diet, which included some milk and beef, as well as on the test and control diets are given in Table 8.

Table 6. *Mean daily intake of calories and nutrients of the children on the crèche diet*

Food category	Calories (Cal.)	Protein (g)	Fat (g)	Carbohydrate (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Vitamin A (i.u.)	Thiamine (mg)	Riboflavin (mg)	Nicotinic acid (mg)	Vitamin C (mg)	Vitamin D (i.u.)
Cereals	650	17·1	3·3	138	21	168	3·6	0	0·34	0·16	5·8	0	0
Sugar and jam	120	0	0	30	19	19	0·2	0	0	0	0	0	0
Legumes	37	3·0	0·1	6	5	41	0·9	24	0·08	0·02	0·3	0	0
Vegetables	27	0·7	0	6	16	15	0·4	789	0·03	0·05	0·3	7·8	0
Coconut and coconut oil	48	0·5	4·3	2	3	13	0·3	0	0·01	0	0·1	0	0
Meat and milk (sweetened)	84	2·2	3·2	11	70	59	0·6	92	0·02	0·01	0·1	0·2	0
Total	960	23·5	10·9	193	134	315	6·0	905	0·48	0·24	6·6	8	0
(U.S.A.) National Research Council: Food and Nutrition Board (1948) recommendations:													
1–3 years	1200	40	—	—	1000	—	7	2000	0·6	0·9	6	35	400
4–6 years	1600	50	—	—	1000	—	8	2500	0·8	1·2	8	50	400

Table 7. *Mean daily intake of calories and nutrients of the children on the test and control diets*

Food category	Calories (Cal.)	Protein (g)	Fat (g)	Carbohydrate (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Vitamin A (i.u.)	Thiamine (mg)	Riboflavin (mg)	Nicotinic acid (mg)	Vitamin C (mg)	Vitamin D (i.u.)
Cereals	469	11·5	2·5	100	14	140	3·8	0	0·30	0·13	4·8	0	0
Sugar and jam	244	0	0	61	43	43	0·6	0	0	0	0	0	0
Legumes	160	12·3	0·6	26	57	176	3·6	36	0·26	0·14	1·2	0	0
Vegetables	40	1·4	0·2	8	53	25	0·9	1704	0·03	0·04	0·2	38·2	0
Coconut and coconut oil	129	1·2	12·1	4	6	35	0·7	0	0·04	0	0·1	0	0
Cereal (extra to 3½–6 years)	136	3·3	0·8	29	4	38	0·8	0	0·09	0·04	1·5	0	0
Supplements:													
Protovit	—	—	—	—	—	—	—	1040	0·42	0·21	2·1	10·3	208
Ca-D-Redoxon	—	—	—	—	165	85	—	—	—	—	—	8·5	150
Total on test diet:													
1–3 years	1042	26·4	15·4	199	338	504	9·6	2780	1·05	0·52	8·4	57	358
3½–6 years	1178	29·7	16·2	228	342	542	10·4	2780	1·14	0·56	9·9	57	358
Skim milk in control diet	49	5·1	0·1	7	177	150	0·1	9	0·06	0·23	0·2	1·0	0
Total on control diet:													
1–3 years	1191	31·5	15·5	206	515	654	9·7	2789	1·11	0·75	8·6	58	358
3½–6 years	1227	34·8	16·3	235	519	692	10·5	2789	1·20	0·79	10·1	58	358

Table 8. *Mean amounts of essential amino-acids and of total protein taken by the children on the crèche, test and control diets, compared with adult maintenance requirements*

	Adult maintenance requirements* (g/day)				Intake on the diets (g/day)				
					Crèche diet	Test diet		Control diet	
Amino-acid	Rose (1949)	Harte & Travers (1947)	Mitchell (1950)	Baptist (1953)	1–6 years	1–3 years	3½–6 years	1–3 years	3½–6 years
Lysine	0·80	0·8	0·99	1·00	0·95	1·29	1·41	1·70	1·82
Threonine	0·50	1·0	0·56	1·04	0·80	0·93	1·05	1·18	1·30
Methionine	1·10	0·5	1·05	0·31	0·40	0·37	0·42	0·49	0·54
Cystine	—	0·3	—	0·54	0·34	0·30	0·34	0·33	0·38
Tryptophan	0·25	0·4	0·32	0·25	0·31	0·27	0·32	0·34	0·39
Isoleucine	0·70	1·2	1·12	1·37	1·06	1·24	1·39	1·55	1·70
Leucine	1·10	1·7	1·86	1·99	1·80	2·09	2·35	2·62	2·89
Valine	0·80	1·1	1·18	1·55	1·32	1·59	1·81	1·81	2·03
Phenylalanine	1·10	1·4	2·42†	1·21	1·16	1·39	1·56	1·62	1·79
Tyrosine	—	1·0	—	0·92	1·10‡	1·07	1·26‡	1·34	1·53‡
Arginine	—	1·2	1·18	1·36	1·42	1·89	2·13	2·06	2·30
Histidine	—	0·5	0·55	0·47	0·48	0·57	0·63	0·69	0·75
Total protein	—	—	—	—	23·5	26·4	29·7	31·5	34·8

* g/day for average (70 kg) man with a basal metabolism of 1700 Cal.
† Includes tyrosine. ‡ Minimal value.

DISCUSSION

Growth in relation to nutrients in the diet. The contributions made by the various categories of foodstuff to the nutrients in the diet are shown in Tables 6 and 7. No account has been taken of the losses, chiefly of vitamins, that must have occurred during the process of cooking. The outstanding features of the normal crèche diet were low intakes of calcium, thiamine and riboflavin, with insufficient total calories. Protein and fat contributed about 10% each and carbohydrates about 80% of the total calories.

In the test diet total calorie intake was increased by increasing the food intake, the percentage of the total calorie intake contributed by major individual components of the diet remaining approximately the same. A marked increase in the intakes of protein, thiamine, riboflavin and calcium was obtained from the legumes which more than offset the low values due to the absence of the meat and milk proteins present in the crèche diet. The vitamin C intake was increased by the inclusion of fresh orange juice. Together with the supplements the intake of nutrients on the test diet was optimal except for riboflavin and calcium, which were about two-thirds and one-third respectively of the intakes recommended by the (U.S.A.) National Research Council: Food and Nutrition Board (1948). However, no noticeable signs of riboflavin deficiency were observed in the subjects at the end of the period; in fact the signs seen at the commencement of the study in some children had disappeared completely at the end.

On this high-legume diet, it was found impracticable to increase any further the intake of leafy and non-leafy vegetables. Thus the latter contributed only about 5% of the total protein intake (see Table 7).

The chief contribution of the skim milk given to the control group (see Table 5) was to increase by 17% the intake of protein, by 50% that of calcium and by 40% that of riboflavin over those on the test diet. During the experimental period there was a distinct improvement in growth in both test and control groups, as shown by the fact that the mean rate of increase in weight was significantly greater than in the pre-experimental period. The same improvement did not occur in the mean rate of increase in height. The experiment had not, however, been designed to show the differences in effect between the experimental diets and the crèche diet. It is not possible, therefore, to attribute to the experimental diets the improvement in the rate of increase in weight. But, as has been shown, the results indicate no significant difference between the control and test children when all age groups are considered together. Indeed such slight significance as was revealed statistically when the children aged $3\frac{1}{2}$–6 years were considered was in favour of the test diet. The results obtained appear to justify the conclusion that for short periods a source of the animal protein factor (if such exists) is not essential for growth and development. The evidence obtained from the increased vitality and active growth of all the children suggests that a vegetarian diet, properly constituted, might suffice for normal growth in children, though the margin of safety would be small for calcium and riboflavin.

In view of the short duration of the experiment and the possibility that the human child might store appreciable amounts of APF, the results obtained are not conclusive about the essential nature or otherwise of this entity. The point of action of this growth factor associated with animal proteins is not known. A part of its activity appears to be associated with one of the active forms of vitamin B_{12} and another part with the essential amino-acids, lysine, tryptophan and methionine, in which animal proteins are rich. There can be little doubt that the test diet was low in vitamin B_{12} activity. If vitamin B_{12} is essential for growth in children, it seems likely that the test subjects were drawing on body stores of this substance. The lack of any significant difference between test and control subjects suggests either that this was so, or that vitamin B_{12} is not essential in amounts greater than present in vegetable foods. A longer experimental period would perhaps have been more revealing, but unfortunately was not practicable under the prevailing circumstances. As far as amino-acid content is concerned, it is apparent that a suitable mixture of vegetable proteins need not be deficient in any of the essential amino-acids in which animal proteins are rich.

Intake of essential amino-acids. The figures in Table 8 indicate that whereas the intake on the crèche diet was about equal to the adult maintenance requirement, the intake on the test diet was somewhat in excess, and the intake on the control diet well in excess. Total intake figures for each amino-acid are probably misleading for children, and a more correct picture is obtained if the figures are calculated on the basis of intake of amino-acid per kg average body-weight (Table 9). In Table 9 only the figures for the children aged $2\frac{1}{2}$–3 years and $5\frac{1}{2}$–6 years are given. The figures calculated by Dean (1953) for infants using the analytical values for human milk reported by Block & Mitchell (1946–7) have been recalculated on the basis of the same content of protein in human milk as used by Dean (1953) according to the recent analyses of Soupart, Moore & Bigwood (1954) by the ion-exchange technique.

Comparison of the amino-acid intake ($2\frac{1}{2}$ to 3-year group) on the crèche diet with the infant requirement (Table 9, columns 1 and 9) indicates that the intake could not have been far short of the actual requirements, except perhaps for lysine requirement in the younger children.

Table 9. *Mean intakes of essential amino-acids (mg/kg body-weight/day) and of total protein (g/kg body-weight/day) by the children of from $2\frac{1}{2}$ to 3 years and from $5\frac{1}{2}$ to 6 years old on the crèche, test and control diets*

	Crèche		Test		Control		Infant requirements		
Mean weight at mid-period (kg) ...	9·72	15·99	10·97	17·95	10·57	16·36	*	†	‡
Age group (years) ...	$2\frac{1}{2}$–3	$5\frac{1}{2}$–6	$2\frac{1}{2}$–3	$5\frac{1}{2}$–6	$2\frac{1}{2}$–3	$5\frac{1}{2}$–6	1	1	1
Lysine	98	59	118	78	161	111	170	162	131
Threonine	82	50	85	56	112	80	87	104	98
Methionine	41	25	34	23	46	33	85	50	41
Cystine	35	21	28	19	31	23	—	76	48
Tryptophan	32	19	25	18	32	24	30	42	40
Isoleucine	109	66	101	77	147	104	90	170	110
Leucine	185	113	190	131	248	177	425	220	205
Valine	136	83	145	101	171	124	161	198	110
Phenylalanine	119	73	127	87	153	110	169	126	85
Tyrosine	111	68	97	70	127	94	—	118	94
Arginine	146	89	172	118	195	141	—	96	64
Histidine	49	30	52	35	65	46	—	64	44
Total protein (g)	2·42	1·47	2·41	1·65	2·98	2·13	—	2·25	2·25

* The figures in this column are those estimated by Albanese (1950) to be the infant essential amino-acid requirements for normal nitrogen retention and body-weight gain.

† The figures in this column are those calculated by Dean (1953) from the data of Czerny & Keller (1925) on positive nitrogen retention and body-weight gain in infants receiving breast milk as the only food. They represent the approximate essential amino-acid requirements for normal growth by a child of 5 kg body-weight receiving 150 g breast milk/kg body-weight/day. The amino-acid composition of human milk used in this calculation was that of Block & Mitchell (1946–7).

‡ The figures in this column have been calculated by us as those in the preceding column, except that the analytical values for human milk used are those of Soupart *et al.* (1954).

Examination of the figures in Tables 8 and 9 suggests that the limiting factor in growth on the crèche diet was probably not one of essential amino-acids (lysine and threonine being the ones most likely to be deficient) but of caloric intake. It is apparent that with the increase in caloric intake as well as protein intake on the test diet with the consequent increase in intakes of lysine and threonine, the essential amino-acid needs were met, as shown by the greatly accelerated growth. The further increase in calories, protein and essential amino-acids in the control diet did not produce a significant difference in the rate of growth. Consequently the intake of essential amino-acids by the lower age group on the test diet may be taken as a close approximation to the requirements of children in this age group.

The intake of the sulphur acids and tryptophan on the test diet was slightly lower than on the crèche diet. Nevertheless, better growth was obtained on the test diet, indicating that these amino-acids could not have been short. Also the additional intake on the control diet did not produce any better growth than on the test diet. Hence it seems likely that the intake of these three amino-acids on the test diet was close to the actual requirement of children aged $2\frac{1}{2}$–3 years.

In the group aged $5\frac{1}{2}$–6 years, the intake of essential amino-acids of the children on the test diet, when reckoned per kg average body-weight, was much less than that of the younger children. The extra rice portion consumed by the older children did not provide sufficient amino-acids to compensate for the increase in average body-weight. That in general they were not subject to any shortage of essential amino-acids is shown by the fact that the children on the control diet in the same age group, whose intake was larger, did not grow significantly better.

On the data available the figures shown in Table 10 are advanced as approximate essential amino-acid requirements of children on rice diets under tropical conditions in the age groups 1–4 years and 4–6 years. It is obvious that these figures do not pin-point the requirements, but that a sliding scale must exist decreasing from infancy through childhood.

Table 10. *Suggested essential amino-acid requirements of children on rice diets*

Amino-acid	Essential amino-acid requirements (mg/kg body-weight) 1–4 years	4–6 years
Lysine	125	100
Threonine	100	75
Methionine	40 } 75	35 } 60
Cystine	35	25
Tryptophan	30	20
Isoleucine	100	75
Leucine	200	150
Valine	150	100
Phenylalanine	125	100
Tyrosine	125	100
Arginine	100	75
Histidine	50	40
Total mixed protein (g/kg body-weight/day)	2·75	1·75

In addition to the conclusions which we sought to obtain about the indispensabilty or otherwise of animal protein certain other conclusions arise which deserve discussion.

Vitamin A. In several instances the clearing-up of signs of vitamin A deficiency as evidenced by phrynoderma was very refractory, though the children were receiving adequate amounts of vitamin A on the experimental diets (see Table 7). The cause of phrynoderma is disputed. Aykroyd & Rajagopal (1936–7) and Wilson & Widdowson (1942) could find no relationship between the incidence of Bitôt's spots and phrynoderma, nor could Aykroyd & Krishnan (1936–7) find any relationship between vitamin A and carotene intakes and the incidence of phrynoderma. Hume & Krebs (1949) have concluded that the tendency to develop keratinized hair follicles is not specifically related to vitamin A nutrition. It seems likely that the hyperkeratosis observed in these subjects may have had some other cause than vitamin A deficiency.

Thiamine, riboflavin and nicotinic acid. The crèche diet was low in thiamine and riboflavin, but the amounts were appreciably higher in the test diet; the amount of riboflavin was, however, still far short of the official recommendation ((U.S.A.) National Research Council: Food and Nutrition Board, 1948). This suggests that either this standard of riboflavin requirement is much too high or that the analytical figures for the riboflavin content of foodstuffs are underestimated. The low riboflavin content of the unsupplemented test diet suggests that riboflavin deficiency is an inherent deficiency in tropical diets which are lacking in animal protein.

Calcium. Nicholls & Nimalasuriya (1939) have found the intake of calcium in 5-year-old children of the labouring class in Ceylon to be as little as 0·2 g. Low calcium intakes in Ceylonese have also been reported by Bibile, Cullumbine, Watson & Wikremanayake (1949). However deficient such intakes of calcium may appear to be, the fact remains that the majority of children in Ceylon do grow to adult size. To explain this, these authors advanced the hypothesis that calcium requirements are individually and racially adaptable to low calcium intakes. Calcium adaptation, therefore, must be interpreted as the ability of the individual to achieve positive calcium balance on low calcium intakes. That it takes place at the expense of a diminished rate of skeletal growth is borne out by the generally poorer physique of Ceylonese compared with Europeans or Americans. With adequate exposure to sunlight, calcification is evidently complete enough to prevent manifestations of rickets. There seems to be no reason to suppose that such ability is limited to any particular race. Our results show that during the 15-week (pre-experimental) period when the daily calcium content was 130–150 mg the average percentage gain in height per week over the initial height was 0·12. On the test diet the calcium content increased to 330–350 mg inclusive of supplements, and the average percentage gain in height was 0·13. In the control group which received an additional amount of calcium from skim milk the average percentage gain in height was 0·15. As many as four children in this control group grew as much as 3·0 cm each. Although these results are not statistically significant, it appears likely that a further increase in calcium intake up to optimum level (accompanied perhaps by adequate amounts of well-balanced protein) would lead to a further improvement in height and general physique in Ceylonese. In experiments on rats, Sherman (1952) has found that growth and development are expedited, that adult vitality is maintained at a higher level for a longer time and that the lives of normal adults are 10% longer on a higher calcium intake. The most striking observation noticed in our subjects was their increased vitality on transferring to the test diet.

Cost. As a matter of practical importance, it was found that the average cost of the prepared food during the test period was approximately 40 cents (7·2*d.*)/child/day; all food was purchased at unsubsidized current prices.

SUMMARY

1. After a preliminary observation period on a mixed diet containing animal protein, the heights and weights of twenty-three Ceylonese children aged 1–6 years attending a municipal crèche in Colombo were studied after they had been given an entirely vegetable diet or the same diet with a supplement of skim milk.

2. No statistically significant difference was found between the two groups as a whole. However, the children between the ages of $3\frac{1}{2}$ and 6 years on the entirely vegetable diet gained somewhat more in weight but decidedly less in height, not significantly so, however, perhaps in view of the short period of the trial. Moreover,

it is felt on the available evidence that an increase in the calcium intake of Ceylonese children on rice diets would lead to improvement in height and general physique.

3. The rate of increase of growth on a mixed diet containing animal protein but deficient in calories was significantly smaller ($P < 0{\cdot}01$) than on an entirely vegetable diet with higher calorie intake.

4. The essential amino-acid content of the diets has been computed and approximate requirements for growth of children are suggested.

5. It is concluded that a suitable mixture of vegetable proteins can support growth in children.

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The Effect of Injections of Oestradiol Dipropionate into Immature Pullets upon the Manganese Content of the Blood Plasma and of some Tissues

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It is well known that many of the changes taking place as a hen comes into lay can be simulated by the injection of sex hormones into immature pullets. Injections of oestrogen will evoke an increase in oviduct size and changes in the composition of the blood, although the ovaries remain quiescent. The manganese in the egg is found mainly in the yolk, with only small amounts in the albumen and shell (Romanoff & Romanoff, 1949). It was therefore of interest to ascertain the effects of injections of oestrogen into immature pullets upon the amount of manganese in the blood plasma and in some other tissues.

Most hormone experiments are carried out by injecting equal doses of hormone at equal intervals, or by the implantation of a pellet. The gradual absorption of the latter sets free a quantity of hormone which increases rapidly at first and decreases gradually later. The daily oestrogen production of a pullet coming into lay, when plotted against time, would probably give a sigmoid curve; i.e. it would not follow either of the usual ways of administering hormones to immature pullets. The scope of the experiment was therefore extended to compare the effects of a given weight of oestrogen administered as gradually increasing doses with those obtained when the oestrogen was given as a number of equal doses.

EXPERIMENTAL

Twelve pullets, all 11 weeks old and of about the same weight at the beginning of the experiment, were housed in individual all-metal cages and fed on the same mash *ad lib.* so that any differential effect of the oestrogen upon appetite could be measured by the resultant difference in growth rate. The oestrogen used was oestradiol dipropionate, injected in solution in arachis oil into the pectoralis muscles. The experiment