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STUDIES ON THE EFFECTS OF PROTEIN DEPLETION AND OF REALIMENTATION WITH DIETS CONTAINING DIFFERENT LEVELS OF PEANUT PROTEIN OR CASEIN ON THE COMPOSITION OF LIVER, MUSCLE AND BODY OF YOUNG ALBINO RATS

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In view of the inadequate supply of milk and other protein-rich foods of animal origin in many developing countries, investigations have been carried out by several workers on the use of protein-rich foods of vegetable origin e.g. oilseed meals and legumes in the treatment and prevention of protein malnutrition in children.[1-4] The proteins of vegetable origin are, in general, deficient in one or more of the essential amino acids and hence possess a lower nutritive value than animal proteins.[5] It is known, however, that deficiencies of amino acids in individual vegetable proteins could be made up by the use of blends of proteins in suitable proportions.[6,7]. Recent investigations have shown that blends of isolated proteins from peanut and soya bean along with skim milk or casein, possess a high protein efficiency ratio and are highly effective in the treatment of protein malnutrition.[8] Peanut protein, by itself, though effective in curing the clinical signs and symptoms of protein malnutrition, has been reported to be inferior to milk proteins or blends of peanut protein or skim milk powder in regenerating serum proteins.[9] Scrimshaw *et al.*,[10] reported that when a vegetable protein blend (INCAP 9 B) was fed at high levels of protein in diet (20 to 26%) it provided adequate amounts of essential amino acids for promoting optimal growth in rats and for optimum N retention in children. Peanut protein when fed at 20% level has been found to promote good growth in rats.[11] It was therefore considered of interest to study the value of peanut protein at different levels as compared with casein in meeting the protein needs of protein depleted rats. The results of studies on the changes in tissue constituents of albino rats fed on a protein-free diet and after realimentation with diets containing 10, 15 and 20% protein as peanut protein isolate or casein are reported in this paper.

Experimental

Materials: Peanut protein isolate used in this study was prepared from edible quality peanut meal according to Anantharaman *et al.*[12] It contained 14·52% nitrogen. The protein was powdered in a hammer mill to pass through 60 mesh sieve and used in the diets. Casein used in the experiment was a commercial sample of good quality, prepared from buffalo milk. It contained 13·62% nitrogen.

Animal experiments: Young male albino rats (Wistar strain) from the laboratory colony, weighing about 65-70g (about 40 days old) were grouped according to a randomized block design, using litters into nine groups of twelve rats each and were housed in individual metal screen bottom cages. One group of rats was sacrificed to determine initial body and tissue composition. Another group of rats (positive control) received a purified diet containing 20% protein as casein and adequate amounts of other essential nutrients. The remaining seven groups of rats were fed on a protein-free purified

Table I. *Composition of experimental diets*

Ingredient (%)	N-free diet***	Peanut protein diets			Casein diets		
		10%	15%	20%	10%	15%	20%
Casein	—	—	—	—	11.8	17.7	23.6
Peanut protein isolate	—	11.0	16.5	22.0	—	—	—
Salt mixture*	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitaminized starch**	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Peanut oil	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Corn starch	87.0	76.0	70.5	65.0	75.2	69.3	63.4

* Hubbel, Mendel and Wakeman salt mixture[24]
** Chapman *et al*[25]
*** Contained 0.082% N.

diet for a period of 10 days. The animals lost on the average about 20 g body weight. One group of depleted rats (negative control) was sacrificed and the remaining six groups were rehabilitated by feeding for a period of 10 days on diets containing 10,15 and 20% protein from peanut or casein and adequate amounts of other essential nutrients. The composition of the experimental diets is given in Table I. Records of the increase in body weight and daily food intake of individual rats were maintained. At the end of the rehabilitation period, the animals were anaesthetized with ethyl ether. Blood was drawn by heart puncture and the serum was separated. The total serum protein content was determined according to King and Wootton.[13] The electrophoretic pattern of the serum proteins was studied by agar-gel method of Giri.[14] The liver was quickly removed, washed with normal saline to remove adhering blood and wiped between filter papers. Portions of the liver were taken for the estimation of ribonucleic acid by the method of Albaum and Umbreit[15] with modifications suggested by Rege and Sreenivasan[16] and deoxyribonucleic acid content by the method of Dische and Schwartz[17]. The moisture content of the liver was determined by drying to a constant weight at 90-95°C. The dried samples were powdered and aliquots taken for the estimation of total nitrogen by the micro-Kjeldahl method and total lipids by the method of Hawk and Elvehjem.[18] The hydroxyproline content of the moisture and lipid free liver material was determined by the method of Neuman and Logan.[19] The collagen nitrogen was calculated on the assumption that all the hydroxyproline in the carcass is present in the collagen and rat collagen contains 14% hydroxyproline and 18% nitrogen. A similar procedure was followed by Dickerson and Widdowson[20] in determining the collagen content of the tissues of pig and poultry.

The gastrocnemii and the quadriceps femoris muscles were removed as completely as possible and weighed. As far as possible, the muscle was analysed within a few hours after removal. Muscle samples which could not be analysed on the same day were freeze dried and stored in sealed sample tubes at 0°C. Moisture, lipids, nitrogen, ribonucleic acid, deoxy-ribonucleic acid and hydroxyproline contents of the muscle were determined according to methods used for liver. Creatine content was determined according to Folin and Wu.[21] Hydroxyproline was estimated on the moisture and lipid free muscle.

The contents of the gastrointestinal tract were removed by squeezing and the whole carcass was minced and weighed. The minced carcass was freeze dried and analysed for moisture, nitrogen, lipids and creatine, according to methods used for liver and muscle. Hydroxyproline was estimated on the moisture and lipid free material. The results of histological examination of the liver and muscle from the different groups of animals will be reported in a separate paper.

Results

The results are presented in Tables II-IV.

Growth of animals (Table II): Protein fasting of growing albino rats for a period of 10 days resulted on the average in about 30% loss of the body weight. Realimentation of the depleted rats with diets containing 10,15 and 20% protein from peanut or casein for a period of 10 days resulted in a considerable increase in the growth of rats. At 10 and 15% protein levels, peanut protein was not as effective as casein in promoting growth in depleted

Table II. *Increase in body weight† of protein depleted rats realimented on diets containing 10, 15 and 20% peanut protein or casein*

Diet	Initial weight (g)	Gain in weight (g)	Diet intake (g)	Feed efficiency ratio*
Peanut protein (10%)	47.1	25.5	92.7	0.272
,, ,, (15%)	46.8	42.6	103.7	0.409
,, ,, (20%)	47.0	53.5	106.4	0.502
Casein (10%)	46.5	45.1	95 3	0.467
,, (15%)	46.3	55.4	97.1	0.571
,, (20%)	46.8	57.6	101.5	0.565
Standard error of the mean (55 d.f.)		± 2.35		± 0.154

† Mean values of 12 male rats per group ; period of realimentation, 10 days
* Expressed as increase in weight per g of diet intake

rats. The 20% peanut protein diet promoted a growth rate (53·5g) nearly equal to that (55·4g) of a 15% casein diet, while the growth rate on 15% peanut protein diet (42.6g) was nearly equal to that (45·1g) on 10% casein diet.

Serum proteins (Table III) : Feeding of albino rats on a protein-free diet for a period of 10 days resulted in a significant decrease in the total serum protein content. The γ-globulin content of the serum of the depleted rats was higher and the albumin content lower than that of normal rats. Peanut protein at 20% level was nearly as effective as casein at 15% level in increasing the serum proteins to normal levels.

Effect of protein depletion and repletion on the weight and nitrogen content of body, liver and muscle (Tables IV and V) : The mean loss in body weight of the rats (27·2%) due to protein depletion was followed by a corresponding loss (30·6%) in the body nitrogen. Realimentation of the depleted rats on diets containing 10,15 and 20% protein from peanut or casein resulted in a considerable increase in the body nitrogen of rats with corresponding increases in body weight. The mean losses in total muscle mass and muscle nitrogen of rats (22·9 and 23·5% respectively) as a result of protein depletion were slightly lower than those observed for body weight and body nitrogen. The loss of nitrogen from liver (41·4%) was higher than that observed for the muscle or body. During protein realimentation, the liver gained nitrogen at a faster rate than the muscle and body. The results show that the rates of losses and gains of nitrogen from liver are greater than those observed with muscle or the whole body during the depletion and repletion periods of 10 days each.

Table III. *Composition of blood of rats depleted on nitrogen-free diet and after rehabilitation on diets containing 10, 15 and 20% of peanut protein or casein**

Diet	Total serum protein (%)	Electrophoretic serum protein pattern					
		Albumin	α_1 globulin	α_2 globulin	β_1 globulin	β_2 globulin	γ globulin
		(as % of total serum protein)					
Initial—control (before depletion)	5.50	55.5	12.2	7.2	5.8	14.6	4.7
N-free	4.55	39.6	18.9	11.7	11.2	11.1	7.4
Repleted on							
Peanut protein (10%)	5.50	54.4	12.7	7.4	4.3	16 3	4.9
,, (15%)	5.67	57 8	13.4	7.6	5.2	11.9	4.2
,, (20%)	6.08	57.8	12.7	7.2	5.6	12.4	4 2
Casein (10%)	5 78	56.2	13.6	7 6	5.0	13.3	4.3
,, (15%)	6.07	57.7	12.9	8.1	5.4	11.8	4.1
,, (20%)	6.13	58.2	13.1	7.9	5.3	11.4	4.3
Casein (20%) diet (Positive control)	6.13	58.0	12.6	7.1	5.5	12.2	4.5
Standard error with 40 d.f.	± 0.09	± 0.10	± 0.07	± 0.07	± 0.07	± 0.07	± 0.10

*Values are mean of 6 males in each group

Table IV. *Loss of body, liver and muscle weights and nitrogen contents in young albino rats fed on protein-free diet for 10 days*

	Body weight (g)	Body nitrogen (mg)	Liver weight (g)	Liver nitrogen (mg)	Muscle mass* (g)	Muscle nitrogen (mg)
Initial (before depletion)	66.5	1882	2.48	73.4	24.9	793
After depletion	48.4	1307	1.84	43.1	19.3	607
Loss due to depletion						
Total	18.1	575	0.64	30.4	5.7	186
As % of initial	27.2	30.6	25.8	41.4	22.9	23.5

* Calculated on the assumption that the creatine estimated in the whole body is present entirely in the muscle and that muscle contains on average 4 mg creatine per gram of fresh muscle.

Retention of protein (Table VI): The mean retentions of protein in rats receiving diets containing 10 and 15% peanut protein were less than those observed in rats fed similar diets containing casein. The mean protein retention on 20% peanut protein diet was nearly of the same order as that observed with 15% casein diet. There were no significant differences in the protein retained per 100g gain in weight of the rats receiving the different diets, showing thereby that the increase in weight was followed by a corresponding increase in body protein.

Composition of liver and muscle (Tables VII and VIII): There was a slight decrease in the nitrogen content and slight increase in the fat content of the livers of depleted rats. No appreciable change was observed

Table V. *Increase in weight and nitrogen content of body, liver and muscle of protein depleted albino rats fed for 10 days on diets containing varying levels of peanut protein or casein*

	Body weight		Body nitrogen		Liver weight		Liver nitrogen		Muscle mass		Muscle nitrogen	
	(a) (g)	(b) (%)	(a) (mg)	(b) (%)	(a) (g)	(b) (%)	(a) (mg)	(b) (%)	(a) (g)	(b) (%)	(a) (mg)	(b) (%)
Depleted rats (Initial)	48.4	100	1307	100	1.84	100	43	100	19.3	100	606	100
Realimented on												
Peanut Protein (10%)	73.9	152	2028	155	3.74	203	99	229	28.4	147	914	151
,, (15%)	91.0	188	2549	195	4.72	256	131	303	35.4	184	1122	185
,, (20%)	101.9	210	2934	224	5.08	280	145	337	40.7	211	1288	212
Casein (10%)	93.5	193	2590	199	4.78	259	132	305	35.3	184	1124	185
,, (15%)	103.8	214	2968	227	5.44	295	157	362	39.2	204	1255	207
,, (20%)	106.0	219	3053	233	5.54	300	175	407	40.4	210	1300	231

a : Total b : as % initial.

Table VI. *Intake and retention of protein* in protein depleted rats after rehabilitation on diets containing 10, 15 and 20% of peanut protein or casein*

Diet	Protein intake (g)	Gain in body weight (g)	Gain in body wt. per g protein intake	Initial body protein (g)	Final body protein (g)	Protein retained (g)	Protein retained	
							as % intake	(g) per 100g. gain in wt
Peanut protein (10%)	9.26	25.5	2.75	8.11	12.55	4.44	48.0	17.5
,, ,, (15%)	15.55	42.6	2.68	7.89	15.71	7.82	50.3	18.8
,, ,, (20%)	21.29	53.5	2.49	7.96	18.01	10.05	47.2	19.0
Casein (10%)	9.52	45.1	4.62	7.86	15.93	8.07	84.7	18.3
,, (15%)	14.56	55.4	3.72	7.89	18.22	10.33	71.0	19.1
,, (20%)	22.30	57.6	2.58	7.84	18.94	11.10	49.8	19.3
Standard error with degrees of freedom		±2.35 (55 d.f.)	±0.03 (55 d.f.)				±1.11 (55 d.f.)	±0.11 (55 d.f.)

* Values are mean of 12 males in each group

in the creatine content of the muscle. The decrease in the weight of the liver and muscle was followed by a corresponding decrease in the total protein contents of these tissues and in the total creatine content of the muscle. Realimentation of the protein depleted rats on the different diets resulted in a significant increase in the liver and muscle weights, total protein content of liver and protein and creatine contents of muscle.

RNA and DNA content of muscle and liver: Depletion of the growing rats resulted in a decreased concentration of

Table VII. *Composition of liver of rats* depleted on a nitrogen-free diet and after rehabilitation on diets containing 10, 15 and 20% peanut protein or casein*

Diet	Weight of liver (g)	Composition of Liver							Collagen nitrogen as% of total nitrogen
		Moisture (%)	Nitrogen (%)	Fat (%)	RNA (mg/g)	DNA (mg/g)	Hydroxy proline (%)	Collagen nitrogen (%)	
Initial control (before depletion)	2.48	69.6	2.96	3.22	10.1	2.8	0.035	0.048	1.61
N—free	1.84	71.3	2.34	3.82	8.6	3.3	0.047	0.057	2.42
Repleted on									
Peanut protein (10%)	3.74	70.4	2.64	3.77	9.6	2.6	0.034	0.043	1.61
,, (15%)	4.72	69.6	2.77	3.85	9.3	2.5	0.032	0.040	1.45
,, (20%)	5.08	69.7	2.86	3.45	9.3	2.4	0.035	0.044	1.53
Casein (10%)	4.78	70.6	2.77	3.28	9.5	2.5	0.031	0.040	1.45
,, (15%)	5.44	71.1	2.88	3.58	9 8	2.5	0.033	0.044	1.53
,, (20%)	5.54	70.7	2.93	3.00	9.6	2.4	0.037	0.047	1.61
Casein 20% diet (positive control)	7.75	69.2	3.15	3.07	9.2	2.3	0.042	0.051	1.61
Standard error with 40 d.f.		±0.30	±0.02	±0.24	±0.09	±0.04			

* Values are mean of 6 males in each group

Table VIII. *Composition of muscle of rats* depleted on a nitrogen-free diet and after rehabilitation on diets containing 10, 15 and 20% peanut protein or casein*

Diet	Weight of paired quadriceps femoris (g)	Weight of paired gastrocnemii (g)	Composition of gastrocnemii								
			Moisture (%)	Nitrogen (%)	Fat (%)	RNA (mg/g)	DNA (mg/g)	Creatine (mg/g)	Hydroxy proline (%)	Collagen nitrogen (%)	Collagen nitrogen as % of total nitrogen
Initial control (before depletion)	0.71	0.57	75.1	3.18	2.7	0.62	0.41	3.65	0.095	0.123	3.86
N—free	0.56	0.44	75.1	3.15	1.9	0.41	0.50	3.77	0.101	0.132	4.19
Repleted on											
Peanut protein											
,, 10%	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	4.11
,, 15%	1.05	0.84	75.5	3.17	2.9	0.65	0.32	3.96	0.086	0.112	3.55
,, 20%	1.17	1.03	75.6	3.17	2.8	0.66	0.35	4.09	0.089	0.112	3.54
Casein 10%	1.11	0.92	75.7	3.18	2.9	0.65	0.33	3.99	0.090	0.116	3.63
,, 15%	1.31	1.15	75.7	3.20	2.7	0.66	0.35	4.27	0.086	0.111	3.47
,, 20%	1.55	1.29	76.0	3.22	2.7	0.68	0.35	4,14	0.085	0.104	3.23
Casein 20% (positive control)	2.16	1.82	74.4	3.41	2.6	0.70	0.40	4.30	0.085	0.110	3.23
Standard error with 40 d.f.		±0.21	±0.64	±0.025	±0.21	±0.007	±0.004	±0.122			

* Values are mean of 6 males in each group

Table IX. *Composition of body of rats‡ depleted on a nitrogen-free diet and after rehabilitation on diets containing 10, 15 and 20% peanut protein or casein*

Diet	Body weight (g)	Composition of body						Collagen nitrogen as % of total nitrogen	Muscle mass*
		Moisture (%)	Nitrogen (%)	Fat (%)	Hydroxy proline (%)	Creatine (mg/g)	Collagen nitrogen (%)		
Initial control (before depletion)	66.5	68.5	2.83	8.6	0.465	1.50	0.600	21.2	37.5
N—free	48.4	68.9	2.70	8.1	0.488	1.59	0.630	23.3	39.8
Repleted on									
Peanut protein 10%	73.9	61.8	2.75	14.7	0.484	1.54	0.626	22.7	38 4
,, 15%	91.0	61.5	2.79	16.8	0.419	1.55	0.528	19.2	38.9
,, 20%	101.9	61.6	2.88	16.1	0.430	1.56	0.555	19.3	39.9
Casein 10%	93.5	62.3	2.77	15.7	0.433	1.50	0.559	20.0	37.3
,, 15%	103.8	62.9	2.86	15.7	0.420	1.51	0.540	18.9	37.8
,, 20%	106.8	62.7	2.88	15.6	0.412	1.52	0.532	18.5	38.1
Casein (20%) (positive control)	112.0	58.3	3.38	15.5	0.582	1.62	0.751	22.3	39.8
Standard error with 40 d.f.		±0.61	±0.024	±0.55		±0.40			

‡ Values are mean of 6 male rats in each group
* As percentage of body weight; calculated on the assumption that all the body creatine is present in the muscle and that the muscle contains on an average about 4 mg creatine per gram of fresh muscle

RNA in the muscle and liver; there was, however, a slight increase in the proportion of DNA in these tissues. On realimentation with the different experimental diets, the RNA and DNA contents in the muscle and liver were brought to normal levels.

Hydroxyproline and collagen content: The collagen N as per cent of total N ranged as follows: Carcass, 18·5–23·3; liver, 1·45–2·42 and muscle, 3·23-4·19. The results show that only a slight increase in the collagen content occurred as a result of protein fasting and that realimentation with different protein diets brought the collagen content to normal levels.

Creatine content and muscle mass: The mean creatine content (mg/g) of the body of the different groups of rats (Table IX) ranged from 1.50 to 1·62 while that of the muscle ranged from 3·65 to 4·30. The muscle mass (expressed as per cent of total body weight) was calculated on the assumption that the creatine found in the whole body is present entirely in the muscle and that muscle contains on an average 4·0 mg creatine/g of fresh muscle. The values thus obtained ranged from 37·5-39·9 %. The results show that muscle mass when expressed as percent of body weight, was not affected to any appreciable extent as a result of protein fasting and refeeding.

Discussion

Protein fasting of growing albino rats over a period of 10 days resulted in about 30% decrease in the body weight. This was followed by a corresponding decrease in weights of liver and muscle. Protein fasting also resulted in a considerable decrease in the ribonucleic acid content of the liver and muscle. The liver lost and gained nitrogen more rapidly than the muscle or the whole body during depletion and repletion for 10 days.

The collagen nitrogen expressed as percentage of total nitrogen before and after protein depletion were as follows: Liver, 1·61 and 2·42; muscle, 3·86 and 4·19 and whole body, 21·2 and 23·3. The results show that the increase in the collagen content of the liver, muscle and body of the albino rat as a result of protein depletion was small. Mendes and Waterlow,[22] however, reported a high increase in the collagen content of the muscle of rats

receiving a low protein Jamaican diet for a period of 28 days. In view of this, further work on the collagen contents of muscle, liver and whole body of rats fed on protein deficient and low protein diets needs to be carried out.

The total muscle mass of the different groups of animals (expressed as percentage of body weight) calculated from the creatine content of the body varied from 37·5-39·9. These values are of the same order as those (30-40%) reported by Hamoir and McKenzie[23] for vertebrates. Since no simple method is available for the estimation of total muscle mass in the body, the method (based on the creatine content) used in this study is likely to prove useful for the estimation of muscle mass in the body of experimental animals fed on various diets.

The results reported here show that peanut protein at 20% level is almost as effective as casein at 15% level in meeting the protein requirements of protein depleted rats while at 10 and 15% levels, it is inferior to casein. These results are in conformity with those of Scrimshaw *et al.*,[10] who reported that a vegetable protein blend (INCAP 9B) was almost as effective as milk in initiating a cure of kwashiorkor and in promoting nitrogen retention in children when fed at high levels of protein intake.

Summary

1. The changes in body weight and composition of body, liver and muscle of albino rats fed on a protein-free diet for 10 days and after realimentation for 10 days on diets containing 10,15 and 20% protein as peanut protein isolate or casein have been studied.

2. The muscle mass in the body of albino rats fed on different diets has been estimated from the creatine contents of muscle and of the whole body of the animals. The collagen N contents of the whole body, muscle and liver have been determined from the hydroxyproline content on the assumption that rat collagen contains 14% hydroxyproline and 18% nitrogen.

3. Peanut protein at 15 and 20% levels was almost as effective as casein at 10 and 15% levels respectively in meeting the protein requirements of protein depleted rats.

4. The results indicate that protein blends based on protein-rich foods of vegetable origin and having a lower nutritive value than animal proteins will have to be fed at a higher level of protein intake than milk proteins for effectively overcoming protein deficiency.

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