23 APs from 1.31 mm (mean, range 0.81–2.00) to 0.36 mm (range 0.14–0.66). R_0 was increased in 8 out of these 9 cases, although R_0 should be underestimated because of the progress of uncoupling will increase the leakage current fraction of the applied current pulse. A tentative calculation of r_i from λ and R_0 revealed that it was increased by about 10 times by Sr APs. The increase of r_i is considered to reflect the increase of intercellular coupling resistance.

$[Na]_o$ was essential to the recovery of coupling obtained by normal Tyrode perfusion, since similar recovery was not obtained by Ca-containing, Na-free Tyrode, but by Ca-deficient, Na-containing Tyrode. The uncoupling induced by electrophoretically injected $[Na]_i$[3] and the recovery of uncoupling by $[Na]_o$ are usually explained by assuming that the Na–Ca exchange system, which transports Sr as well as Ca, participates in the regulation of $[Ca]_i$ and $[Sr]_i$[10].

The present uncoupling by Sr APs could be due to the high $[Sr]_i$ produced by large Sr current, presumably by slow uptake of Sr by intracellular stores and by decreased Sr efflux under Na-free condition. Although the coupling under normal condition is high, it might be variable depending on $[Ca]_i$ which is determined by Ca current, Ca stores and Na–Ca exchange system.

10 H. Reuter and N. Seitz, J. Physiol., Lond. *195*, 451 (1968).

Transport of sodium, water, 3-O-methyl-glucose and L-phenylalanine in vitro in biotin-deficient rats intestine

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

Institute of General Physiology, University of Camerino (Italy), 3 March 1977

Summary. In biotin-deficient rats, a decreased intestinal transport of Na^+, H_2O and L-phenylalanine, and no transport differences of 3-O-methyl-D-glucose were observed. The lower Na^+ and L-phenylalanine transport appears to be referable to a decreased energy availability and probably not to the lack of a carrier.

It has been demonstrated that incorporation of amino acids into proteins of liver, intestinal mucosa, pancreas and skin in vivo and in vitro, is markedly decreased in biotin deficiency, and that a single injection of biotin to the biotin deficient rats stimulates amino acid incorporation, both in vivo and in vitro, more than 2fold[1–3]. The evaluation of the amino acids incorporation into liver proteins in vivo and in vitro indicated that the synthesis of some proteins was highly stimulated, while the synthesis of other proteins was not stimulated at all[2]: such a specificity has already been described[4,5]. The biotin-mediated stimulation was suppressed following treatment with inhibitors of protein or RNA synthesis, like puromycin, ethionine or actinomycin D. The effect of biotin on protein synthesis was 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 acids incorporation by normal rat liver ribosomes, if compared 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].

Table 1. Sodium, water and L-phenylalanine transport: values obtained from everted intestinal sacs of normal control and biotin-deficient rats

Groups	Transport		
	Water ($ml/g^{-1}\ h^{-1}$)	Sodium ($\mu moles/g^{-1}\ h^{-1}$)	L-phenylalanine ($\mu moles/g^{-1}\ h^{-1}$)
Normal rats (6)	6.34 ± 1.14	911 ± 165	62.2 ± 7.5
Biotin-deficient rats (7)	3.27 ± 0.94*	470 ± 137*	43.3 ± 10.7*

Incubating fluid: Krebs-Henseleit bicarbonate (pH 7.4) + 10 mM L-phenylalanine.
Numbers in parenthesis indicate the number of animals. Values are expressed as mean + SD. * $p < 0.001$; significant difference from normals (Student's t-test).

In biotin deficiency, the energy production is impaired by decreased utilization of glucose and by decreased oxidative phosphorylation[9]; also the lipid content of mitochondria is significantly decreased[9]. The liver acetyl CoA carboxilase activity, as well as the in vivo incorporation of acetate-1-^{14}C into liver phospholipid fraction of biotin-deficient rats, was less than 50% of normal levels. The cholesterol synthesis is also altered[10]. The biotin-deficient rat liver mitochondria showed decreased phosphorylation efficiency and poor respiratory control, as compared to normal rat liver mitochondria, when NAD^+-linked substrates were oxidized. No difference 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 was localized at site I[11,12]. The locus of damage in energy conservation is

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. it. 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., in press.
9 K. Dakshinamurti, V. V. Modi and S. P. Mistry, Proc. Soc. exp. Biol. Med. *127*, 396 (1968).
10 K. Dakshinamurti and P. R. Desjardins, Can. J. Biochem. *46*, 1261 (1968).
11 K. Dakshinamurti, M. A. Sabir and C. Bhuvaneswaran, Archs Biochem. Biophys. *137*, 30 (1970).
12 C. Bhuvaneswaran and K. Dakshinamurti, Archs Biochem. Biophys. *142*, 665 (1971).
13 C. Bhuvaneswaran and K. Dakshinamurti, Int. J. Biochem. *5*, 819 (1974).

at site I and related to the synthesis of the high-energy intermediate rather than to its utilization[12]. Further evidence suggests that the damage is at the energy conservation site I[13].

In the chick liver, the biotin deficiency results in a decrease in the incorporation of ^{32}P into RNA and DNA[14]. In view of the effect of biotin deficiency on protein synthesis, lipogenesis, glucose metabolism and oxidative phosphorylation, we investigated the possibility of a relation between biotin and the intestinal transport of Na^+, H_2O, L-phenylalanine and 3-O-methyl-glucose.

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

Following 12 h fasting and s.c. Nembutal administration (10 mg/100 g b.wt), a 10 cm long tract of jejunum, starting from about 15 cm from the pylorus, was removed and everted according to Wilson and Wiseman technique[15]. One extremity was tied up, the other was connected with a glass cannula and 2 ml of serosal solution were introduced into the intestinal sac. This was diped into 50 ml of mucosal solution gassed with a mixture of 95% O_2 and 5% CO_2; following 5 min preincubation the preparation was incubated 30 min at 28 °C. The mucosal medium was: 50 ml Krebs-Henseleit bicarbonate[16], pH 7.4, added with L-phenylalanine (10 mM) and L-phenyl (2, 3-^3H) alanine (0.5 µCi/ml), or with 3-O-methyl-D-glucopyranose (5.55 mM) and 3-O-methyl-D-(1-^3H) glucose (0.5 µCi/ml); the same solution used as mucosal medium, but with (^{14}C) polyethylene glycol (0.2 µCi/ml) added, was utilized as serosal medium.

Table 2. Sodium, water and 3-O-Methyl-D-glucose transport: values obtained from everted intestinal sacs of normal control and biotin-deficient rats

Groups	Transport Water ($ml/g^{-1}\ h^{-1}$)	Sodium ($\mu moles/g^{-1}\ h^{-1}$)	3-O-methyl-D-glucose ($\mu moles/g^{-1}\ h^{-1}$)
Normal rats (6)	6.63 ± 0.91	969 ± 161	119.9 ± 11.4
Biotin-deficient rats (7)	4.42 ± 0.96*	635 ± 138*	132.8±12.8 NS

Incubating fluid: Krebs-Henseleit bicarbonate (pH 7.4) + 5.55 mM. 3-O-Methyl-D-glucose.
Numbers in parenthesis indicate the number of animals. Values are expressed as mean ± SD. * $p < 0.01$; significant difference from normals (Student's t-test). NS: No significant difference from normals.

Table 3. Sodium, water and L-phenylalanine transport: values obtained from everted intestinal sacs of normal control and biotin-deficient rats

Groups	Transport Water ($ml/g^{-1}\ h^{-1}$)	Sodium ($\mu moles/g^{-1}\ h^{-1}$)	L-phenylalanine ($\mu moles/g^{-1}\ h^{-1}$)
Normal rats (6)	6.33±0.20	908±150	59.7±6.5
Biotin deficient rats (7)	6.36±0.38 NS	913±170 NS	62.3±9.9 NS

Incubating fluid: Krebs-Henseleit bicarbonate (pH 7.4) + 10 mM L-phenylalanine and 5.55 mM D-glucose.
Numbers in parenthesis indicate the number of animals. Values are expressed as mean ± SD. NS: No significant difference from normals (Student's t-test).

At the beginning and at the end of the incubation, the radioactivity of the mucosal and of the serosal medium was determined by means of a liquid scintillation counter Nuclear-Chicago mod. 725; scintillation fluid: 4 g of PPO, 300 mg of POPOP, 700 ml of toluene and 300 ml of methylcellosolve. The net intestinal transport of water was evaluated on the basis of the dilution of the (^{14}C) polyethylene glycol in the serosal fluid; the water transport was referred to 1 g of dry intestinal tissue and to 1 h ($ml\ g^{-1}\ h^{-1}$).

The net intestinal transport of Na^+ was evaluated from the concentration in the incubation medium (143,5 mM) and from the transport of water, since the transported fluid is isotonic. The net transport of L-phenylalanine and 3-O-methyl-D-glucose was calculated from the following relation:

$$\frac{(V_f \cdot C_f - V_i \cdot C_i) \cdot 2}{P} = \mu moles\ g^{-1}\ h^{-1}.$$

V_f and V_i: serosal fluid amounts evaluated at the end and at the beginning of the experiment; C_f and C_i: concentrations evaluated in the serosal fluid at the end and at the beginning of the experiment; P: dry weight of the intestinal tract: at the end of the experiment, the intestinal tract was wiped with filter paper and dryed by heating (100 °C) until constant weight was obtained.

Results and discussion. The experiments were carried out in order to verify the possible existence of a relation between biotin and the intestinal transport of Na^+, H_2O, L-phenylalanine and 3-O-methyl-D-glucose.

The data of table 1 demonstrate that biotin-deficient rats, as compared with normal control rats, show a decrease in intestinal transport, statistically significant ($p < 0.001$), of Na^+, H_2O and L-phenylalanine.

Table 2 indicates that between the biotin-deficient and normal control rat intestine, no transport differences of 3-O-methyl-D-glucose can be observed; the data demonstrate, as previously described (table 1), a decreased Na^+ and H_2O transport, but with smaller statistical significance ($p < 0.01$).

The data of table 3 relate to intestinal Na^+, H_2O and L-phenylalanine transport in the presence of D-glucose (5.55 mM). Such data show that the addition of D-glucose to Krebs-Henseleit bicarbonate solution, restores Na^+, H_2O and L-phenylalanine transport values of deficient rats to the values of normal control rats. The lower Na^+ and L-phenylalanine transport observed in biotin-deficient rat intestine appears to be referable to a decreased energy availability and probably not to the lack of a carrier. Such an hypothesis is supported from the data referred in table 3, showing that Na^+ and L-phenylalanine intestinal transport of biotin-deficient rats reaches the same values observed in normal control animals when D-glucose has been added to the incubation medium. The normal values of the transport of 3-O-methyl-D-glucose, in deficient rats, could be explained if it is accepted that the active outflow mechanism at the level of the serosal side of the intestinal cells[17–19] is more efficient than the active transport mechanism of Na^+ and L-phenylalanine.

14 A. S. Atwal, A. R. Robblee and L. P. Milligan, J. Nutr. *101*, 1555 (1971).
15 T. H. Wilson and G. Wiseman, J. Physiol. *123*, 116 (1954).
16 H. A. Krebs and K. Henseleit, Z. Physiol. Chem. *210*, 33 (1932).
17 A. Faelli, G. Esposito and V. Capraro, Archs Sci. Biol. *50*, 234 (1966).
18 G. Esposito, A. Faelli and V. Capraro, Pflügers Arch. *340*, 335 (1973).
19 G. Esposito, T. Z. Csaky, Archs J. Physiol. *226*, 50 (1974).