Changes Occurring in the Proteins as a Result of Processing Groundnuts under Selected Industrial Conditions

2. Nutritional Changes

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With increasing production of edible groundnuts, conservation of the nutritive value of the meal gains in importance. Much information is available concerning the optimal conditions for producing meals of good quality from soya beans and cottonseeds but, hitherto, comparable experiments on groundnuts have not been reported. Soya beans and cottonseeds contain only about 20 % of oil and can therefore be efficiently expelled, pressed or solvent-extracted in one operation. This is not so with groundnuts, which contain about 50 % of oil and must therefore be expelled in at least two stages. In each of these a 'cooking' operation is followed by pressing or solvent extraction. Thus groundnut meal is usually subjected to much more severe heat treatment than soya-bean meal or cottonseed meal.

The fundamental issue is the effect of the treatment applied to extract the groundnut oil on the nutritive value of the protein-rich residue. Some heat treatment is beneficial because sulphur-containing amino-acids are made available and bound biotin is set free. Furthermore, it is very probable that a trypsin inhibitor present in the untreated nuts is destroyed by heating. Exposure to higher temperatures (especially in the presence of steam) significantly reduces the nutritive value of groundnut protein. The purpose of the present paper is to give evidence in support of the above statements.

Previous work on the effect of heat on proteins

Seegers & Mattill (1935) and Fixsen (1934-5) reported decided decreases in nutritive value when casein, meat, liver, kidney, heart, cereals and fish meals were heated to high cooking temperatures for a long time. On the other hand, Osborne & Mendel (1917) and Shrewsbury, Vestal & Hauge (1932) found that heated soya beans brought about a definite improvement in the growth of animals previously given a similar diet containing 'raw' soya beans. Both groups of workers showed by experiments on rats that heating increased the palatability and the nitrogen absorption. The true digestibility coefficient was raised by about 4 %. Johnson, Parsons & Steenbock (1939) observed that the amount of nitrogen absorbed by the rats was almost the same from raw and from heated soya beans but the nitrogen retention was higher with heated beans. Only 11 % of sulphur was retained from the raw beans as compared with 25 % from the heated beans. The difference led the authors to conclude that cystine exists in the raw soya beans in a bound form but, after heat treatment, becomes available to the animal.

They also showed that the improvement is best attained with controlled moderate heating and that drastic heat treatment denatures the protein and reduces its nutritive value.

Hayward, Steenbock & Bohstedt (1936) made it clear by both growth and nitrogen-balance methods that the modern 'expeller' process of obtaining oil from seeds much improves the nutritive value of the proteins of soya-bean meal. The digestibility increased by only about 3 %, whereas the increase in the biological value was about 12 %. They agreed that heating made available for absorption and metabolic use some essential protein component, but that overheating could do more harm than good.

Ham, Sandstedt & Mussehl (1945) reported the presence of a heat-labile trypsin inhibitor in raw soya-bean meal, and suggested that its destruction accounted for the increased nutritive value of heated meal. They found that the trypsin inhibitor, when added to food, retarded the growth of chicks. Evans, McGinnis & St John (1947) showed that the autoclaving of raw soya-bean meal at 100–130° for $\frac{1}{2}$ hr. increased the digestibility of the cystine- and methionine-containing proteins for the chick. Similar increases were observed by in vitro proteolysis with trypsin and erepsin.

Information regarding the amino-acids of the groundnut is not complete, but there is considerable evidence of a relative deficiency of the sulphur-containing amino-acids, cystine and methionine (Grau, 1946; Traill, 1950). The arachin fraction (which accounts for 80 % of the total globulin) is markedly deficient in methionine (White & Beach, 1937–8; Baernstein, 1937–8), but the conarachin fraction is much nearer to being a complete protein. Smuts & Marais (1940), from feeding experiments involving supplementation with amino-acids, found that groundnut meal is not deficient in cystine, tryptophan or lysine, but may be deficient in methionine.

In the present study, two methods have been used to determine the efficiency of protein: the growth method of Osborne, Mendel & Ferry (1919) and the nitrogen-balance method of Mitchell (1923–4 a–c) and Mitchell & Carman (1926). The two methods are not necessarily comparable (Fixsen, 1934–5).

A short report of the work has already been published (Lord & Wakelam, 1949; Cama & Morton, 1949).

<div align="center">EXPERIMENTAL AND RESULTS</div>

<div align="center">*Types of meals*</div>

In the present work, four types of groundnut meal obtained in different ways have been studied. They will be referred to throughout as meals A, B, C and D.

Meal A was a reference meal obtained by ether extraction of nuts crushed in the laboratory. It was light coloured and had a raw beany odour.

Meal B was a product of screw-pressing (expelling) in three stages with slight 'cooking'. It was light brown in colour.

Meal C was produced by screw-pressing (expelling) in two stages followed by solvent extraction. The solvent was removed from 2-ton batches by steaming for 30–40 min. at the rate of 2000 lb./hr. steam. The material was then dried over belts. Meal C had been subjected to maximal heat and steam treatment and was dark brown in colour.

Meal D was obtained under the same conditions as meal C except that the solvent

was removed by heating under reduced pressure using minimal quantities of open steam (much less than 100 lb./hr.). This meal had the same light brown colour as meal B.

Lord & Wakelam (1950) have recorded the results of chemical studies on the four meals on which we have carried out nutritional tests.

First growth test

The objects of this test were: (*a*) to see if groundnut meal was qualitatively adequate, i.e. gave rise to no deficiency symptoms; and (*b*) to determine the optimal level of protein for growth in rats given diets in which groundnut meal provided the protein. It should be observed that the criterion for optimal level is not maximal growth but the most efficient utilization of protein consistent with normal growth and the absence of any deficiency symptoms. Protein levels of 9, 14, 20 and 29% were used.

Methods

Experimental diets. The groups of rats on experimental diets containing groundnut meal were compared with groups kept on the stock diet (Table 1) modified by admixture with sufficient maize starch to reduce the level of protein from 22·5 to 14·5%. The stock diet was so diluted to see if at a protein level of 14·5% its good palatability and growth-promoting value could be maintained.

A basal diet in which caseinogen was used to provide a protein level of 9% was compared with a diet in which groundnut meal was used to give the same protein level.

In addition to the main experiments, subsidiary experiments on pairs of rats were carried out to test the effects of supplementing the diets with sulphur-containing amino-acids. These tests had particular relevance to the appearance of the deficiency symptoms to be described later. The composition of the diets employed in this test is shown in Tables 1 and 2. The salt mixture of Osborne & Mendel (1919) was used to prepare the basal diets.

Table 1. *Percentage composition of the stock diet as made specially for rat colonies and pressed into cubes by Lever Bros. and Unilever Ltd.*

Wheat germ	2·8	Coconut-cake meal	16·8
Skim milk powder	3·3	Groundnut-cake meal	5·6
Dried yeast	3·7	Maize	10·3
Fine bran	29·3	Fish meal	5·6
Broad bran	7·5	Dried-blood meal	1·4
Molasses	11·2	Limestone	0·9
		Sodium chloride	0·7
		Bone flour	0·9
Protein (N × 6·25)	22·5		
Oil	5·3		
Moisture	10·8		

Vitamin supplements. Vitamins A and D were supplied as cod-liver oil incorporated in the diet and the following components of the vitamin B complex were dissolved in water and the solution mixed with the solid ration: riboflavin 0·5, aneurin hydrochloride 0·3, pyridoxin 0·3, nicotinic acid 2·0 mg./kg. and choline chloride 2·0 g./kg.

20-2

Table 2. *Percentage composition of diets used in the first growth test*

	Protein level				
	9 %	9 %	14 %	20 %	29 %
Groundnut meal	20·8	—	31·3	47·0	62·7
Casein	—	9·0	—	—	—
Sucrose	10·0	10·0	10·0	10·0	10·0
Salt mixture	3·0	3·0	3·0	3·0	3·0
Sodium chloride	1·0	1·0	1·0	1·0	1·0
Groundnut oil	5·7	9·0	5·0	1·5	—
Cod-liver oil	1·0	1·0	1·0	1·0	1·0
Maize starch	58·5	64·0	48·7	36·5	22·3
Protein	9·4	8·5	13·8	20·1	28·8
Oil	9·1	10·3	10·1	9·3	11·4
Moisture	6·4	7·6	6·7	6·4	6·3

Test animals. Six groups of young albino rats with initial weights between 60 and 80 g. were used; each was divided into subgroups of five males and five females, as nearly equal as possible in weight within a subgroup. All the rats in any subgroup were fed to appetite communally so that, although their individual weights at each stage of the experiment were known, no record of individual consumption of groundnut meal was possible, only the total amount eaten by the subgroup being recorded. The test lasted 28 days.

Results

Protein efficiency. The protein efficiency for any particular diet was measured by the regression coefficient of the weight of the subgroup on the total amount of protein consumed by the subgroup.

If x and y denote respectively the cumulative totals on each day of the experiment of protein eaten and increase in weight from the beginning of the experiment, n denotes the number of observations and b is the regression coefficient, then

$$b = \frac{s(xy) - \left[\dfrac{s(x)\,s(y)}{n}\right]}{s(x^2) - \dfrac{[s(x)]^2}{n}}.$$

It is to numbers calculated in this way that the term regression coefficient is applied throughout. The numerical results are shown in Table 3, together with protein efficiency as indicated by increase in weight over protein eaten. There is very good agreement with regression coefficients.

The following observations summarize the findings:

(1) A diet in which groundnut meal provided a protein level of 9% did not maintain normal growth in rats (Fig. 1) and typical deficiency symptoms were early observed.

(2) Protein efficiency (Table 3 and as seen also from the regression coefficient) was almost the same for the 14 and 20% diets and also for the 'diluted' stock-diet, but it was lower for the 29% diet.

(3) Addition of methionine to the diets containing groundnut meal at protein levels of 9 and 14% very considerably improved the protein efficiency and prevented the

Table 3. *First growth test. Statistical interpretation of growth during 4 weeks in relation to protein eaten by rats receiving diets containing groundnut meal B at different protein levels*

Diet no.	Protein level (%)	No. of rats		Regression coefficient (as defined on p. 300)		Total increase in weight x		Total protein eaten y		Protein efficiency x/y		First appearance of alopecia in male rats (days)	First appearance of 'spectacled-eye' in male rats (days)
		♀	♂	♀	♂	♀ (g.)	♂ (g.)	♀ (g.)	♂ (g.)	♀	♂		
1	9	5	5	0·502	0·342	47	71	116·1	133·9	0·404	0·530	23	25
1*	9	—	2	—	1·24	—	69	—	52·3	—	1·31	—	—
2	9	5	5	1·55	1·21	97	104	85·8	102·3	1·13	1·02	28	32
3 (casein)	14	5	5	0·771	1·07	147	236	193·3	186·7	0·763	1·265	26	26
3†	14	—	2	—	1·31	—	85	—	66·5	—	1·280	Hair slightly sparse round neck and mouth	—
3*	14	—	2	—	1·47	—	140	—	87·6	—	1·600	—	—
4	20	5	5	0·666	0·954	209	367	276·5	350·4	0·757	1·047	—	—
5	29	5	5	0·533	0·757	222	401	387·6	474·2	0·574	0·847	—	—
6 (stock diet diluted with starch)	14·5	5	5	0·803	1·21	240	372	269·4	308·5	0·892	1·205	—	—

• Supplemented with 0·4 % methionine.   † Supplemented with 0·4 % cystine.

appearance of the deficiency syndrome, but addition of cystine improved the protein efficiency of the 14% diet to a less degree than methionine, and deficiency symptoms were observed.
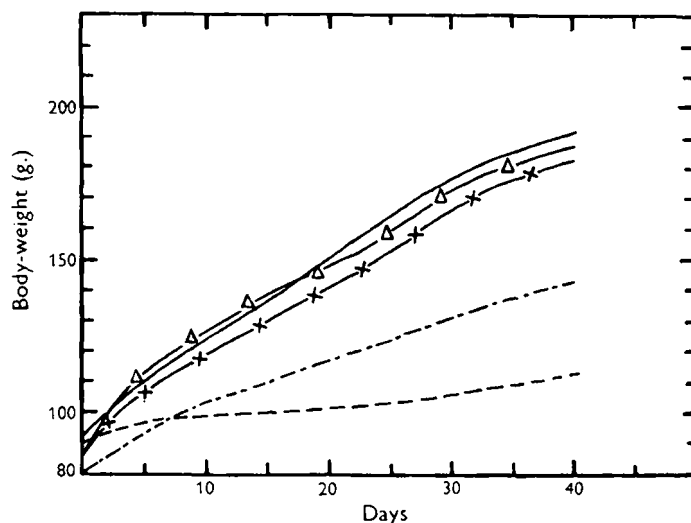

Fig. 1. Growth curve of rats fed on groundnut meal at different protein levels. – – – –, 9% protein level; – · – · –, 14% protein level; ×–×, 20% protein level; △–△, 29% protein level; ————, 'diluted' stock-diet at 14·5% protein level.

(4) Rats given the caseinogen diet at a protein level of 9% showed much better protein efficiency, but the deficiency symptoms (alopecia and 'spectacled-eye') were clearly shown.

(5) When given to provide a protein level of 20%, groundnut meal maintained 'normal' growth in young rats and deficiency symptoms were not seen. At a protein level of 29%, growth was good, but the protein efficiency was considerably reduced.

Deficiency symptoms. When groundnut meal was given to rats at protein levels lower than 20%, alopecia and 'spectacled-eye' developed first in the males and considerably later in the females. These symptoms were most evident with meals subjected to little or no heat treatment. The following signs of nutritional deficiency were noted: lessened activity, awkward gait, humped back, increased shedding of hair, soiled fur, paws and tail, haloes round the eyes and a characteristic sitting posture (Pl. 1).

A striking feature, observed in male rats only, was an early deep-yellow pigmentation of the epidermis, followed by alopecia and 'spectacled-eye'. The rats sat in a humped, kangaroo-like position. At a later stage, these rats were seen to eat each other's hair.

The above symptoms are typical of deficiency of biotin and inositol (Rosenberg, 1942) and were also recorded by Parsons (1931) as a result of diets rich in egg-white.

Nielsen & Elvehjem (1941) and Spitzer & Phillips (1946) observed alopecia and 'spectacled-eye' in rats on diets containing soya-bean meal. The syndrome was prevented by supplements of biotin and/or inositol. Interesting facts noted by these workers, who used soya-bean meals, and confirmed by us with groundnut meals are that

for the rat there is an optimal level of biotin intake (1–4 μg./rat/day) and that large doses tend to produce alopecia and 'spectacled-eye', perhaps due to hypervitaminosis. The syndrome did not develop when the soya-bean meal diets were supplemented with either cystine or methionine.

In our experiments, all animals in the deficient groups were later transferred to the stock diet supplemented with Marmite at a 2% level. The animals not only recovered from the deficiency state, but also caught up in weight with the control group maintained on the stock diet, as may be seen from Fig. 2. No permanent damage appeared to have been done. After complete cure, the animals given meals A, B, C and D at 12% protein level were allowed to breed. The litters were normal and healthy, and there were no symptoms of alopecia or 'spectacled-eye'. Rats of the third generation were also quite normal.
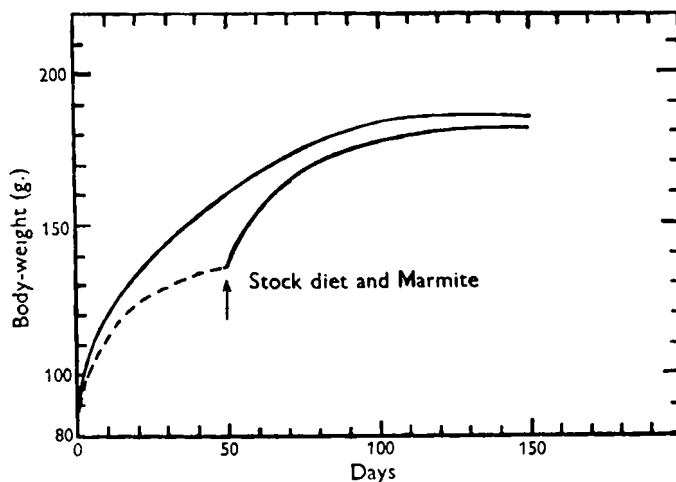

Fig. 2. Effect of Marmite and stock diet on rats given a groundnut-meal diet. – – – –, groundnut meal at 12 % protein level; ————, stock diet at 22·5 % protein level.

Biotin-avidin antagonism. Biotin deficiency symptoms may be due to unavailability of biotin bound with protein (e.g. avidin). In order to distinguish between vitamin deficiency and avidin-like inhibition, an experiment was carried out with the diets containing groundnut meal to provide protein levels of 9, 12 and 15%. The alopecia-'spectacled-eye' syndrome appeared first on the 9% protein diet, later on the 12% diet and (up to 4 weeks) not at all on the 15% diet. Methionine supplements gave protection at the three protein levels. These experiments demonstrate genuine deficiency here as more important than any possible antagonism.

Second growth test

The work so far described fixed the optimal protein level for groundnut meal near 20% and made it possible to undertake a comparative test of the four meals.

Methods

Diets. The diets (Table 4) were planned to compare four types of groundnut meal. Vitamin supplements were given as in the first growth test (p. 299).

Animals. Four groups of albino rats (five males and five females in each) were used. As before, all the rats in any subgroup were fed to appetite communally so that the total, rather than individual, consumption of meal was known.

Results

The numerical results are shown in Table 5. Regression coefficients were calculated as in the first growth test.

The test showed that various oil-extraction processes exert a considerable effect on the nutritive value of the protein of the residual groundnut meal.

The regression coefficients were highest for meals B and D which had undergone controlled heat and steam treatment. More drastic treatment (meal C), though assisting extraction of oil, had a very deleterious effect upon the nutritive value of the protein. Meal A, which had not been subjected to any heat treatment, was, however, definitely inferior to meals B and D, but meal C was inferior even to meal A. Table 5 shows that the four meals came out in the same order in respect of nutritive value for male and female rats.

The present investigation would be incomplete if growth-tests only were used. Accordingly, nitrogen-balance experiments were done.

First metabolic test

Methods

General. The technique first proposed by Thomas (1909), and later developed and adapted to growing rats by Mitchell (1923–4 *a–c*) and Mitchell & Carman (1924), was used. The procedure is considered to give the most satisfactory picture of protein utilization by the body.

It has long been realized that protein requirements for growth and maintenance are different, and that the lower the protein concentration, the higher is the biological utilization of absorbed nitrogen.

Diets. In the experiments described below, meal A was used first. The protein levels were 9, 16 and 22% in the experimental diets, and a nitrogen-free diet was used to estimate the endogenous urinary and faecal nitrogen for each rat. The diets are shown in Table 6.

Animals. A group of six albino rats (three males and three females), weighing from 80 to 100 g. and about 3 months old, was selected. The rats were fed on the nitrogen-free diet for 7 days, and urine and faeces were collected from the 4th to the 7th day of the experimental period, both days inclusive. To prevent decomposition of urine, 20 ml. of 5% sulphuric acid containing 5% phenol and 1% thymol were placed in the collecting flask. The metabolic cage, funnel, ball separator and flask (Pl. 2) were washed down daily with small quantities of distilled water to collect all the urine. The faeces collected over the same period were dried in an air oven to constant weight after moistening with a few drops of 5% oxalic acid, and the entire sample was digested. In order to reduce sampling error, three portions were analysed for total nitrogen.

Cages. Our metabolism cage is similar to the Hopkins cage (Ackroyd & Hopkins, 1916) with the difference that the food container is directly attached to the cage and the

Table 4. *Percentage composition of diets used in the second growth test*

	Type of meal			
	A	B	C	D
Groundnut meal	47·7	40·5	39·5	37·5
Sucrose	10·0	10·0	10·0	10·0
Salt mixture	3·0	3·0	3·0	3·0
Sodium chloride	1·0	1·0	1·0	1·0
Groundnut oil	2·3	5·0	7·5	8·5
Cod-liver oil	1·0	1·0	1·0	1·0
Maize starch	35·0	39·5	38·0	39·0
Protein	19·0	20·5	20·0	19·4
Oil	10·1	9·9	9·5	9·7
Moisture	6·7	5·9	5·3	5·5

Table 5. *Second growth test. Statistical interpretation of growth during 4 weeks in relation to protein eaten by rats receiving diets containing 20% protein derived from groundnut meal processed in four different ways*

Type of meal	No. of rats		Regression coefficient (as defined on p. 300)				Total increase in weight *x*		Total protein eaten *y*		Protein efficiency *x/y*		First appearance of alopecia in male rats (days)
			Value		Standard error								
	♀	♂	♀	♂	♀	♂	♀ (g.)	♂ (g.)	♀ (g.)	♂ (g.)	♀	♂	
A	5	5	0·841	0·869	0·012	0·017	221	225	267·7	266·0	0·828	0·842	Depigmentation of fur observed after 32 days on diet
B	5	5	0·865	0·913	0·010	0·012	204	253	244·8	270·3	0·824	0·937	No symptoms
C	5	5	0·827	0·867	0·013	0·020	176	196	230·0	240·0	0·767	0·816	No symptoms
D	5	5	0·911	1·071	0·015	0·017	234	255	250·0	245·0	0·936	1·041	No symptoms

Table 6. *Percentage composition of diets used in the first metabolic test*

| | Diets with groundnut meal A Protein level | | | |
	9 %	16 %	22 %	Nitrogen-free diet
Groundnut meal	24·2	34·4	53·2	—
Sucrose	10·0	10·0	10·0	10·0
Salt mixture	3·0	3·0	3·0	3·0
Sodium chloride	1·0	1·0	1·0	1·0
Groundnut oil	2·6	4·6	0·4	10·0
Cod-liver oil	1·0	1·0	1·0	1·0
Maize starch	58·2	46·0	31·4	75·0
Protein	8·9	16·3	21·8	—
Oil	10·0	9·5	9·6	11·5
Moisture	7·4	6·8	6·2	6·6

rat has not to find its way to the feeding pot through a narrow conical tube. This arrangement avoids inconvenience to the rat in turning and a possible loss of excreta if the rat remains in the tube instead of in the cage.

All the nitrogen determinations were made by the method of the Association of Official Agricultural Chemists (1930).

At the end of the period on the nitrogen-free diet, the three experimental diets were tested, following exactly the same procedure as for the nitrogen-free diet. In our experiments, the treatments were given in the same order for all the six rats. Macrae, Henry & Kon (1943) using twelve rats preferred to employ the Latin Square design in which the order is varied from rat to rat. This has statistical advantage. Other recent workers (Goyco & Asenjo, 1947) have used the more convenient simple design.

Results

The results are shown in Table 7.

Table 7. *First metabolic test. Biological values and true digestibility coefficients of groundnut meal A at three protein levels*

Protein level (%)	Biological value (%)	True digestibility coefficient (%)
9	46·9	86·1
16	40·9	88·5
22	28·7*	88·9
Error (i.e. standard deviation of mean of six from analysis of variance)	1·65	0·53

* Five rats only.

Second metabolic test

The object of this test was to compare meals A, B and C in respect of their biological values and true digestibility coefficients.

Methods

Diets. Three diets were prepared containing the groundnut meals A, B and C respectively, at a 9 % protein level. In the preceding test the rats did not eat enough of the nitrogen-free diet and lost weight. To correct this defect, a ration was used in this and in the following (third) metabolic tests containing dried, defatted whole egg, to supply about 4 % of crude protein (Mitchell & Carman, 1926). The nitrogenous material of dried whole egg is apparently completely digestible and at this low concentration the protein is probably completely utilized in metabolism.

Another change in the diets for this and the third metabolic tests was the addition of roughage to the low egg-nitrogen ration. For this purpose, the almost nitrogen-free commercial product Cellu-flour (obtained from British Celanese Ltd.) was used in amount approximately equivalent to the roughage content of groundnut meal. The composition of the diets used is given in Table 8.

Procedure. The standardizing diet was fed at the beginning and end of the experiment, and the general procedure of the first metabolic test was followed except that the collecting period was increased from 4 to 5 days.

Table 8. *Percentage composition of diets used in the second metabolic test*

	Diets with groundnut meal Type of meal			
	A	B	C	Standardizing diet
Groundnut meal	21·0	18·0	17·0	—
Sucrose	10·0	10·0	10·0	10·0
Salt mixture	3·0	3·0	3·0	3·0
Sodium chloride	1·0	1·0	1·0	1·0
Groundnut oil	6·0	7·0	8·0	9·0
Cod-liver oil	1·0	1·0	1·0	1·0
Maize starch	58·0	60·0	60·0	66·8
Dried, defatted whole egg	—	—	—	6·2
Cellu-flour	—	—	—	3·0
Protein	9·0	8·7	8·9	4·1
Oil	10·7	9·7	10·5	10·4
Moisture	6·7	6·9	6·8	7·5

Results

The results are shown in Table 9.

Table 9. *Second metabolic test. Biological values and true digestibility coefficients of differently processed groundnut meals*

Type of meal	Protein level (%)	Biological value (%)	True digestibility coefficient (%)
A	9	43·3	90·2
B	9	47·8	93·4
C	9	42·8	93·0
Error (i.e. standard deviation of mean of six from analysis of variance)		1·64	0·46

Third metabolic test

The object of this test was to check the effect of heat and steam treatment on meals B, C and D and to compare the biological value and true digestibility coefficient of processed groundnut meal with that of caseinogen. The enhancement of the biological value and true digestibility coefficient by supplementation of the unheated meal A with caseinogen was also tested.

Methods

Diets. Basal diets were prepared containing meals B, C and D at a protein level of 9%. A basal diet containing caseinogen at the same protein level was also prepared. Finally, a basal diet containing two parts of meal A to one part of caseinogen was prepared, also at 9% protein level. The compositions of these diets and of the standardizing diet are given in Table 10.

Table 10. *Percentage composition of diets used in the third metabolic test*

	Diets with groundnut meals Type of meal			Caseinogen diet	Diet with caseinogen and meal A	Standard-izing diet
	B	C	D			
Groundnut meal	18·2	17·8	16·8	—	14·4	—
Sucrose	10·0	10·0	10·0	10·0	10·0	10·0
Salt mixture	3·0	3·0	3·0	3·0	3·0	3·0
Sodium chloride	1·0	1·0	1·0	1·0	1·0	1·0
Groundnut oil	6·7	8·3	8·9	9·0	7·1	8·8
Cod-liver oil	1·0	1·0	1·0	1·0	1·0	1·0
Maize starch	60·1	58·9	59·3	64·0	59·3	67·5
Dried, defatted whole egg	—	—	—	—	—	5·7
Cellu-flour	—	—	—	3·0	—	3·0
Caseinogen*	—	—	—	9·0	4·2	—
Protein	9·7	10·3	9·5	8·5	9·1	3·8
Oil	10·5	10·9	9·7	10·3	10·1	10·6
Moisture	7·2	6·8	7·6	7·6	7·04	7·2

● 'Ashless' Extracted Casein obtained from Glaxo Laboratories Ltd.

Procedure. The standardizing diet was given as before at the beginning and at the end of the experiment, but the six rats were from the same litter, and the collecting period was increased from 5 to 7 days.

Results

The results are shown in detail in Table 11.

Statistical interpretation of nitrogen-balance experiments

The findings were examined by analysis of variance and by the *t* test of 'Student' (1908, 1925) and the results are given below. The following notation was adopted:

S*   = significant between 5 and 10% levels.
S**   = significant between 1 and 5% levels.
S*** = significant at 1% level.
N.S. = not significant.

Table 11. *Biological values and digestibility coefficients of meals B, C and D, of caseinogen, and of a 2 : 1 mixture of meal A and caseinogen. Daily nitrogen intakes, urinary nitrogen excretions and faecal nitrogen excretions. (Average on 7 days)*

Rat no.	Body-weight Initial (g.)	Body-weight Final (g.)	Food intake (g.)	Nitrogen intake (mg.)	Faecal nitrogen (mg.)	Urinary nitrogen (mg.)	Biological value For individual animal (%)	Biological value Mean for group (%)	Digestibility coefficient For individual animal (%)	Digestibility coefficient Mean for group (%)
Low egg-nitrogen ration, nitrogen content 0·700 % (first standardizing period)
1	101	105	9·990	—	11·3	28·7	—	—	....	—
2	100	103	9·870	—	14·2	26·4	—	—	—	—
3	100	104	9·817	—	12·5	25·6	—	—	—.	. . —
4	124	126	9·980	—	12·2	32·8	—·	—	—	—
5	130	134	10·000	—	10·9	32·0	—	—	. .	—
6	130	132	9·968	—	12·5	35·9	—	—	- ·	.—
Caseinogen, nitrogen content 1·458 %
1	105	117	9·987	145·6	15·7	76·0	67·5		96·8	
2	103	113	9·975	145·4	14·9	84·0	61·0		99·3	
3	104	116	9·830	143·3	14·1	86·0	58·5	62·6	98·8	98·6
4	126	134	9·883	144·0	14·1	91·2	59·4		98·7	
5	134	142	9·979	145·5	13·5	84·5	63·6		98·3	
6	132	144	9·957	145·2	12·6	86·0	65·7		99·9	
Meal B, nitrogen content 1·542 %
1	117	120	9·957	153·5	24·3	117·0	39·0		91·3	
2	113	119	9·981	153·9	23·9	112·5	42·0		93·3	
3	116	120	9·921	152·9	23·9	112·0	41·3	41·0	92·4	91·9
4	134	140	9·982	153·9	24·8	113·7	44·2		92·0	
5	142	148	9·992	154·0	24·8	116·2	40·8		91·1	
6	144	148	9·330	143·8	24·5	117·0	38·7		91·2	
Meal D, nitrogen content 1·570 %
1	120	126	9·887	155·2	22·6	116·8	41·0		92·3	
2	120	124	9·828	154·3	23·3	115·2	41·0		93·3	
3	121	125	9·907	155·5	23·9	118·7	38·2	43·0	92·5	92·7
4	140	147	9·994	156·9	24·6	113·8	46·0		92·5	
5	148	155	10·000	157·0	22·2	115·2	44·0		93·0	
6	148	152	9·920	155·7	24·5	112·0	48·1		92·4	
Meal C, nitrogen content 1·570 %
1	126	136	9·798	153·8	24·2	118·8	39·4		91·0	
2	124	130	9·508	149·2	22·0	119·0	36·8		93·5	
3	125	138	9·727	152·7	21·8	116·0	40·8	39·4	93·5	92·3
4	147	152	9·966	156·4	25·6	120·2	41·6		91·9	
5	155	166	9·956	156·3	22·1	122·0	39·7		93·2	
6	152	160	9·627	151·1	26·0	122·0	38·2		91·0	
Mixture (2 : 1) of meal A and caseinogen, nitrogen content 1·541 %
1	136	144	9·959	153·4	24·0	107·2	48·6		91·2	
2	130	142	9·893	152·4	25·3	100·2	51·4		91·6	
3	138	146	9·794	150·9	23·1	98·0	54·0	52·8	92·5	91·8
4	152	164	9·930	153·0	23·6	103·0	54·2		93·2	
5	166	172	9·987	153·9	24·7	105·0	50·2		91·5	
6	160	170	9·821	151·3	26·9	94·0	58·7		90·7	
Low egg-nitrogen ration, nitrogen content 0·700 % (second standardizing period)
1	144	148	9·992	—	10·4	36·1	—	—	—.	. -
2	142	146	9·915	—	12·2	33·7	—	—	—	- ·
3	146	152	9·970	—	12·0	35·1	—	—	—	- ·
4	164	166	9·949	—	13·5	38·7	···—	—	.. —	- ·
5	172	178	9·922	—	11·8	35·1	—	—	....	—
6	170	176	9·845	—	13·0	35·3	—	—	—	—.

First metabolic test

Analysis of variance of effect of concentration of protein in the diet on the biological value

Source of variation	Degrees of freedom	Variance	Variance ratio
a Between diets	2	467·52	$a/c = 28·75$ (S***)
b Between rats	5	38·01	$b/c = 2·33$ (N.S.)
c Error	9	16·26	—
d Total	16	—	—

Analysis of variance of effect of concentration of protein in the diet on the true digestibility coefficient

Source of variation	Degrees of freedom	Variance	Variance ratio
a Between diets	2	15·54	$a/c = 9·141$ (S***)
b Between rats	5	5·25	$b/c = 3·088$ (S*)
c Error	9	1·70	—
d Total	16	4·54	—

One rat escaped from the metabolic cage during the test at 22 % protein level, and in consequence the biological value and the true digestibility coefficient were obtained on

Table 12. *Net protein values of foods quoted from literature and obtained in present experiments*

Food product	Protein level (%)	Crude-protein content (%)	Biological value (%)	Digestibility coefficient (%)	Net protein value (%)	Workers
Raw soya flour	10·3	42·5	59·4	84·8	21·4	
Partly exploded soya flour	10·3	42·0	75·2	95·6	30·2	
Fully exploded soya flour	10·0	41·8	71·2	93·4	27·8	Mitchell, Hamilton & Beadles (1945)
Coconut meal	10·0	20·7	70·7	86·1	12·6	
Sunflower-seed meal	10·0	55·4	64·5	94·3	33·7	
Dried food yeast	8·0	49·2	48·8	88·3	21·2	Goyco & Asenjo
Dried brewer's yeast	8·0	50·8	69·3	85·5	30·1	(1947)
Caseinogen	8·0	—	68·0	—	—	Kon (1928)
	8·0	—	66·0	—	—	Morgan (1931)
	10·0	—	65·0	—	—	Hughes & Hauge (1945)
						Present study
Meal A	9·0	45·6	47·0	86·1	18·4	
	16·0	45·6	40·9	88·5	16·5	First metabolic test
	22·0	45·6	28·7	88·9	11·6	
	9·0	45·6	43·3	90·2	17·8	
Meal B	9·0	48·7	47·8	93·4	21·8	Second metabolic test
Meal C	9·0	50·0	42·8	93·0	19·9	
Caseinogen	9·0	90·0	62·6	98·6	55·6	
Meal B	9·0	48·7	41·0	91·9	18·4	
Meal C	9·0	50·0	39·4	92·3	18·2	Third metabolic test
Meal D	9·0	53·7	43·1	92·7	21·5	
Mixture (2:1) of meal A and caseinogen	9·0	60·0	52·8	91·8	29·1	

five rats only. In order to complete the analysis of variance, the missing value was 'estimated' from the formula of Yates (1933) (cf. Snedecor, 1946):

$$x = \frac{tT + bB - S}{(t-1)(b-1)},$$

where t = number of treatments, b = number of blocks, T = sum of items with the same treatment as the missing item, B = sum of items in the same block as the missing item, S = sum of all observed items.

Second metabolic test

Analysis of variance of the biological values of groundnut meals A, B and C

Source of variation	Degrees of freedom	Variance	Variance ratio
a Between diets	2	45·738	$a/c = 2·81$ (N.S.)
b Between rats	5	29·440	$b/c = 1·81$ (N.S.)
c Error	10	16·281	—
d Total	17	—	—

Analysis of variance of the true digestibility coefficients of groundnut meals A, B and C

Source of variation	Degrees of freedom	Variance	Variance ratio
a Between diets	2	18·10	$a/c = 14·25$ (S***)
b Between rats	5	2·09	$b/c = 1·64$ (N.S.)
c Error	10	1·27	—
d Total	17	—	—

Comparison of meals by the t test of 'Student' (1908, 1925)

Difference tested	Meals compared		
	A and B	A and C	B and C
Biological value:			
Mean difference	4·5	0·5	5·0
Significance	S***	N.S.	N.S.
True digestibility coefficient:			
Mean difference	3·2	2·8	0·4
Significance	S***	S**	N.S.

Third metabolic test

Analysis of variance of the biological values of groundnut meals B, C and D

Source of variation	Degrees of freedom	Variance	Variance ratio
a Between diets	2	19·75	$a/c = 3·05$ (S*)
b Between rats	5	7·56	$b/c = 1·17$ (N.S.)
c Error	10	6·47	—
d Total	17	—	—

Analysis of variance of the true digestibility coefficients of meals B, C and D

Source of variation	Degrees of freedom	Variance	Variance ratio
a Between diets	2	0·93	$a/c = 2·51$ (N.S.)
b Between rats	5	1·54	$b/c = 4·16$ (S**)
c Error	10	0·37	—
d Total	17	—	—

Comparison of meals by the t test of 'Student' (1908, 1925)

	Meals compared			
Difference tested	C and D	B and C	B and D	D and 2:1 mixture of A and caseinogen
Biological value:				
Mean difference	3·6	1·6	2·1	—
Significance	S*	N.S.	N.S.	—
True digestibility coefficient:				
Mean difference	—	—	—	0·9
Significance	—	—	—	S*

From the analysis of variance in biological value in the second and third tests, it is evident that the 'between-rats' variance is not significant even at the 10% level. In the third test, in which the rats were litter-mates, the 'between rats' variance was especially

Second metabolic test

$$29·440 = 3s_r^2 + s^2$$
$$16·281 = \qquad s^2$$
$$\overline{13·16\ \ = 3s_r^2}$$
$$4·39\ \ = s_r^2$$

Third metabolic test

$$7·56 = 3s_r^2 + s^2$$
$$6·47 = \qquad s^2$$
$$\overline{1·09 = 3s_r^2}$$
$$0·36 = s_r^2$$

small. An analysis of the 'components of variance' following Snedecor (1946, p. 260) shows clearly that it is advantageous to use rats from the same litter.

DISCUSSION

In the commercial extraction of groundnuts, heat treatment leads to a high yield of oil. The heat may be applied for the purpose of liberating oil or for recovery of solvent, but in either case there is risk of damage to protein. This can be reduced by restricting exposure to heat and moisture to a desirable minimum which can only be found by experience. There is no doubt that moderate heating of groundnuts has a genuinely beneficial effect on the nutritive value of the residual meal. We shall deal with this point first.

Improvement in nutritive value brought about by controlled heating. It is known that groundnut meal obtained by extraction with solvents of low boiling-point (e.g. light petroleum) contains a trypsin inhibitor (Borchers, Ackerson & Kimmett, 1947; Borchers, Ackerson, Sandstedt & Kimmett, 1947) which can be concentrated (Lord & Wakelam, 1950). The deficiency syndrome recorded earlier in this paper is consistent with poor availability of biotin and of sulphur-containing amino-acids. Meal A, which had undergone little or no heat treatment, was inferior in promoting growth and for utilization of absorbed nitrogen. Its low true digestibility coefficient in the second metabolic test is consistent with the presence of a possible trypsin inhibitor. In the third metabolic test, where meal A was supplemented with caseinogen, the true digestibility coefficients were: caseinogen 98·6, meal D 92·7, meal A with one-third replaced by the protein equivalent of caseinogen, 91·8. If the true digestibility coefficients of meals A and D had been identical, the caseinogen-reinforced meal ought to have had a coefficient greater than 92·7 but less than 98·6. In fact, it was below 92·7,

and the finding, statistically significant between 5 and 10% levels, can only mean inhibition.

Meals B and D had undergone less heat treatment than meal C. As compared with meal A, meals B and D were slow to produce the deficiency syndrome and were thus qualitatively superior. Controlled heating made more readily available the biotin and the sulphur-containing amino-acids.

The second metabolic test permits a comparison of meals A and B, and the results show that the latter was significantly superior to the former. The third metabolic test shows that reinforcement of meal A with caseinogen failed to improve it to the expected extent as compared with meals B and D. This confirms the finding based on the growth test that meals B and D were better than meal A (see Table 5). The beneficial effect of moderate heat is thus fully established.

Adverse effect of excessive heating. Meal C had undergone considerable heat treatment and exposure to open steam. It did not readily produce the deficiency syndrome, and from that point of view was better than meal A and not easily distinguishable from meals B and D. Judged by the true digestibility coefficient (Tables 9 and 11) meal C had lost the possible trypsin inhibitor. By the criterion of biological value, meal C was inferior to meals B and D. In the comparison of meals C and D, in the third metabolic test, this inferiority was significant between the 5 and 10% levels. The results of the growth tests were confirmatory, since the regression coefficients show that meal C was significantly the worst at 1% level for both males and females. In the extraction of oil from groundnuts excessive exposure to heat or steam must therefore be avoided lest the protein should be seriously damaged.

Protein level as a variable in the first metabolic test. The results of the first metabolic test show that the lowering of the biological value with increase in protein level is very highly statistically significant. This decrease shows that the greater the influx of absorbed amino-acids, the less readily are they utilized as such, i.e. with increasing concentration of protein in the diet, a larger proportion of the absorbed amino-acids undergoes oxidative deamination.

The net protein value (Mitchell & Carman, 1924) (Table 12) also decreases with increase in protein level, but on the other hand there is a statistically significant increase in the true digestibility coefficient with increase in protein level (Table 7).

General considerations. It cannot be expected that in an investigation comparing one type of groundnut meal with another, the differences will be very obvious. The proteins under study are all groundnut proteins, although they may be modified. As will be seen from Table 12, the biological values observed at a 9% protein level fell between 39·4 and 47·8%; and as compared with the proteins listed at the head of Table 12, the mixture of proteins in groundnut meal was always nutritionally defective. The major cause was presumably the known deficit of sulphur-containing amino-acids (Grau, 1946; Traill, 1950). This can be accentuated by excessive heating and steaming.

The deficiency symptoms were essentially due to the unavailability of sulphur-containing amino-acids because supplements of methionine and cystine were effective in the first growth test (Table 3). This is in accord with the findings of White & Beach (1937–8) and Baernstein (1937–8). Meal A, even at a protein level of 20%, gave rise to

N IV 4   21

the deficiency syndrome in an incipient form, and this observation supports the view that biotin-protein complexes are contributory causes (cf. Chu, 1948). The syndrome might also be due to the presence of a possible trypsin inhibitor.

Lightbody & Lewis (1929 a, b) presented evidence that the protein content of the diet affects the growth of hair at levels of protein intake which also cause stunted growth. A relationship between hair growth and the sex glands has been suggested by Bengtson (1931). Furthermore, Salmon (1947) emphasized that casein at a protein level lower than 18% was deficient in labile-methyl groups, thus giving rise in rats to deficiency of cystine and methionine. From all these facts it is clear that the deficiency symptoms shown markedly by male rats in the present study were due to insufficient methionine in a low-protein diet. It is significant that these rats at a later stage were seen to eat each other's hair, but only after alopecia had appeared. Supplementation of groundnut meal with caseinogen considerably enhanced the nutritive value. Similar complementary relationships between the proteins of dairy products and those of bread and potato have been demonstrated (Henry & Kon, 1946). Recently, Henry & Kon (1949) observed an interesting example of supplementation of soya-flour protein by the proteins of plain and milk bread. There is convincing evidence (Evans *et al.* 1947) that soya beans contain a trypsin inhibitor, and very strong evidence (Borchers *et al.* 1947; Borchers & Ackerson, 1950; Lord & Wakelam, 1950) that such an inhibitor is also present in groundnuts. Its destruction is no doubt responsible in part for the beneficial effect of heating.

It is highly desirable that further studies on this problem include sulphur-balance tests analogous to the nitrogen-balance tests (cf. the work of Johnson *et al.* (1939) with soya-bean meals).

The biological tests recorded in the present paper fit in well with the analytical findings of Lord & Wakelam (1950), and also with similar work on soya-bean meals already cited. The increase in growth effect, in biological value and in the digestibility of the cystine- and methionine-containing proteins is probably connected with heat denaturation and its effect on the —SH and —S—S— groups. It has thus been suggested that the opening up of the protein molecule accounts for the appearance of active groups on denaturation. There is thus an increase in —SH groups at the expense of —S—S— groups (Neurath, Greenstein, Putnam & Erickson, 1944).

SUMMARY

1. Groundnut meal given at a 9% protein level failed to maintain 'normal' growth in rats, and typical deficiency symptoms were observed which could be prevented by addition of methionine and biotin. Similar symptoms were observed in animals given casein at the 9% protein level.

2. Rats grew normally when properly processed groundnut meal was given at a 20% protein level, and deficiency symptoms did not appear.

3. Controlled heat and steam treatment enhanced the nutritive value of groundnut meals in comparison with unheated or overheated meals.

4. The lower nutritive value of unheated groundnut meal was mainly due to the un-

Plate 1
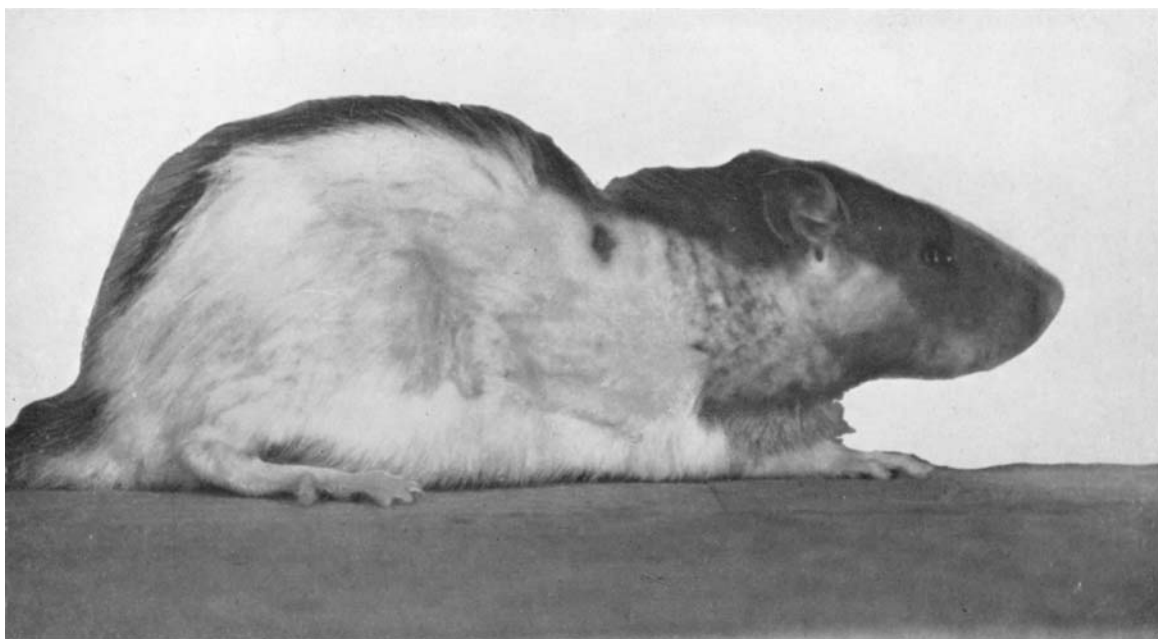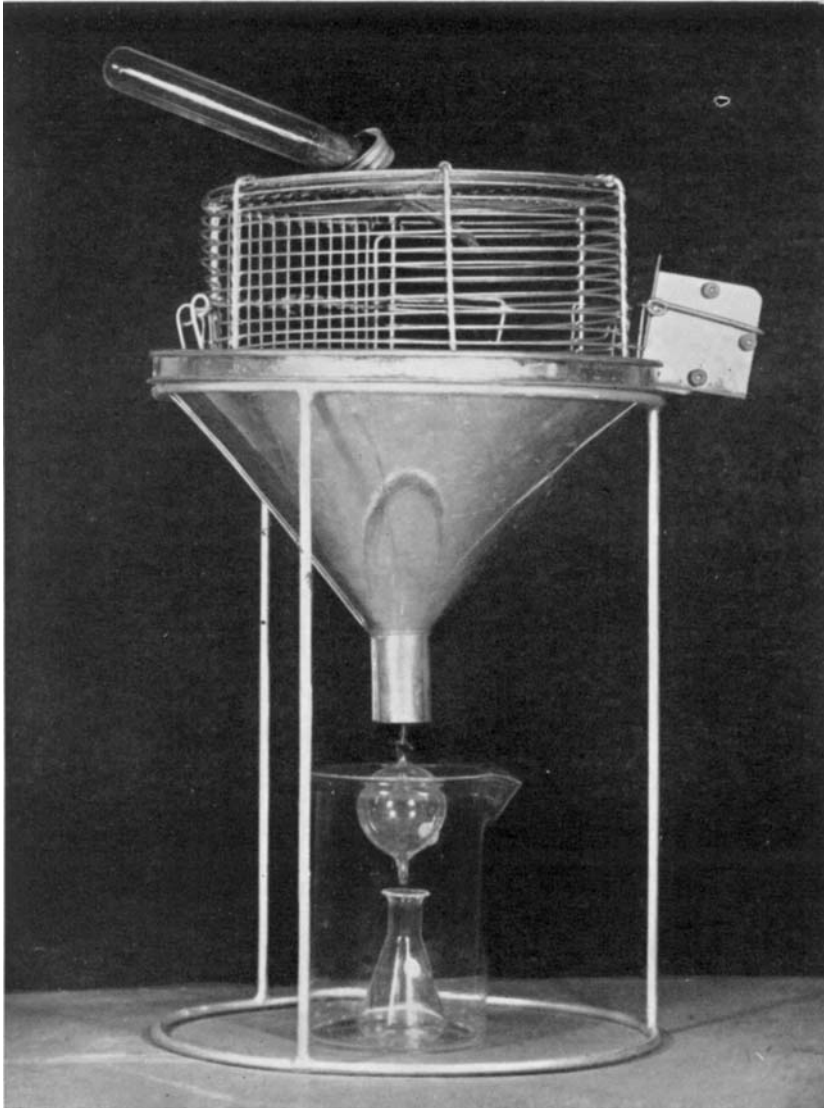
availability of sulphur-containing amino-acids and biotin, but also in part to the presence of a trypsin inhibitor.

5. The biological values of the groundnut meals tested were between 39·4 and 47·8% at a 9% protein level in comparison with 63% for caseinogen tested at the same level. There was considerable enhancement of the nutritive value when groundnut meal was supplemented with caseinogen.

EXPLANATION OF PLATES

PLATE 1

Photograph illustrating signs of deficiency on diets containing less than 20 % protein derived from groundnut meal (see p. 314).

PLATE 2

Metabolic cage used in nitrogen-balance experiments.

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The Energy Metabolism of Man During Overfeeding

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Studies on man show that a considerable retention of nitrogen occurs when healthy human subjects on adequate diets receive additional carbohydrate (Cuthbertson & Munro, 1937; Cuthbertson, McGirr & Munro, 1937; Basu & Basak, 1939), or additional fat (Cuthbertson & Munro, 1937), or a supplement consisting of protein, fat and carbohydrate (Cuthbertson, McCutcheon & Munro, 1937 a, b). Retention of nitrogen has also been observed in other species when they receive surfeit carbohydrate (Hoppe, 1856; Voit, 1869 b; Larson & Chaikoff, 1937; Lathe & Peters, 1949), or surfeit fat (Voit, 1869 a). The common factor in these experiments appears to be the excessive intake of energy, and it is therefore of some theoretical interest to know whether nitrogen retention due to surfeit feeding is accompanied by an increase in basal energy metabolism.

Treichler & Mitchell (1941) and Mukherjee & Mitchell (1949) have established that an increase in the total food intake of the adult rat leads to a significant rise in basal metabolic rate, but the evidence for man is less satisfactory. As Krauss & Küppers (1931) point out in a review of this field, most of the experiments involving overfeeding have been made on previously undernourished subjects. There are, however, three experiments in the literature carried out on healthy adults. Müller (1911) gave a student a diet rather low in protein and energy content (39 Cal./kg. body-weight) and then raised the subject's energy intake to 2750–3000 Cal., chiefly through a very considerable increase in his protein intake. During the 28 days of this surfeit diet 210 g.