Effects of biotin deficiency on serum proteins and plasma amino acids

F. Petrelli, S. Coderoni, P. Moretti, M. Paparelli and D. Barra

Institute of General Physiology, University of Camerino, I-62032 Camerino (Italy) and Institute of Biological Chemistry, University of Roma, I-00100 Roma (Italy), 16 February 1978

Summary. In biotin-deficient rats, a decrease of total proteins, attributable to a decrease of albumin and a_1-globulin fractions, a decrease of the pre-β-lipoproteins and an increase of the α-lipoproteins, was observed, together with a rise of total amino acids. Such a situation may be related to the influence of biotin on the synthesis of RNA and proteins.

The incorporation of amino acids into proteins of liver, intestinal mucose, pancreas and skin in vivo and in vitro, is markedly decreased in biotin deficiency; a single injection of biotin to the biotin-deficient rat stimulates amino acid incorporation, both in vivo and in vitro[1-3]. The evaluation of the amino acid incorporation into liver proteins in vivo and in vitro indicates that the synthesis of some proteins is highly stimulated, while the synthesis of other proteins is not stimulated at all[2]: such a specificity has already been described[4,5]. The biotin-mediated stimulation is suppressed following treatment with inhibitors of protein or RNA synthesis, like puromycin, ethionine or actinomycin D. The effect of biotin on protein synthesis is preceded by a stimulation of the incorporation of orotic acid into nuclear and ribosomal RNA, as early as 2-4 h after biotin treatment. In the presence of nuclear RNA from biotin-treated rats, higher levels of amino acid incorporation by normal rat liver ribosomes in comparison with the incorporation in the presence of similar RNA isolated from biotin-deficient rats[1], were obtained. Further evidence suggests that the synthesis of other RNA fractions is stimulated by biotin[6-8].

In biotin deficiency, the energy production is impaired as the consequence of a decreased utilization of glucose and of a decreased oxidative phosphorylation[9]. The biotin-deficient rat mitochondria show decreased phosphorylation efficiency as well as poor respiratory control, as compared to normal rat liver mitochondria when NAD^+-linked substrates are oxidized. No differences between biotin-deficient and normal rat liver mitochondria in both the parameters referred to above were seen when succinate was the substrate: such results indicate that the observed loose coupling is localized at site I[10-12]. The locus of damage in energy conservation at site I is related to the synthesis of the high-energy intermediate rather than to its utilization[11].

In the liver of the biotin-deficient chick[13] and rat[14], a decreased incorporation of ^{32}P into RNA and DNA is observed. The biotin deficiency impaired interactions between histone proteins and DNA[8] may be related to the observed modifications of the phosphorylation, acetylation and methylation rates of the histones[15].

In view of the effect of biotin on protein and RNA synthesis, glucose metabolism, oxidative phosphorylation and chemical modifications of histone proteins and interactions between histone proteins and DNA, we have investigated the effect of biotin deficiency on some haematic parameters: protein and amino acid levels.

Material and methods. Sprague-Dawley female rats, average initial weight 45 g, were utilized. Biotin deficiency was obtained by feeding a diet free of biotin and with added avidin (György and Rose). Control animals received the same diet and in addition i.p. injections of an aqueous solution of biotin (200 µg/rat, on alternate days).

Following 12 h fasting and s.c. Nembutal administration (10 mg/100 g b.wt), the blood was removed by abdominal aorta puncture. All blood samples were taken at the same time in the morning on each experimental day, in order to avoid any variation due to the circadian periodicity of plasma amino acids[16].

An aliquot of blood was utilized for the determination of the serum proteins. The protein content was determined by the Weichselbaum's method[17] modified by Josephson and Gyllenswärd[18]. The proteins were fractionated by cellulose acetate (cellogel) electrophoresis using Tris-HCl buffer (0.05 M), pH 7.8. The strip was stained for a least 5 min with 0.5% Ponceau S in 5% trichloracetic acid, destained with 5% acetic acid solution and scanned at 510 nm in a densitometer. The serum lipoproteins were fractionated by cellulose acetate (cellogel) electrophoresis using Tris-HCl buffer (0.05 M), pH 7.8. The strip was stained for at least 3 h with Sudan Black B, washed in water and scanned at 530 nm in a densitometer.

An Aliquot of blood was immediately centrifuged in the presence of sodium citrate, the supernatant plasma removed and deproteinized by 50 mg of 5-sulfosalicylic acid per ml of plasma. The deproteinized supernatant fluid was stored at $-20\,^{\circ}C$ until analyzed. Amino acid analyses were carried out by means of a LKB 3201 instrument, equipped with a single column. The method of Spackman et al.[19] for analysis of protein hydrolisates was used. The elution volume of citrulline, under our experimental conditions, was checked by the inclusion of this amino acid in the calibration mixture.

Results and discussion. The results reported in table 1 represent the values of the protein concentrations in the serum in 6 sets of pooled samples, each of which was obtained from 2 animals. The data demonstrate that biotin-deficient rats, as compared with normal control rats, show a statistically significant decrease in albumins ($p < 0.0001$)

Table 1. Concentration of proteins in the serum of biotin-deficient and normal rats

Protein fractions	Normal rats (%) Mean	SD	Biotin-deficient rats (%) Mean	SD	p
Albumin	3.608	0.219	2.427	0.304	<0.001
a_1-Globulin	1.249	0.100	0.893	0.077	<0.001
a_2-Globulin	0.492	0.066	0.471	0.076	NS
β-Globulin	1.310	0.108	1.462	0.171	<0.05
γ-Globulin	0.787	0.095	1.021	0.195	<0.02
Total proteins	7.446	0.323	6.275	0.358	<0.001

Analyses were carried out using pooled serum samples from 2 animals. Values are the mean of 6 sets and are expressed as g/100 ml of serum ± SD. Statistical significant, Student's t-test.

Table 2. Lipoprotein fractions in the serum of biotin-deficient and normal rats

Lipoprotein fractions	Normal rats (%) Mean	SD	Biotin-deficient rats (%) Mean	SD	p
α-Lipoprotein	13.07	3.93	14.30	2.50	NS
Pre-β-lipoprotein	43.43	7.88	58.85	2.50	<0.02
β-lipoprotein	43.50	9.88	26.85	2.89	<0.02

Analyses were carried out using pooled samples from 4 animals. Values are the means of 3 sets and are expressed as a percentage of total lipoproteins ± SD. Statistical significant, Student's t-test.

and α_1-globulins ($p < 0.001$) and a small, but significant, increase in β-globulins ($p < 0.05$) and γ-globulins ($p < 0.02$). The concentration of total proteins is lower in biotin-deficient than in normal rats. It appears to be the consequence of a decrease of the albumin and α_1-globulin fractions.

Table 2 reports the results concerning the determination of the lipoprotein fractions in serum of normal and biotin-deficient rats, expressed as a percentage of total lipoproteins. The data demonstrate that biotin-deficient rats, as compared with normal rats, show a statistically significant decrease of the pre-β-lipoproteins ($p < 0.02$) and an increase of the β-lipoproteins ($p < 0.02$).

In biotin deficiency, a significant rise of total amino acids is present. The results reported in table 3 represent the values of the amino acid concentrations in the plasma in 3 sets of pooled samples, each of which is derived from 4 animals. The concentrations of histidine, isoleucine, leucine, phenylalanine, citrulline, tyrosine, valine and taurine are higher in biotin-deficient rats, and the concentrations of glutamic acid and of the group including asparagine, glutamine, serine and threonine, are lower than in normal rats. In biotin-deficient rats, the plasma urea concentration also increases.

Table 3. Concentration of free amino acids and related substances in plasma of biotin-deficient and normal rats

Amino acid	Normal rats Mean	SD	Biotin-deficient rats Mean	SD	p
Alanine	50.67	13.61	49.00	12.81	NS
Arginine	16.67	5.13	20.00	8.20	NS
Aspartic acid	–	–	–	–	–
Cystine	36.50	6.36	41.00	21.38	NS
Glutamic acid	10.50	3.28	–	–	<0.001
Glycine	20.67	3.21	20.75	4.64	NS
Histidine	7.60	0.51	11.00	1.00	<0.01
Isoleucine	8.57	2.48	30.75	7.37	<0.01
Leucine	14.33	3.79	86.33	19.65	<0.001
Lysine	74.00	20.42	82.75	34.05	NS
Methionine	3.90	1.15	5.55	1.18	NS
Phenylalanine	5.93	1.00	10.35	3.44	<0.05
Citrulline	7.63	1.55	42.25	8.30	<0.001
Asparagine Glutamine Serine Threonine	113.67	17.67	51.50	16.05	<0.01
Tryptophan	–	–	–	–	–
Tyrosine	5.67	1.69	22.00	7.44	<0.02
Valine	21.33	1.53	124.33	25.15	<0.001
Taurine	17.50	0.50	39.33	8.08	<0.001
Total amino acids	415.14		636.89		
Non-amino acids					
Urea	17.67	1.53	25.00	1.00	<0.001

Analyses were carried out by means of one of the methods for the analysis of protein hydrolisates[19], using pooled plasma samples from 4 rats. Values are the means of 3 sets of pooled samples expressed as *nmoles/100 ml plasma ± SD*. *Statistical significant*, Student's t-test.

The concentrations of alanine, arginine, cystine, glicine, lysine and methionine in biotin-deficient and normal rats are similar.

The results of the present experiments demonstrate that biotin deficiency causes in the rats a decrease of total proteins attributable to a decrease of albumin and α_1-globulin fractions. A decrease of the pre-β-lipoproteins and an increase of the α-lipoproteins is also present.

Moreover, a significant rise of total amino acids is present in plasma of biotin-deficient rats as compared with normal rats: this is mainly due to an increase of essential amino acids.

Such a situation might be related to the influence of biotin on the synthesis of RNA and proteins; the incorporation of amino acids into proteins is markedly decreased in the biotin-deficient rat[1-3]; the synthesis of some proteins, and of nuclear and ribosomal RNA is also decreased[4-8]. The treatment with biotin gives rise to higher levels of amino acid incorporation and of protein and RNA synthesis[6-8].

The results reported here also suggest that the liver of the biotin-deficient rat is more active than normal rat liver with regard to amino acid catabolism, since both the citrulline and urea levels of the plasma (table 3) increase significantly.

1 K. Dakshinamurti and S. Litvak, J. biol. Chem. *245*, 5600 (1970).
2 R.L. Boeckx and K. Dakshinamurti, Proc. Can. Fed. Biol. Soc. *15*, 200 (1972).
3 R.L. Boeckx and K. Dakshinamurti, Biochem. J. *140*, 549 (1974).
4 K. Dakshinamurti and C. Cheah-Tan, Archs Biochem. Biophys. *127*, 17 (1970).
5 K. Dakshinamurti, L. Tarrago-Litvak and H.C. Hong, Can. J. Biochem. *48*, 493 (1970).
6 F. Petrelli, G. Marsili and G. Centioni, Boll. Soc. ital. Biol. sper. *49*, 1332 (1973).
7 R.L. Boeckx and K. Dakshinamurti, Biochim. biophys. Acta *383*, 282 (1975).
8 F. Petrelli, G. Marsili and P. Moretti, Biochem. exp. Biol. *12*, 461 (1976).
9 K. Dakshinamurti, V.V. Modi and S.P. Mistry, Proc. Soc. exp. Biol. Med. *127*, 396 (1968).
10 K. Dakshinamurti, M.A. Sabir and C. Bhuvaneswaran, Archs Biochem. Biophys. *137*, 30 (1970).
11 C. Bhuvaneswaran and K. Dakshinamurti, Archs Biochem. Biophys. *142*, 665 (1971).
12 C. Bhuvaneswaran and K. Dakshinamurti, Int. J. Biochem. *5*, 819 (1974).
13 A.S. Atwal, A.R. Robblee and L.P. Milligan, J. Nutr. *101*, 1555 (1971).
14 F. Petrelli, S. Coderoni, P. Moretti and M. Paparelli, Biochem. exp. Biol., in press.
15 F. Petrelli, S. Coderoni, P. Moretti and M. Paparelli, Molec. Biol. Rep., in press.
16 R.D. Feigin, A.S. Klainer and W.R. Beisel, Metabolism *17*, 764 (1968).
17 T.E. Weichselbaum, Am. J. clin. Path *16*, 40 (1946).
18 R. Josephson and C. Gyllensvärd, Scand. J. clin. Lab. Invest. *9*, 29 (1957).
19 D.H. Spackman, S. Moore and W.H. Stein, Analyt. Chem. *30*, 1190 (1958).