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EFFECTS OF PROTEIN MALNUTRITION ON THE THYROID FOLLICULAR CELL SIZE OF ALBINO RATS

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In this study, the effect of protein malnutrition and its reversal was observed in rats. A total of 80 male albino rats (Wistar strain) were used in this study. The animals of various groups were given isocaloric diet but the protein content of the diet varied from 2.5 to 20%. It was observed that protein malnutrition had an adverse though reversible effect on the thyroid follicular cell size of the albino rats. It is suggested that a similar study should be conducted to see the effects of PEM on the thyroid hormones as well to correlate our study with the function of the gland.

INTRODUCTION

Protein Malnutrition is the most important public health problem of the world[1]. It effects nearly 150 million children under the age of five years[2]. The name "Protein Energy Malnutrition" (PEM) is usually applied to this condition because it is caused by gross deficiency of two important elements in our food i.e. Energy and Protein. The condition is firmly rooted in the cultural pattern, general socio- economical condition of the population in the nature and availability of food. In Pakistan alone about 50% of the deaths in an average pediatric ward are caused by the status of PEM either directly or indirectly[3].

In our country the condition is more common in pre school children. On average, about 50% of our children suffer from some degree of PEM, but in poor communities, the ratio may rise to 70%. Children suffering from this condition get sick or fall ill easily or may even die on account of superimposed infection[4].

If the protein of diet is seriously deficient in one or more of the essential amino acids, nitrogen equilibrium, cannot be sustained, no matter how complete and excellent the diet may be in all other aspects[1]. The protein requirements therefore, must be considered on the basis of quality not merely quantity[5].

To create awareness regarding the importance of balanced nutrition, we observed the weight change of animals in various experimental groups malnourished for a shorter duration as well as reversal of protein malnutrition. Serum albumin level is estimated to confirm that protein malnutrition had actually occurred.

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The effect of protein malnutrition was also observed on the histological appearance of Thyroid gland of the albino rat by measuring the follicular cell size of the gland.

MATERIALS AND METHODS

Eighty (80) male albino rats (Wistar strain) of 2 weeks age were used in the present study. They were kept in animal house of the zoology department of Punjab University under standard conditions. The animals were given a period of two weeks to acclimatize with the environment. During this period, they were supplied with food (commercial diet) and water ad libitum. The experiment was started when the animals were 4 weeks old.

The animals were divided randomly into 3 groups before the commencement of the experiment. They were grouped as 'Control', 'Experimental group-I' and 'Experimental group-11'. The Control group comprised of 40 animals while the Experimental groups I and II contained 20 animals each. The Control group was furthers divided randomly into 8 sub-groups containing 5 animals each. They were named as Control A to H. The Experimental groups I and II were also divided randomly into 4 sub-groups with 5 animals. each. They were named as El A to E1D and E2A to E2D. These animals were weighed at the onset of the experiment and each sub-group was kept in a separate cage. The diet given to all the animals was 'isocaloric' but the protein content of the diet of different groups varied from 20% to 2.5%.

The experimental time of different groups also varied from 3 to 10 weeks. The animals of both Experimental groups were given 2.5% protein in their ration for 3,4,5 and 6 weeks for sub groups la, Ila; Ib,IIb; Ic,IIc and Id, IId respectively. The animals of Experimental group I were then killed. The animals of the Control a to d sub groups were also killed with them for comparison. Each of the sub groups of Experimental II animals were reverted to the ration containing 20% protein for 4 weeks. Thereafter they were also killed along with the animals of the relevant Control sub groups for comparison. The animals of Experimental group II

were also compared with those of the Experimental I group.

After specified period each animal was deeply anaesthetized with ether. The thorax and neck were opened by a midline incision blood was collected directly from aorta, while the animal was still breathing. It was analyzed for the estimation of serum albumin level in the biochemistry laboratory of the Sheikh Zayed hospital Lahore. The thyroid gland of the animal was dissected out. It was examined for gross appearance and preserved in the Bouin's solution for shifting to the histology laboratory of the Ayub Medical College Abbottabad. After tissue treatment 5p thick sections were taken and stained with H&E technique. The size of the follicular cells surrounding thyroid follicles was measured randomly. After screening all the slides, results were compared statistically with the control group.

RESULTS

The sub-groups of Experimental-I group were compared with the control group A to D for statistical significance while the sub-groups of Experimental-11 group were compared with the Control group E to H for statistical significance. The sub-groups of Experimental-II group were also compared with the respective sub-groups of the Experimental-I group for statistical significance. The results are summarized in the tables 1 to 5. The values in the tables are expressed as mean + Standard Deviation.

Table 3 shows the weight changes, Serum albumin and Cell size of the A to D of the experimental groups I and II.

TABLE-1: FINAL AGE, INITIAL WEIGHT AND FINAL WEIGHT OF THE CONTROL AND EXPERIMENTAL GROUPS

(The values are expressed as Mean ± SD)

GROUP	SUB GROUP	AGE AT KILLING (weeks)	INITIAL WEIGHT (grams)	FINAL WEIGHT (grams)
CONTROL	*A*	07	55.42±3.15	99.3016.18
	B	08	56.70±3.04	111.0214.69
	C	09	58.75±1.93	127.7117.75
	D	10	58.9611.47	141.9018.82
	E	11	60.3111.58	156.0919.20
	F	12	54.2811.70	170.28+9.77
	G	13	57.0712.74	184.47111.25
	H	14	51.3811.35	198.66112.25
EXPERIMENTAL -1	A	07	51.3211.53	91.04+3.72
	B	08	56.8612.61	104.92+3.97
	C	09	54.36+2.46	97.08+4.30
	D	10	57.6511.12	105.7314.19
EXPERIMENTAL -2	A	11	57.3611.73	148.1415.87
	B	12	59.2310.95	164.5914.16
	C	13	51.2311.13	158.4615.44
	D	14	53.6514.41	168.03+4.36

TABLE-2: WEIGHT CHANGE, SERUM ALBUMIN, FOLLICLE SIZE, CELL SIZE AND NUMBER OF FOLLICLES IN THE CONTROL GROUP (SUBGROUPS A-D)
(The values are expressed as Mean ± SD)

PARAMETER	UNIT	CONTROL GROUP			
		A	B	C	D
WEIGHT CHANGE	grams	43.8813.02	54.32±4.35	68.96±4.05	82.94±3.32
SERUM ALBUMIN	mg/dl	3.55±0.35	3.34±0.29	3.46±0.25	3.45±0.26
CELL SIZE	pm	7.67±1.86	7.40±1.11	7.51±1.40	7.50±1.00

TABLE-3: WEIGHT CHANGE, SERUM ALBUMIN, FOLLICLE SIZE, CELL SIZE AND NUMBER OF FOLLICLES IN THE CONTROL GROUP (SUBGROUPS E-H)
(The values are expressed as Mean ± SD)

PARAMETER	UNIT	CONTROL GROUP			
		E	F	G	H
WEIGHT CHANGE	grams	95.78±2.77	116.00±8.40	127.4016.27	147.28+8.06
SERUM ALBUMIN	mg/dl	3.60±0.32	3.55±0.29	3.5410.23	3.5410.22
CELL SIZE	pm	7.62±0.62	7.64+0.61	7.6610.53	7.6210.48

TABLE-4: WEIGHT CHANGE, SERUM ALBUMIN, FOLLICLE SIZE, CELL SIZE AND NUMBER OF FOLLICLES IN THE EXPERIMENTAL 1 GROUP (SUBGROUPS A-D)
(The values are expressed as Mean ± SD)

PARAMETER	UNIT	EXPERIMENTAL 1			
		A	B	C	D
WEIGHT CHANGE	grams	39.72+1.37 *	48.06+1.89*	42.7212.40***	48.0813.60***
SERUM ALBUMIN	mg/dl	3.20+GC10	3.0910.14	2.7010.23**	2.5010.28***
CELL SIZE	pm	6.17+0.97	6.10+0.27*	4.3710.73**	3.9310.61***

*: p value <0.05 when compared with the controls
**: *p value < 0.01 compared with the controls*
***: *p value <0.001 compared with the controls*

TABLE-5: WEIGHT CHANGE, SERUM ALBUMIN, FOLLICLE SIZE, CELL SIZE AND NUMBER OF FOLLICLES IN THE EXPERIMENTAL 2 GROUP (SUBGROUPS A-D)
(The values are expressed as Mean ± SD)

PARAMETER	UNIT	EXPERIMENTAL 2			
		A	B	C	D
WEIGHT CHANGE	grams	90.78±3.06 ***, †	105.36±5.36***,†	107.2316.18 ***,†††	114.38+7.07***,†††
SERUM ALBUMIN	mg/dl	3.64±0.38 *	3.82=0.43 **	3.92+0.47 ***	4.14+0.62 ***,†
CELL SIZE	pm	9.3111.29 **, †	9.9311.33 ***,††	10.2511.71 ***,†	11.5011.31 ***,†††

* : P<0.05 when compared with Exp: 1
** : P<0.01 when compared with Exp: 1
*** : P<0.001 when compared with Exp: 1
† :<0.05 when compared with Controls E, F, G &H
†† :<0.01 when compared with Controls E, F, G &H
††† :<0.001 when compared with Controls E, F, G &H

DISCUSSION

The protein deficient diet is known to produce abnormal structural changes in a number of organs in man as well as in experimental animals. The effects of PEM on the structure of some of the glands like submandibular gland, pancreas and parotid gland[6] have been reported but very little work has been done to study the effects of PEM on the structure of the thyroid gland. Our study was an anatomical one. Mainly the histological findings were stressed upon but due consideration was also given to the weight changes of the animal and their serum albumin level in order to see the actual extent of PEM. As there is clear sexual dimorphism in the thyroid follicles of the rat[7], all the animals used in this study were of the same sex. The age of the animals at the time of commencement of the experiment was 4 weeks because in normally growing rats, the thyroid appears most active at about 4 weeks of age[8].

We observed the weight changes of the animals in various sub-groups. We found that when exposed to protein malnutrition, the animals showed a significant decrease in weight. The animals facing prolonged malnutrition showed more decrease as compared to the ones malnourished for a shorter duration. Upon reversal from protein malnutrition, the animals showed an increase in the weight but even this weight was significantly less than the control group of the same age. This suggests that the protein content of the diet is a major determinant of feed efficiency, as judged by the weight gain in the normal growing rats. This was in agreement with the findings of Andik (1963), Decastro and Boyd (1967) cited by Orien *et al.,* [8] Heard and Stewart,[9] and Fleagle *et al.,*[10].

The serum albumin levels were measured in order to confirm that protein malnutrition had actually occurred. The results were in accordance with our expectations. The serum albumin levels decreased according to the degree of malnutrition. The animals kept on protein malnourished diet for prolonged periods showed significant decrease as compared to those who faced protein malnutrition for shorter periods. However, there was certainly a decrease as compared to the control group. Upon retrieval from protein malnutrition, there was again an increase in the concentration of serum albumin. This was significantly more as compared to the normal control group. These findings were also observed by Onura *et al.,*[11] who observed that both total proteins as well as serum albumin were moderately depressed in marasmic-Kwashiorkor children. The same changes were also observed by Ashraf *et al.*[12] Truswell *et al.,* (1966) and Wittman *et al.,* (1967), cited by Allyene *et al.,* [13].

The cell height was observed to decrease in the malnourished group as compared to the control.

This observation was in concordance with the results of Orien *et al.,* [8] who observed a decrease in the cell height of the malnourished rats. Within the malnourished group, the decrease in height was more in animals subjected to malnutrition for prolonged periods as compared to the ones subjected to malnutrition for shorter intervals. When these animals were reverted to normal diet, the cell height increased significantly indicating increased activity. This increase in the cell height was significantly more than the cell height of the comparative controls. Trowell *et al.,* (1954) (cited by Alleyne *et al.,*[13] reported small thyroid follicles, with follicular cells cuboidal or flattened and inactive with diminished colloid. This is in agreement with our findings in which diminished colloid and a decrease in the mean cellular height has been shown in the malnourished rats. Our findings are however, in total disagreement with those of Sterling, (1962) (cited by Alleyne *et al.,*)[13] who reported that the thyroid gland was found to have no abnormality in PEM. Our data suggests that protein content of the diet is a major determinant of normal structure and function of the thyroid gland in growing rats.

An interesting observation during the course of study was that with the passage of time, the protein malnourished animals developed voracious appetite. In addition to this, these animals also showed increased activity. This observation has also been observed by Young *et al.,*[14]. Who reported a relative hyperphagia in the rats fed on a low protein and high carbohydrate diet.

From this study, we conclude that the Protein Malnutrition primarily causes structural changes in the thyroid gland and it is suggested that the studies should be designed to correlate the morphological changes with the functional changes in the thyroid gland. In addition, the effect of changes in the thyroid gland on the other dependent organs and systems like submandibular gland should also be studied.

CONCLUSION

It is concluded that protein malnutrition has an adverse though reversible effect on the microscopic structure of the thyroid gland of the albino rat. Correlating our results with the already established effects of protein malnutrition on the thyroid function, it is suggested that the structural changes are mainly responsible for the functional changes in the thyroid gland and its target organs.

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