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PROTEIN METABOLISM IN ADULT RABBITS FED DIETS WITH DIFFERENT PROTEIN CONTENT: BLOOD AND TISSUES BIOCHEMICAL PARAMETERS.

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INTRODUCTION

The blood protein levels (particularly that of albumin) have been extensively used as index of protein malnutrition. However, in this context, the reliability of plasma albumin concentration has been questioned since it may be buffered quite effectively in several cases of protein malnutrition (Waterlow - 1969, Coward et al. 1977).

In the present study the effects of different protein intakes upon blood proteins was investigated in adult rabbits in order to evaluate the reliability of such indexes when studying protein adequacy.

Total serum protein, albumin, transferrin and fibrinogen were considered. To provide more direct information about the state of protein metabolism in the liver, soluble protein and free tryptophane concentration were measured in this organ. These parameters were also investigated in the semimembranous muscle in order to elucidate the differential response of liver and skeletal muscle to either protein deficiency and excess.

Furthermore to better define the alterations of nitrogen metabolism urea and other catabolites were also determined in the plasma of our animals.

MATERIALS AND METHODS

Twenty adult male NZW rabbits were used. After adaptation on a standard diet (STD) the animals were weight-sorted in four group of five, 15 animals were transferred to three experimental diets: the 'high protein' diet (HP) had a Crude Protein content of 27.7% (dry matter), the 'moderate low protein' diet (MLP) a CP content of 8.1% and the 'low protein' diet a CP content of 4.8%. The 'control' group continued on the STD diet (18.3%, CP). Details of the animals, diet composition and food intake as been referred previously (Greppi et al. 1984).

The experimental diets were fed for a 40 days period. At the end of the experimental period, animals, under ethyl-urethane anaesthesia, were laparatomyzed and blood was drawn from the inferior vena cava. After blood collection the animals were killed by decapitation, liver and semimembranous muscle specimens were rapidly excised and frozen immediately with solid CO_2. Tissues sample were homogenyzed with 9 volumes of ice cool physiological solution. The supernatant obtained by centrifugation was used for free tryptophane determination following the methods od Denkla and Dewey (1967) with modifications of Stefanini and Biggio (1975). Aliquots of the same supernatant were used for protein determination according the method of Lowry et al. (1951).

The usual procedures were employed for the determination of blood biochemicals parameters. Statistical analysis of the differences between means was performed by Duncan's Multiple Range Test.

RESULTS

Blood parameters. Fibrinogen levels were significantly lower in both LP and MLP groups when compared to the controls, whereas a significant depression in albumin and transferrin levels was observed only in the LP group. Total serum protein concentration was not significantly altered, although in the LP group total protein fell below 6 g per 100 ml of serum. Creatinine and ammonia plasma concentrations were not significantly affected by varying the protein intake, while uric acid was significantly elevated in the LP group. Urea con-

centration showed an excellent correlation with the protein content of the diet (X%). The regression could be expressed by the following equation: $Y=1.36 + 13.05 X$, where Y is expressed as mg per 100 ml of plasma ($r=0.918$, $n=20$, $p<0.001$). Out of the catabolic parameters investigated only urea concentration was significantly elevated in the HP group (Table 1).

Tissues parameters. Soluble protein content was significantly reduced in the liver of rabbits fed the LP diet but uneffected in the liver of animals fed the MLP and the HP diets. Skeletal muscle did not show any significant change in soluble protein content.

Free tryptophane concentration was significantly reduced in the liver of the rabbits fed the LP and the MLP diets. The response of free tryptophane in the skeletal muscle was less evident and a significant depression occurred only in the LP group (Table 2).

DISCUSSION AND CONCLUSIONS

The present results illustrate that albumin and transferrin serum levels are fairly well conserved in the blood of adult rabbits submitted to a mild restriction of the protein intake, whereas they are significantly depressed when protein intake is severely reduced. Fibrinogen plasma level, on the contrary, seem to respond more sensitively to the dietary protein levels. Thus this parameters could be an useful index of marginal or impending protein malnutrition.

A close relationship between the rate of albumin synthesis and the serum level of tryptophane and branched chain amino acids has been reported by Yap et al. (1975). Baertl et al. (1974) showed that serum albumin concentration is correlated with the serum level of tryptophane in severely malnourished infants and children. Even though plasma tryptophane was actually reduced in the case of rabbits fed the LP diet (Corti et al. 1984) we could not detect any difference in the liver concentration of the free amino acid when the LP and the MLP groups were compared. Furthermore free tryptophane attained the highest levels in the HP group whereas albumin concentration in this group was even lower than in the MLP group.

Thus our results fail to demonstrate a primary role of tryptophane availability on albumin synthesis and albumin concentration in the serum.

These observations are analogous to those by Yap et al. (1981) who postulated that in fasted adult rabbits mechanisms other than reduced tryptophane availability at the site of protein synthesis inhibit albumin synthesis in the liver. The possibility exists, however, that different mechanisms are involved in albumin synthesis inhibition which occurs in different form of malnutrition.

Beside the fall in blood protein concentrations which occurred in the LP group, a failure in the liver synthetic activity was indicated by the low soluble protein concentration in the liver of the rabbits fed the low protein diet. The soluble protein liver content attained the maximum level in the HP group, in this group, however, blood protein concentrations, particularly transferrin and albumin, tended to be depressed.

These findings could indicate that in the liver of the rabbitd fed the high protein diet the normal pattern of protein synthesis was altered.

Skeletal muscle protein content, unlike the liver, was not affected by varying the protein content of the diet. This finding agree with the channelling mechanism wich under adverse conditions cause movement of nitrogen towards the muscle leaving the liver depleted (Munro 1969). The occurrence of this mechanism under condition of protein depletion has been described in rats fed low protein diets by Coward et al. (1977).

Differencial responses of liver and muscle protein to dietary protein deprivation have been observed by Yalaguki and Kandatsu (1967) and by Millward(1970).

Blood urea. The urea production in the hepatocytes has been shown to be correlated with the protein content of the diet (Briggs and Freeland 1977).The increase of ureogenesis which occurs in the liver of rats given high protein diets is reflected by elevation of liver urea (Peret et al. 1981) and blood urea (Munchow and Bergner 1968, Eggum 1970). Out of the catabolic parameters, plasma urea exibited the best correlation with the protein content of the diet. Thereafter plasma urea might be regarded as a highly reliable index of protein nutritional status. In spite of the large differences in the dietary protein influx, ammonemia attained similar levels among the experimental groups. This is in agreement with previous observations on metabolic adaptation to high protein intakes (Wergelad and Harper 1964) showing that rats adapted to high protein intakes increase their capacity to convert ammonia into urea. Measurement of urea cycle enzymes activity and urea intermediates concentration in the liver could eluci-

date the mechanism involved in such adaptation especially as far as species differences are concerned.

The significant rise of uric acid in the plasma of rabbits fed the LP diet indicate that alteration of purine catabolism was likely to occur. This observation provides additional evidence about occurrence of serious metabolic alterations in the case of the animals fed the low protein diet. Creatinine plasma levels do not appear to be influenced by protein intake.

Table 1. Blood parameters

each value is the mean ± SEM of five animals, different number of animals given in parentheses, means in the same line and without common superscripts differ significantly for $p < 0.05$

		diet			
		LP	MLP	STD	HP
serum	total protein (g%)	5.8 ± 0.2^{a} (4)	6.3 ± 0.2^{a}	6.4 ± 0.3^{a}	6.1 ± 0.3^{a}
	albumin (g%)	3.7 ± 0.2^{b} (4)	4.4 ± 0.2^{a}	4.5 ± 0.2^{a}	4.1 ± 0.2^{ab}
	transferrin (mg%)	312 ± 28^{b} (3)	342 ± 12^{ab}	381 ± 25^{a}	313 ± 27^{ab}
plasma	fibrinogen (mg%)	125 ± 30^{b} (4)	160 ± 33^{b}	317 ± 50^{a}	315 ± 71^{a} (4)
	ammonia (mg%)	185 ± 32^{a}	150 ± 25^{a}	145 ± 15^{a}	121 ± 20^{a}
	urea (mg%)	18.3 ± 1.7^{a}	24.1 ± 1.5^{a}	34.9 ± 1.6^{b}	47.4 ± 3.7^{c}
	uric acid (mg%)	0.45 ± 0.08^{a}	0.23 ± 0.03^{b}	0.19 ± 0.02^{b}	0.39 ± 0.09^{ab}
	creatinine (mg%)	1.82 ± 0.08^{a}	1.80 ± 0.05^{a}	1.70 ± 0.13^{a}	1.60 ± 0.09^{a}

Table 2. Tissues parameters

each value is the mean ± SEM of five animals, means in the same line and bearing different superscripts differ significantly for $p < 0.05$.

	diet			
	LP	MLP	STD	HP
liver soluble protein (mg/g fresh tissue)	50.3 ± 6.5^{a}	70.2 ± 6.8^{ab}	81.8 ± 10.1^{b}	87.0 ± 16.3^{b}
muscle sol. protein (mg/g fresh tissue)	44.4 ± 7.6^{a}	45.7 ± 2.8^{a}	41.1 ± 3.7^{a}	39.4 ± 1.6^{a}
liver free tryptophane (µg/g fresh tissue)	42.3 ± 9.6^{a}	42.7 ± 9.4^{a}	74.2 ± 7.7^{b}	78.6 ± 13.2^{b}
muscle free tryptophane (µg/g fresh tissue)	3.09 ± 0.60^{a}	3.41 ± 0.56^{ab}	5.01 ± 0.37^{b}	3.56 ± 0.46^{ab}

of the diet. Free tryptophane and soluble protein concentrations in the liver and the semimembranous muscle were considered too. Free tryptophane levels were significantly reduced in the liver of rabbits fed the low protein and the moderate low protein diet, whereas in the skeletal muscle a significant decrease occurred only in the case of animals fed the low protein diet. Soluble protein content was depressed only in the liver of rabbits fed the low protein diet.

RIASSUNTO

Sono stati studiati gli effetti della somministrazione di quattro diverse diete, caratterizzate da tenori proteici del 4.8, 8.1, 18.3 e 27.7%, sulla concentrazione, nel sangue di conigli adulti, di: proteine totali, albumina, transferrine, fibrinogeno, urea, creatinina e acido urico. Una significativa diminuzione dei livelli serici di albumina e transferrine è stata riscontrata nel caso dei conigli alimentati con la dieta a basso tenore proteico; la concentrazione di fibrinogeno, invece, è risultata significativamente più bassa sia nel plasma degli animali che ricevevano la dieta a basso tenore proteico, sia in quello degli animali alimentati con la dieta con un tenore proteico moderatamente basso. Una significativa elevazione della concentrazione di acido urico è stata riscontrata nel plasma degli animali soggetti alla dieta ipoproteica. La correlazione tra livelli plasmatici di urea e tenore proteico della dieta è risultata eccellente (r = 0.92). Sono state anche prese in considerazione le concentrazioni di triptofano libero e di proteine solubili nel fegato e nel muscolo semimembranoso. Una caduta dei livelli di triptofano libero è stata riscontrata sia nel gruppo alimentato con la dieta isoproteica sia in quello che riceveva la dieta moderatamente isocalorica.

Nel caso del muscolo, invece, una corrispondente riduzione è stata riscontrata solo negli animali soggetti alla dieta isoproteica. Per quanto riguarda il contenuto in proteina solubile dei tessuti si è registrata una significativa riduzione solo nel fegato dei conigli alimentati con la dieta isoproteica.

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SUMMARY

Total serum protein, albumin and transferrin, plasma fibrinogen, urea,uric acid and creatinine were determined in the blood of adult rabbits fed diets with 4.8, 8.1, 18.3 and 27.7% of Crude Protein for a 40 days period. Albumin and transferrin were significantly depressed in the serum rabbits fed the low protein diet, whereas fibrinogen was significantly reduced in the plasma of animals fed either the low and the modetate low protein diet. Uric acid was significantly higher in the plasma of rabbits fed the low protein diet. Plasma urea exibited an excellent correlation (r = 0.92) with the protein content