

UTILIZATION OF SOYA-MILK PROTEIN FOR THE  
FORMATION OF BLOOD PROTEINS.

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INTRODUCTION.

THE biological assay of proteins for their nutritive value is usually done by the growth and nitrogen-balance methods. The methods developed by Osborne, Mendel and Ferry (1919), Thomas (1909), Mitchell and Carman (1926), Mitchell, Burroughs and Beadles (1936) and Chick, Hutchinson and Jackson (1935) are being widely used by all workers in the field of protein nutrition. These methods measure the nitrogen economy of the experimental animal in the matter of utilizing the protein for maintaining the various functions of protein in metabolism. Although this method of assay may not give us an idea as to the value of the protein for satisfying the requirements of the individual metabolic processes, it gives us a fairly comprehensive picture of the total value of the protein for satisfying all the functions of dietary protein put together.

In recent years, however, certain new methods have been developed for assaying the nutritive value of proteins which are based on the capacity of dietary protein for maintaining certain individual vital functions of protein in metabolism. These include the capacity of protein for (i) hæmopoiesis, (ii) plasma-protein production and (iii) liver-protein regeneration (Damodaran and Vijaya Raghavan, 1943; Orten

and Orten, 1946; Seeley, 1943; Kosterlitz, 1944; Harrison and Long, 1945). While no substitute method can claim to be as satisfactory as the nitrogen-balance method for evaluating the total nutritive value of proteins, these later methods mentioned above, in conjunction with the nitrogen-balance method, will add useful additional information to the nutritive value of the proteins.

It was on this consideration that the present work on the nutritive value of soya-bean milk protein was taken up. Our previous work on the nutritive value of the proteins of soya-bean milk by the growth and nitrogen-balance methods has shown that the proteins of soya-bean milk are about 90 per cent as efficient as the proteins of cow's milk regarding their nutritive value (Desikachar, De and Subrahmanyam, 1946). This paper deals with the comparative value of soya-bean milk protein and casein for hæmopoiesis as well as its value for maintaining the serum-protein levels in rats.

### Part I.

#### COMPARATIVE VALUES OF SOYA-BEAN MILK PROTEIN AND CASEIN FOR HÆMOPOIESIS.

The method used was the same as the one developed recently by Damodaran *et al.* (*loc. cit.*) It consists chiefly in producing artificial anæmia in young rats (80 g. to 90 g. in weight) by an intraperitoneal injection of phenyl hydrazine and measuring the capacity of the experimental protein for restoring the normal levels of hæmoglobin and red blood corpuscles. On the fourth day after the injection there is a maximum fall in the percentage of hæmoglobin and in the red cell count. After this period, there is a gradual increase in the hæmoglobin percentage and in the red cell count, both of which reach normal levels on the 11th or 12th day after the injection. The percentage increase in the hæmoglobin and r.b.c. levels over those in the anæmic state gives a measure of the value of the protein for hæmopoiesis.

The soya-bean milk protein for the experiment was prepared by precipitation after adjusting the acidity to pH 4.2. The coagulum was collected after centrifuging, washed with water and dried on sheets of filter-paper in a current of warm air at about 45°C. The casein-like preparation thus obtained was finally powdered and used for the experiment.

The soya-bean milk protein was fed to the rats at 5 per cent level. Casein fed at the same level served as a control for comparison. Six rats were used in each group receiving the milk proteins. The composition of the diets was as follows :—

Starch ...	...	...	...	71 per cent.
Cane sugar ...	...	...	...	10 "
Fat (coco-nut oil) ...	...	...	...	10 "
Protein ...	...	...	...	5 "
Salt mixture (Weeson's, 1932) ...	...	...	...	4 "

A preparation of yeast extract was given to serve as the source of vitamins of the B complex. Sufficient amounts of vitamin A and calciferol were also

## Part II.

SERUM-PROTEIN FRACTIONATION OF THE BLOOD OF RATS  
RECEIVING SOYA-MILK PROTEIN.

It is now an established fact that deficiency of essential amino acids in the dietary protein prevents the maintenance of normal levels of plasma proteins in the blood. Albanese, Holt, Kajdi and Frankston (1943), while studying the effect of tryptophane deficiency on blood formation, observed that deficiency of tryptophane brings about a lowering of the plasma-protein level of the blood, the lower plasma-protein values becoming normal after supplementation with tryptophane. Seeley (*loc. cit.*) and Allison, Seeley, Brown and Anderson (1946) have developed a method of evaluation of proteins in hypoproteinæmic dogs by measuring the capacity of the protein for regeneration of the proteins of the plasma.

It was considered desirable, therefore, to study the serum-protein levels of the blood of rats receiving soya-bean milk protein against a control set receiving casein at the same level. The fractionation of the serum proteins was also carried out to obtain the ratio of the albumin and globulin in the blood as this ratio would be an index to the normal health of the rats.

Two groups of rats (six in each) about 60 g. in body-weight were kept on the casein and soya-bean milk protein diets for two months, the diet being fed *ad libitum*. The protein levels in both the diets were kept exactly at 10 per cent, the composition of the diet being: starch 66 per cent, cane sugar 10 per cent, coco-nut oil 10 per cent, salt mixture 4 per cent and protein 10 per cent. At the end of this period the rats were anaesthetized with ether and about 3 c.c. of blood were removed from each rat by heart-puncture. The serum samples were prepared and analysed for (i) total nitrogen, (ii) non-protein nitrogen and (iii) albumin nitrogen. Globulin nitrogen was calculated by difference. Nitrogen estimations were made by the micro-Kjeldahl method on 0.2 c.c. of plasma according to the methods of Albanese *et al.* (*loc. cit.*). In the albumin determinations, the usual precautions as suggested by Robinson, Price and Hogden (1937) were followed.

The results on the serum-protein fractionation of the blood of individual rats receiving the two proteins are given in Table II:—

TABLE II.  
*Casein group.*

Rat number and sex.	Total nitrogen in mg. per 100 c.c. serum.	Non-protein nitrogen in mg. per 100 c.c. serum.	Protein nitrogen in mg. per 100 c.c. serum.	Albumin nitrogen in mg. per 100 c.c. serum.	Globulin nitrogen in mg. per 100 c.c. serum.	Albumin : globulin.
1	985	50.2	934.8	662.1	272.7	2.42
2	921	46.8	874.2	618.4	255.8	2.41
3	1,041	56.6	984.4	696.7	287.8	2.42
4	962	49.2	912.8	650.6	262.2	2.48
5	954	52.1	901.9	651.1	250.8	2.59
6	920	50.6	869.4	620.6	248.8	2.49
Average ...	964	50.9	913.1	649.9	263.2	2.47

administered. Apart from the salt mixture, a solution of a mineral mixture containing iron, copper and manganese in optimum concentrations was also given.

The data bearing on the hæmoglobin and erythrocyte contents of the blood of the rats on the 4th and 12th days after the injection of phenyl hydrazine are given in Table I:—

TABLE I.  
GROUP 1: 5 per cent casein diet.

Rat number and sex.	HÆMOGLOBIN IN G. PER 100 C.C. BLOOD.			R.B.C. IN MILLIONS PER C.MM. BLOOD.		
	4th day.	12th day.	Percentage increase.	4th day.	12th day.	Percentage increase.
1	8.5	11.5	35.3	3.58	5.07	41.6
2	8.5	11.2	31.8	3.72	5.11	37.4
3	8.2	11.5	40.3	3.81	5.22	37.0
4	8.5	11.2	31.8	3.60	5.69	58.1
5	8.2	11.0	34.1	3.66	5.80	58.5
6	8.5	12.2	43.6	3.41	4.91	46.3
Average ...	8.4	11.5	36.2	3.63	5.30	45.5

GROUP 2: 5 per cent soya-bean milk protein diet.

1	8.5	11.5	35.3	3.40	5.47	40.3
2	9.0	12.0	33.3	3.72	5.26	41.4
3	8.5	11.8	38.8	3.67	5.27	43.6
4	8.5	11.2	31.2	3.50	5.02	49.1
5	9.2	12.2	32.6	4.08	5.87	43.8
6	8.8	11.5	30.7	3.92	5.93	51.2
Average ...	8.8	11.7	33.5	3.80	5.47	44.9

These results show that soya-bean milk protein is only slightly less efficient than casein for the regeneration of hæmoglobin and red blood cells. These observations are in conformity with those of our earlier findings by the growth and nitrogen-balance methods.