SHORT COMMUNICATION

The relationship between plasma biotin concentration and circulating leucocyte $\beta$-methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase

K. BARTLETT*, T. HORSBURGH† AND D. GOMPERTZ‡
Royal Postgraduate Medical School, London

(Received 4 June 1979; accepted 20 September 1979)

Summary
1. A newly developed biotin assay was used to monitor plasma and urinary biotin concentration after the oral administration of biotin.
2. In two normal subjects, leucocyte propionyl-CoA carboxylase and $\beta$-methylcrotonyl-CoA carboxylase were markedly stimulated after the administration of biotin.

Key words: biotin, carboxylases, leucocyte.

Abbreviation: CoA, coenzyme A.

Introduction
Inborn defects of propionyl-CoA carboxylase (EC 6.4.1.3), $\beta$-methylcrotonyl-CoA carboxylase (EC 6.4.1.4; Gompertz, 1974) and pyruvate carboxylase (EC 6.4.1.1; De Vivo, Haymond, Leckie, Bussman, McDougal & Pagliara, 1977) have been described. Variants of propionyl-CoA carboxylase deficiency and $\beta$-methylcrotonyl-CoA carboxylase deficiency which respond clinically to large doses of biotin have also been described (Barnes, Hull, Balgobin & Gompertz, 1970; Gompertz, Draffan, Watts & Hull, 1971; Gompertz, Bartlett, Blair & Stern, 1973). Our experience of this group of defects and our recently developed assay for biotin (Horsburgh & Gompertz, 1978) prompted an investigation of the relationship between plasma biotin concentration and leucocyte carboxylase activity in normal individuals. We report that after the administration of biotin, leucocyte propionyl-CoA carboxylase and $\beta$-methylcrotonyl-CoA carboxylase were markedly elevated. Furthermore, there was a lag period of a week between the peak plasma biotin concentration and the peak of carboxylase specific activities.

Methods
Two healthy male adults received 5 mg of biotin orally twice daily for 7 days. Peripheral blood was obtained by venepuncture. Leucocyte pellets were prepared from 20 ml of blood (Mellman & Tedescu, 1965); 10 ml of blood was used for haematological examination and for plasma biotin estimation (Horsburgh & Gompertz, 1978). Blood was taken at weekly intervals for 8 weeks and biotin was administered during the fourth week.

Leucocyte pellets were homogenized by sonication ($2 \times 10^{-6}$ s) in 1 ml of phosphate buffer (5 mmol/l), pH 7.3. Propionyl-CoA carboxylase and $\beta$-methylcrotonyl-CoA carboxylase were assayed as previously described (Gompertz et al., 1971; Gompertz, Goodey & Bartlett, 1973). Myeloperoxidase was measured by the method of Segal &
Peters (1977). Protein was determined by the Lowry method (Lowry, Rosebrough, Farr & Randall, 1951) and DNA was determined by the method of Le Pecq & Paoletti (1966). Haematological examinations were kindly performed by the Haematology Department, Hammersmith Hospital. Twenty-four hour urine specimens were obtained from one of the participants during this study.

Results

Myeloperoxidase, propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase were measured in leucocyte homogenates. The specific activity of myeloperoxidase remained constant over the experimental period for both subjects (subject no. 1, 0.063–0.081 nmol h⁻¹ µg⁻¹ of DNA; subject no. 2, 0.059–0.082 nmol h⁻¹ µg⁻¹ of DNA). However, both propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase were markedly elevated after the administration of biotin (Fig. 1). This response to biotin was apparent irrespective of whether the specific activity was expressed in terms of protein, as shown in Fig. 1, or in terms of DNA (not shown). Moreover, the ratio of leucocyte protein to DNA was the same in both subjects and remained constant (mean ± 1 SD; subject no. 1, 22.9 ± 4.6 µg of protein/µg of DNA; subject no. 2, 22.0 ± 2.3 µg of protein/µg of DNA).

The maximum specific activities of carboxylases were observed a week after the final administration of biotin. The ratios of propionyl-CoA carboxylase to β-methylcrotonyl-CoA carboxylase were constant (mean ± 1 SD; subject no. 1, 3.37 ± 0.57; subject no. 2, 2.85 ± 0.30). The maximum degree of stimulation was the same for both carboxylases: approximately threefold.

Interestingly, the plasma biotin concentrations in both subjects remained significantly elevated for at least 4 weeks after the final administration of biotin. Similarly, neither carboxylase returned to preload activities over this period. The urinary output of biotin rose to a maximum of about 5 mg/24 h during the administration of biotin and then declined to preload outputs 10 days after the final dose of biotin. A total of 55% of the administered dose was recovered.

Leucocyte and erythrocyte counts, packed cell volumes and total haemoglobin values were constant and within normal limits throughout the experimental period for both subjects.

Discussion

The development of a specific and sensitive method for the estimation of biotin in physiological fluids

![Biotin](image-url)
Plasma biotin and leucocyte carboxylases

(Horsburgh & Gompertz, 1978) has allowed us to follow its plasma and urinary concentration after oral biotin administration. Although the plasma concentrations remained elevated for 4 weeks after biotin administration had ended, the urinary output declined to preload values much earlier. Whether this reflects a renal threshold for biotin or binding of biotin to plasma proteins remains unanswered.

The majority of reports concerning biotin metabolism have involved birds or micro-organisms. However, the fate of [14C]biotin administered intraperitoneally to biotin-sufficient and -deficient rats has been investigated (Lee, McCall, Wright & McCormick, 1973). It was found that in both groups about 95% of the label was recovered in the urine within 24 h. In these studies the amount of biotin was high (5 mg/kg), given as a single dose. In the present investigation the dose was approximately 0.07 mg/kg twice daily, orally for 7 days. The difference in our results and those of Lee et al. (1973) could reflect differences in dose, route of administration or species.

Hsia, Rosenberg & Wolf (1977) have shown that both propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase are elevated in leucocytes after the administration of biotin. These workers suggested that this effect could be utilized to detect patients with biotin-responsive defects of these enzymes (Wolf & Hsia, 1978). Our results confirm those of Hsia et al. (1977) but differ in one respect. We have observed an approximately equal effect on both carboxylases, whereas Hsia et al. (1977) reported a four- to nine-fold stimulation of β-methylcrotonyl-CoA carboxylase, compared with a two- to three-fold stimulation of propionyl-CoA carboxylase. The biotin dose reported was 20 mg/day given to adults for an unspecified length of time. The timing of the venepunctures after the administration of biotin was also not reported. We have measured the plasma biotin concentration at each point that blood was taken for preparation of leucocytes. It is apparent that the peak of stimulation occurred after the peak plasma biotin concentration was observed. However, it may well be that higher carboxylase specific activities would have been observed in our study had smaller time intervals been chosen. Furthermore, the numbers of subjects in both our study and that of Hsia et al. (1977) were small, and the detailed elucidation of this response to biotin must await further studies.

The specific activity of myeloperoxidase, an enzyme specific to leucocytes (Maehly, 1955), remained approximately constant, as did the haematological measurements. These results suggest there was no non-specific effect of biotin on leucopoiesis. Moreover it has been demonstrated that the administration of biotin to biotin-deficient rats results in the stimulation of serum albumin synthesis (Boeckx & Dakshinamurti, 1974). In order to avoid any such effect on leucocyte protein, carboxylase specific activities were measured in terms of both protein and DNA. However, the ratio of leucocyte protein and DNA remained constant in both subjects and a stimulation of both carboxylases was observed regardless of how the specific activities were expressed.

The mechanism of the stimulation of carboxylases by biotin is open to conjecture. The net specific activity of any enzyme is the result of synthesis and degradation of enzyme protein. In the case of the carboxylases, biotin is covalently attached to the inactive apoenzyme by an apoenzyme–biotin ligase (holoenzyme synthetase). It is not known whether apoenzyme is turned over independently of holoenzyme, but clearly biotin could either activate synthesis or inhibit degradation at either point. In addition, in Bacillus stea rothermophilus holopyruvate carboxylase synthetase is regulated by a number of allosteric effectors (Cazzulo, Sundaram & Kornberg, 1970), although in chicken liver no such regulation was detected (Madappally & Mistry, 1970). There appear to be no reports of studies in higher animals in the literature. In this connection it is of interest that normal cultured fibroblasts do not exhibit a response to high biotin concentrations (Bartlett & Gompertz, 1976; Weyler, Sweetman, Maggio & Nyhan, 1977).

References


