STUDIES ON 'BALANCED FOOD'

Part II. The Quality of the Proteins of the Food

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Summary

The biological value of the mixed proteins of the 'balanced food' processed in these laboratories was assessed by four different methods. The data clearly indicate that the proteins are quite adequate for growth, maintenance of nitrogen balance, formation of hæmoglobin and regeneration of liver proteins.

The significance of the observations have been discussed.

In a recent communication,[1] the method of processing a cheap 'balanced food' was outlined. The food is meant primarily to meet the requirements of proteins, particularly to the class of population consuming a poor diet. A mixture of oilcake proteins has been employed so as to supply all the essential amino acids.

It was considered desirable to assess the quality of the proteins by some of the well-known methods. The data collected are reported and discussed in this paper.

The evaluation of the protein quality was made by the following four methods: (1) protein efficiency ratio by the growth method of Osborne, Mendel and Ferry[2]; (2) biological value by the nitrogen balance technique;[3] (3) hæmopoietic value[4]; and (4) liver protein regeneration.[5]

Experimental and Results

Protein efficiency ratio.—Two groups of young albino rats of six in each group, four-week old and weighing 40-50 gm., were employed for the study. The distribution of the rats into the two groups was made according to the established conventions with due consideration to the weight, sex and littermate. The rats were housed in independent cages. One group was given casein as the dietary protein at 10% level and these animals served as the controls. The other group received the 'balanced food' at a level which supplied the same amount of protein as the control group. During the experimental period of 8 weeks, weekly growth record of the individual animals was maintained. All the rats were given weighed

quantities of the food* and the unconsumed diet was collected separately so as to obtain the actual food intake. From these data, the protein consumed by each rat and the protein efficiency ratio were calculated. The results are presented in Table I.

TABLE I

Biological value of the balanced food by the growth method

Sex	Initial weight gm.	Final weight gm.	Gain in weight gm.	Average weekly increase in weight gm.	Average weekly food intake gm.	Average weekly protein intake gm.	Protein efficiency ratio
			Control Group (Casein)				
Male ..	40	144	104	13·0	68·6	6·86	1·89
,, ..	46	154	108	13·5	65·3	6·53	2·07
,, ..	50	165	115	14·4	66·5	6·65	2·16
Female ..	39	133	94	11·7	63·1	6·31	1·85
,, ..	52	154	102	12·8	67·3	6·73	1·9
,, ..	35	136	101	12·6	65·3	6·53	1·91
Average ..							1·96 ±0·03
			Experimental Group				
Male ..	40	153	113	14·1	74·9	7·49	1·88
,, ..	45	150	105	13·1	73·0	7·3	1·8
,, ..	40	158	118	14·7	76·3	7·63	1·92
Female ..	45	151	106	13·3	74·5	7·45	1·78
,, ..	51	159	108	13·5	75·0	7·5	1·8
,, ..	56	159	103	12·9	75·8	7·58	1·7
Average ..							1·81 ±0·02

* *Control group diet*

Protein at 10% level (casein) ..	12·9%
Fat	10%
Sugar	10%
Starch	58·1%
Salt mixture (Osborne and Mendel)	4%
Vitaminised starch	5%

Experimental group diet

To provide 10% protein level (balanced food A)	30%
Fat	10%
Sugar	10%
Starch	41%
Salt mixture (Osborne and Mendel)	4%
Vitaminised starch	5%

100 gm. of the vitaminised starch contained: 4 mg. of thiamine, 10 mg. of riboflavin, 20 mg. of nicotinic acid, 4 mg. of pyridoxine, 40 mg. of calcium pantothenate, and 200 mg. of choline chloride.

The above procedure of assessing protein quality has been stated to be somewhat empirical.[6] The actual gain in weight of the animals, taken in proportion to the protein consumed, may not be due to increase in the protein content of the animals. In such studies the diets have been fed on *ad. lib.* basis. Under these conditions, there will naturally be a tendency for the group of animals receiving a better quality of food to consume more diet than the other group. Though the increase in body weight is computed to unit weight of protein intake, the data cannot normally be considered to be satisfactory. As an improvement on this, paired-feeding has been suggested. But even so, diet restriction of the animals having better appetite will undoubtedly have an effect on weight increase.

In spite of the above adverse comments on the method, several investigators, including the originators of the method, have obtained reliable data, particularly when different proteins are compared.

The data presented in Table I show that the proteins of the 'balanced food' compare very favourably with those of casein. Inasmuch as growth of young rats is concerned, the proteins in the balanced food may be considered to be quite adequate. Protein nutrition especially during the growing stage, is fairly important and the food seems to have met this requirement in a fairly satisfactory manner.

Biological value by nitrogen balance technique.—In the previous experiment young rats were employed and as such the value of the food proteins for growth was ascertained. It was, therefore, felt that for the nitrogen balance studies, adult animals could be used so that the adequacy of the food proteins for maintenance requirement could be evaluated. Ordinarily animals of medium weight (80 gm.) are employed for this technique and the nitrogen balance index obtained would include growth as well as maintenance.

Six adult rats, three males and three females, weighing 130-180 gm., were put on a low nitrogen (4% egg protein) diet for one week. After allowing the first three days for adjustment, fæces and urine were collected for the last four days and analysed for nitrogen. From these data the endogenous nitrogen excretion was obtained. After a brief period of rest, the animals were given the experimental diet for one week, and fæces and urine were collected for the last four days. The nitrogen in these were determined. Record of food intake was maintained so that the total protein intake could be calculated. The biological value, digestibility coefficient and other relevant data are recorded in Table II (*a*) and (*b*).

The value of 76·2 for the mixed proteins of the food is higher, though only slightly, than the value of 72·5 obtained for a mixture of groundnut, sesame and soya cake flours in the proportion of 3 : 1 : 1.[7] This may be due to the incorporation of wheat flour (10%) into the food. Though the value for casein is about 84 (there is some variation depending on the quality), it may be stated that a biological value of 76·2 may be considered as highly satisfactory and as such the

TABLE II

Biological value of the 'Balanced food' (A) by the nitrogen balance method

(*a*) Endogeneous fæcal and urinary nitrogen excretion of rats

Rat No.	Sex	Nitrogen excretion	
		Fæcal mgm.	Urinary mgm.
1	Male	95·2	191·2
2	,,	111·7	172·1
3	,,	98·0	175·1
4	Female	99·0	194·5
5	,,	100·0	180·2
6	,,	99·0	169·1

(*b*) Experimental diet* period; Protein level in the diet—10·15%

Rat No.	Food intake gm.	Nitrogen intake mgm.	Nitrogen excretion		Digestibility coefficient mgm.	Biological value
			Fæcal mgm.	Urinary mgm.		
1	50·38	817·5	230·1	350·1	83·5	76·6
2	55·76	905·5	218·3	375·1	88·2	74·6
3	57·54	934·4	216·4	389·4	87·0	73·7
4	59·71	969·7	215·4	395·0	88·0	76·5
5	58·57	951·1	233·1	375·2	86·0	76·1
6	60·00	974·4	230·0	340·1	86·4	79·7
Average ..					86·5±0·4	76·2±0·6

**Experimental diet*

To provide 10% of protein (balanced food A)	30%
Sugar	10%
Groundnut oil	10%
Vitaminised starch	5%
Salt mixture (Osborne & Mendel) ..	4%
Starch	41%

mixed proteins in the 'balanced food' could help very considerably in ameliorating protein malnutrition. The digestibility of the food protein is also very satisfactory.

It is of interest, in this connection, to state that oilcake proteins generally have a fairly low biological value. By a suitable combination of these it has been possible to obtain a protein mixture of fairly high biological value. Thus, it is possible to provide as food proteins, proteins from oilcakes which are not normally used as items of human food. While taste and other factors must govern the practical application of these studies, the scientific information now collected would show that substantial benefits could be derived by the section of population who consume a poor diet. This is certainly one of the ways of overcoming protein malnutrition and the associated unfavourable manifestations of such a condition.

Hæmopoietic value method.—Although early observations[8,9] connected anæmia with protein deficiency, it is perhaps not quite correct to state that anæmia is brought about only by inadequate supply of proteins in the diet. At the same time, it is possible to produce a mild type of anæmia by feeding a diet low in protein. Orten & Orten[10] succeeded in making rats anæmic by this procedure and showed further, that the anæmia responded favourably to an increase in protein supply and not to extra iron or to enhanced caloric intake.

Some investigators reported that certain amino acids played a significant role in hæmopoiesis. Thus, Fontes & Thivolle[11] found tryptophane and histidine to produce a remarkable effect on the rate of increase in hæmoglobin in the case of hæmorrhagic anæmia in dogs. Whipple & Robscheit-Robbins[12] found under similar conditions, cystine, proline and certain other individual amino acids to be extremely effective. A diet lacking or deficient in lysine[13] or tryptophane[14] was found to produce anæmia in rats.

Orten & Orten[15] suggested, on the basis of their experimental data, that normally supplementation with any amino acid or acids should not result in increased hæmotopoiesis, if the diet contained adequate quantities of all the amino acids. When the diet was deficient in some amino acid, supplementation with this amino acid should naturally lead to improved hæmotopoietic response. Such an observation would not justify a conclusion that a particular amino acid was responsible for increasing the hæmoglobin regeneration. Orten *et al.*[16] thus pointed out that rats fed a diet containing human or beef globin, developed anæmia and that addition of iso-leucine, the chief qualitatively deficient amino acid in globin, increased the rate of growth and corrected the anæmia.

Youmans[17] found mild anæmia to be common, but could not trace it to uncomplicated protein deficiency.

Whether only adequate dietary protein supply could prevent the development of anæmia, the experimental evidence thus far recorded would show that an adequate protein could help to a considerable extent in hæmoglobin regeneration, particularly when experimental animals were rendered anæmic. As such, protein quality could be assessed in relation to its capacity for building up hæmoglobin.

The effect of the proteins in the balanced food was therefore tested by thi technique.

EXPERIMENTAL

Of the different methods[10,16,4,18] available for producing anæmic conditi on in experimental animals the method developed by Yeshoda[4] is simple and elegant. Anæmia is developed on the fourth day after an intraperitoneal injection of phenyl hydrazine.

Eighteen young albino rats weighing 80–100 gm. were chosen for the study and formed into three groups according to the usual convention. They were given the following diets:

Diet No. 1

Oilcake mixture 4[7] to provide protein at 5% level	10·4%
Groundnut oil	10·0%
Sugar	10·0%
Vitaminised starch	5·0%
Salt mixture (Osborne & Mendel)	4·0%
Starch	60·6%

Diet No. 2

Balanced fooc (A) to provide protein at 5% level	15·0%
Groundnut oil	10·0%
Sugar	10·0%
Vitaminised starch	5·0%
Salt misture (Osborne & Mendel)	4·0%
Starch	56·0%

Diet No. 3

Casein	5·0%
Groundnut oil	10·0%
Sugar	10·0%
Vitaminised starch	5·0%
Salt mixture (Osborne & Mendel)	4·0%
Starch	66·0%

Two drops of adexolin were given to each rat twice weekly to supply the requirements of vitamins A & D.

The normal R.B.C. and hæmoglobin levels were determined using Sahli-Adam's hæmoglobinometer. The blood was taken from the individual rats by cutting the tip of their tail. The rats were then given an intraperitoneal injection of phenyl hydrazine, the dosage being 2 mgm. for 100 gm. of body weight. On the fourth

day after the injection, R.B.C. and hæmoglobin were assessed, the final levels of these two were determined again on the 12th day. The improvements in R.B.C. and hæmoglobin due to the three different types of diet thus worked out are presented in Table III.

The mixture of proteins supplied by diet No. 1, was included in this study so as to evaluate the hæmoglobin response as influenced by protein of low biological value.[7] It was felt that the data thus obtained would be helpful in correlating the results of biological value and hæmoglobin regeneration.

The results (Table III) show that while casein has the highest hæmopoietic value, the 'balanced food' has also produced an influence comparable to that of casein. The values are significant and hence it may be stated that the balanced food has answered the test of protein quality assessment by this method also as satisfactorily as by the other two earlier methods.

Diet No. 1 has failed to evoke an adequate response and it is consistent with its low biological value.

Liver protein regeneration.—It is well known that fasting results in a loss of body proteins, the extent being governed by the duration of fast. Feeding a low-protein diet also brings about a reduction of body proteins.

The loss of protein occurring under the above circumstances is not contributed equally from the different organs of the body. Some organs and tissues lose comparatively higher amounts than others; while there are some organs which do not lose appreciable quantity of protein. These inequalities in protein catabolism naturally result from the individual characteristics of protein anabolism.

Addis *et al.*[19, 20] found that after a seven-day fast, the liver lost 40% of its original protein content, kidney 20%, heart 18%, and all the other organs and tissues combined lost only 10%. They further pointed out that as the loss suffered by the liver was so much that this might constitute a depot for stored proteins and hence might be used during fasting in the same way as glycogen.

This pronounced sensitivity exhibited by the proteins of the liver finds a close correlation with the degeneration of the liver. Harrison & Lang[5] employed the rapidity with which liver proteins were lost during fasting and restoration on protein feeding as a means of assaying the nutritional adequacy of proteins. Vars and Gurd[21, 22] subjected the rats, for a period of 14 days, to a non-protein diet followed by 69·4% hepatectomy. Rations containing different types of protein were then fed. A close correlation was observed between the quality, as also the quantity, of the food protein and the regeneration of liver protein.

Kosterlitz[23] and Campbell & Kosterlitz[24] outlined a new procedure for assaying the nutritive value of proteins using adult rats. The amount of labile cytoplasm present in the liver depended on the quality and quantity of food protein. Thus, by determining the labile cytoplasm the quality of a protein could be assessed.

TABLE III

Comparative mertis of casein, balanced food (A) and a low quality protein in hæmopoiesis

No.	Hæmoglobin in gm. per 100 c.c. of blood			Percentage increase	R.B.C. in millions per cu. mm. of blood			Percentage increase
	0 day	4th day	12th day		0 day	4th day	12th day	
Diet No. 1—Low quality protein								
1	13·4	9·4	12·2	29·78	5·9	3·9	5·3	35·9
2	12·97	9·5	11·9	25·2	6·1	3·9	5·4	38·46
3	13·1	9·8	12·3	25·5	6·5	4·0	5·3	32·5
4	13·0	8·9	11·6	30·34	5·8	3·8	5·0	31·6
5	13·2	9·2	12·0	30·4	6·1	3·7	5·0	35·1
6	13·4	9·4	12·1	28·7	6·9	3·9	5·2	33·3
Average..	13·17	9·36	12·0	28·32 ±0·6	6·05	3·88	5·2	34·47 ±0·7
Diet No. 2—'Balanced food' (A)								
1	13·4	9·5	12·8	34·7	6·6	3·8	5·4	42·1
2	13·2	9·0	12·3	36·6	6·3	3·7	5·2	40·54
3	13·0	9·32	12·5	34·1	6·3	3·9	5·6	43·6
4	12·93	9·8	12·9	31·6	5·8	3·88	5·7	46·7
5	13·6	9·0	12·1	34·4	6·2	3·92	5·5	40·3
6	13·2	9·0	12·3	36·6	6·3	3·9	5·6	43·6
Average..	13·22	9·18	12·48	34·66 ±0·5	6·23	3·85	5·5	42·8 ±0·6
Diet No. 3—Casein								
1	13·45	9·2	12·95	40·76	6·6	3·87	5·7	47·28
2	13·3	9·37	12·8	36·6	6·34	3·91	5·81	48·59
3	12·99	9·42	12·99	37·9	6·43	3·7	5·4	45·94
4	13·7	9·5	13·1	37·9	5·97	3·92	5·62	43·38
5	13·5	9·23	12·82	38·8	5·89	3·8	5·6	47·37
6	13·4	9·4	13·0	38·3	6·3	3·9	5·79	48·46
Average..	13·39	9·35	12·91	38·38 ±0·4	6·25	3·85	5·65	46·83 ±0·6

EXPERIMENTAL

The liver protein regeneration by the 'balanced food' was studied employing the procedure developed by Harrison & Lang.[5] Thirty adult rats of both sexes, weighing 250–280 gm., were divided into five comparable groups of six rats in each

group. They were fed a synthetic diet of the following composition for a period of one week:

Casein	20%
Starch	47%
Groundnut oil	24%
Vitaminised starch	5%
Salt mixtures (Osborne & Mendel)	4%

One group of rats was continued on the synthetic diet and the remaining four groups of animals were fasted for a 48-hour period, after which the weights of all the rats were taken. At this stage the group on synthetic diet and one group of fasted rats were sacrificed to determine the level of liver nitrogen before and after fasting. The remaining three groups of rats were then put on the following diets:

Diet No. 1

Oilcake mixture No. 4[7] to provide protein at 10% level	20·8%
Groundnut oil	24·0%
Starch	46·2%
Vitaminised starch	5·0%
Salt mixture (Osborne & Mendel)	4·0%

Diet No. 2

Balanced food (A) to provide protein at 10% level	30%
Groundnut oil	24%
Starch	37%
Vitaminised starch	5%
Salt mixture (Osborne & Mendel)	4%

Diet No. 3

Casein	10%
Groundnut oil	24%
Starch	57%
Vitaminised starch	5%
Salt mixture (Osborne & Mendel)	4%

After a feeding period of four days, all the rats in the three groups were killed and their liver protein was determined. The liver should not contain blood as otherwise the value for liver potein would vary markedly depending on the extent of blood retained in the liver. To avoid this, the rat was anæsthetised by giving an intraperitoneal injection of nembutol and washing out the liver blood by circulating physiological saline till the liver was pale and the saline coming out of the abdominal aorta was perfectly colourless.

The dried liver was powdered and suitable aliquot (in duplicate) was employed for nitrogen determination. The relevant data on weight and liver protein increase due to the different diets are given in Table IV (*a*) and (*b*).

TABLE IV

Comparative merits of casein, balanced food (A) and a low quality protein for regeneration of liver protein

(*a*) Weight Data

Group	Weight before fasting (gm.)	Weight after fasting (gm.)	Weight after refeeding (gm.)	Average weight gain during refeeding (gm.)
Control	246	..	..	..
Fasted	248	228	..	..
Diet No. 1 (Mixture No. 4)	237	219	226	7 ± 0·2
Diet No. 2 .. (Balanced food A)	243	224	235	11 ± 0·2
Diet No. 3 (Casein)	243	221	236	15 ± 0·9

(*b*) Liver Nitrogen

Group	Dry weight of liver (gm.)	Total nitrogen in liver (mgm.)	Liver nitrogen per 100 gm. body weight (mgm.)	Liver nitrogen increment over level per 100 gm. body weight (mgm.)
Control	2·435	236·2	96·3	..
Fasted	1·30	156·4	63·1	..
Diet No. 1 .. (Mixture No. 4)	1·572	175·8	74·0	10·9 ± 0·9
Diet No. 2 .. (Balanced food A)	1·82	188·5	78·7	15·6 ± 0·9
Diet No. 3 (Casein)	1·94	205·2	85·4	22·3 ± 0·5

The above observations would show that the quality of the proteins in the 'balanced food' for their capacity to regenerate liver proteins is quite comparable with casein. The regain of the body weights of the rats after fasting as also the increase in liver nitrogen as influenced by the food are of such magnitude to

warrant a safe conclusion that the combined proteins of the food have a pronounced favourable effect on the growth and well-being of the experimental animals.

Diet No. 1 has given the lowest values both in regard to growth and liver protein increase and this is consistent with the low quality of the proteins in this diet.

Discussion

The 'balanced food' has yielded highly favourable results by all the four methods of protein quality assessment. These are the most common procedures that are normally employed for elucidating the quality of a protein. In fact, a judgment of the quality of protein is seldom assessed by four different methods. Generally the nitrogen balance technique with young growing animals would serve to give the requirements for growth and maintenance and this is considered as quite adequate for gaining an insight into the quality of a protein. In view of anticipated practical application of this project of manufacturing the food on a large scale firstly with a view to meeting the food shortage and secondly to reduce the protein malnutrition so widely prevalent, it was considered highly desirable to test the protein rather comprehensively. The data collected show that the proteins in the food have given a satisfactory index concerning their nutritive value. In fact, it is rather rare to expect a particular protein to show such a favourable type of response when tested by several methods.

For, Guggenheim & Buechler-Czaczkes[25] found a protein to give different orders of response as a result of evaluation by different methods. According to these authors, a protein could differ in its relative efficiency according to the method of assessment. While soya proteins were of great value for liver protein regeneration they had very little use for granulocyte formation. Egg proteins were found to be extremely useful for hæmoglobin regeneration and formation of granulocytes as also for promoting growth and maintenance of nitrogen balance, but was inferior to soya protein in respect of liver protein regeneration. Gelatin, the least efficient protein for maintaining nitrogen balance and hæmoglobin regeneration, is more useful for liver protein increment than cereal proteins.

The above observed facts have a definite significance. Each of the methods calls for a particular constitution of protein wherein certain amino acid composition comes into play for accomplishing a specific body function. Maintenance requirement may be different from that of hæmoglobin formation.

It would, therefore, mean that a protein to be termed 'good' need not necessarily satisfy all the tests that may be employed to assess its nutritive value. But, if a protein or a particular combination of proteins, when tested by different methods, has yielded satisfactory results, then it may be stated that this protein source is of high order in its quality.

Thus, the 'balanced food' proteins, blended into a suitable combination, would prove of great benefit under Indian conditions particularly because of inadequate consumption of animal proteins.

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