

## The assessment of biotin status in man and animals

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The metabolic role of biotin in animals is as a prosthetic group in enzymes involved in carboxylation reactions. There are three enzymes of principal importance. Pyruvate carboxylase (PC; *EC* 6.4.1.1) catalyses the conversion of pyruvate to oxaloacetate within the mitochondrion. Oxaloacetate is an intermediate in the biosynthesis of phosphoenol pyruvate and, ultimately, glucose. Thus, PC plays an essential role in gluconeogenesis via pyruvate. Liver and kidney are the tissues with greatest gluconeogenic capacity and they also contain highest activities of the enzyme.

The conversion of pyruvate to oxaloacetate also influences lipogenesis, since acetyl-CoA generated within the mitochondrion must combine with oxaloacetate to form citrate before it can pass into the cytoplasm. Once there, citrate is cleaved to release acetyl-CoA which can then enter the lipogenic pathway.

Lipogenesis also requires the presence of another biotin-dependent enzyme, acetyl-CoA carboxylase (ACC; *EC* 6.4.1.2), for normal function. This enzyme catalyses the conversion of acetyl-CoA to malonyl-CoA, the starting point in the biosynthesis of fatty acids.

The third enzyme is involved in propionate metabolism. Propionyl-CoA carboxylase (PCC; *EC* 6.4.1.3) converts propionyl-CoA to methylmalonyl-CoA which can ultimately enter the citric acid cycle after conversion to oxaloacetate. This pathway is especially important for ruminants which, because of ruminal degradation of glucose, have to rely on it almost entirely for their supply of glucose.

Biotin is thus directly involved in the vitally important metabolic process of glucose and fat synthesis and can also have profound effects on other pathways by its influence on many metabolic intermediates. It is therefore necessary for normal utilization of nutrients, growth of body tissues, reproduction and ultimately, life. This general metabolic role, together with its involvement in certain specific disorders, means that an inadequate supply of biotin can have serious consequences and, in farm animals, can result in considerable economic loss as a result of both decreases in performance and increases in mortality. However, synthetic biotin is a comparatively expensive vitamin and an over-supply to intensively-housed animals can be wasteful. Thus it is of considerable importance to be able to assess the extent to which an animal's need for biotin is being met.

### *Effects of a deficiency*

The initial effects of a deficiency are decreases in the activities of the biotin-dependent enzymes, although not all of these are affected to the same extent. Thus in the livers of deficient chicks and rats the decreases in the activities of PC and PCC are proportionately larger than the decreases in ACC activity (Arinze &

Mistry, 1971), perhaps because the presence of ACC in the cytoplasm allows it freer access to the biotin available.

The decreases in enzyme activities affect the metabolic pathways involved. In gluconeogenesis a loss of PC activity results in an accumulation of pyruvate and lactate. Decreased activity of ACC results in a reduced rate of lipogenesis and abnormalities in fatty acid synthesis.

Biotin deficiency also has effects on other metabolic pathways which do not involve biotin directly since they are not inhibited *in vitro* by avidin. For instance, protein synthesis is impaired (Polsnanskaya, 1957) and this is associated with an inhibition of RNA synthesis (Dakshinamurti & Litvak, 1970).

The direct and indirect biochemical effects of a deficiency bring about characteristic pathological changes in affected animals such as reduced growth and reproductive performance and dermatitis characterized by hyper- and parakeratosis. The dermal lesions are exacerbated by pressure or abrasion and hence are worse on undersurfaces of the feet.

#### *General indications of biotin status*

*Dietary intake.* If an animal's requirement for a nutrient is known, it should be possible to deduce its likely status on the basis of dietary intake. However, not all of the biotin in some common feedstuffs is biologically available (Frigg, 1976, 1977), hence dietary measurements may not give a reliable indication of useful intake. Other factors can influence the supply of biotin. For instance, intestinal micro-organisms can use or synthesize biotin. The direct contribution of intestinally-synthesized biotin to the biotin supply in monogastrics is uncertain, but recent experiences suggest that in poultry and pigs at least it is negligible. However, intake will be increased if an animal indulges in coprophagy routinely, as with the rat, or perhaps more infrequently, the chicken. Thus, method of husbandry, if it affects the extent of coprophagy, will also affect biotin status.

*Clinical signs.* Although the clinical signs of biotin deficiency are characteristic, the lesions can resemble those caused by deficiencies of other B vitamins, such as pantothenic acid, or minerals, such as zinc or sodium. Moreover, they are usually only manifest when the deficiency has become comparatively severe. They are thus of only limited use in identifying and quantifying a deficiency and obviously are of no use in detecting a subclinical deficiency, which nevertheless may result in serious consequences.

The most specific criteria for identifying and quantifying a biotin deficiency are biochemical and there has been considerable progress in developing such methods for several species over the last few years.

#### *Birds*

*Biotin levels.* The most direct method is the measurement of biotin levels in blood or tissues such as liver. Experimentally, good relationships have been demonstrated in chicks between biotin intake, plasma biotin levels and growth

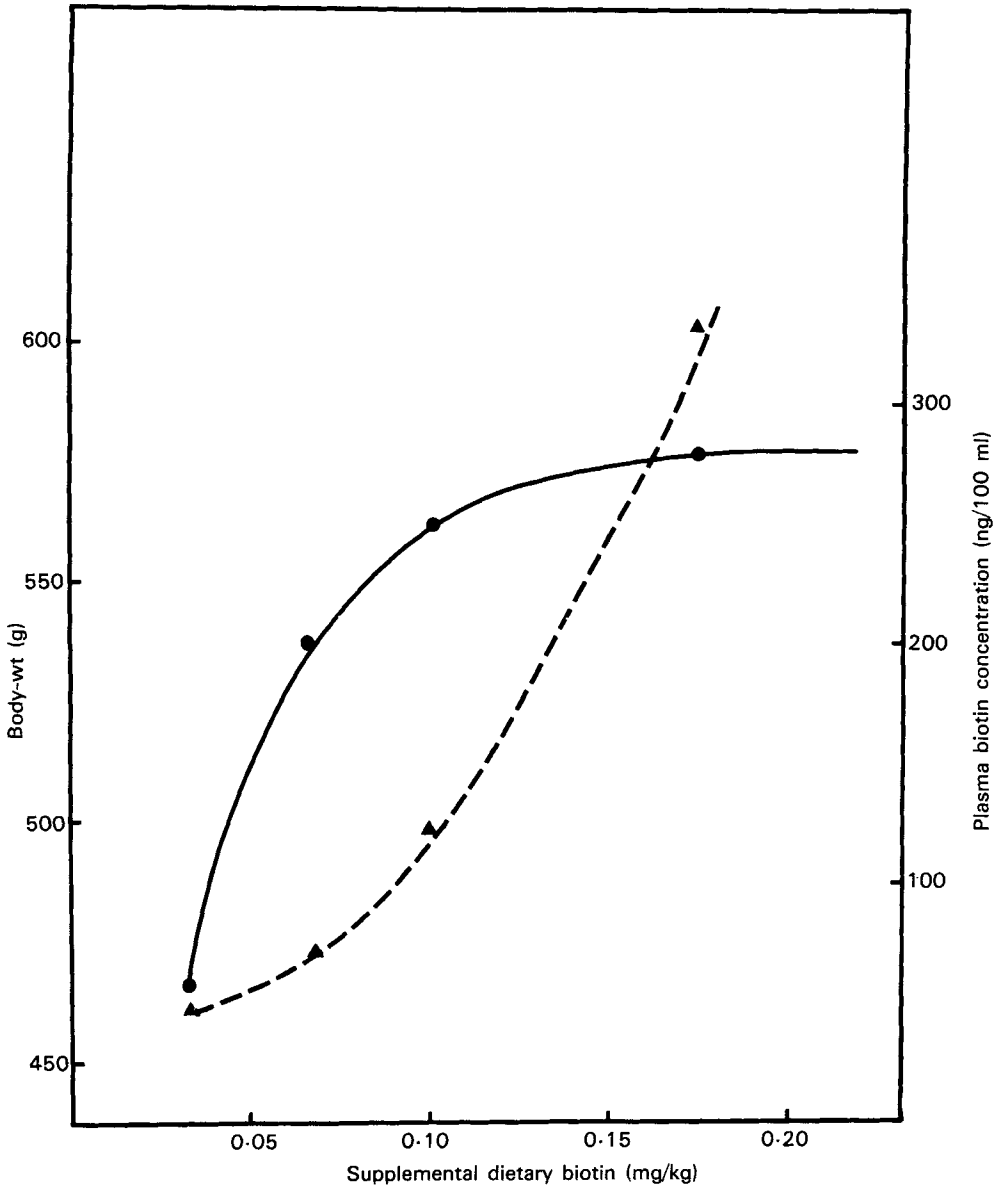


Fig. 1. Responses of body-weight (●—●) and plasma biotin concentration (▲—▲) at 38 d in chicks fed diets containing different levels of supplemental biotin (Frigg *et al.* 1973).

rate and it has been possible as a result to set blood levels that are consistent with a good biotin status in young chicks as is shown in Fig. 1 (Frigg *et al.* 1973).

When birds reach sexual maturity, the hormone-mediated changes initiated for the transfer of nutrients to the egg result in a tenfold increase in plasma biotin levels (Whitehead, 1980) and at present there are not sufficient results to indicate a

satisfactory plasma biotin concentration in breeding hens. However, levels in egg yolk from these birds have been measured and have shown direct relationships with both the biotin intake of the hen and the hatchability of the eggs (Brewer & Edwards, 1972).

Biotin concentration in liver has also been used to give information on biotin status. It has been especially useful in post-mortem studies, such as by Hood *et al.* (1976) on fatty liver and kidney syndrome.

Because of variability between individuals, direct measurements of biotin are of most use on a group basis. Moreover, they do not give information on the distribution of biotin or the function of biotin-dependent pathways. For a more specific indication of biotin status, particularly in relation to likely biotin requirement, measurements of the activities of biotin-containing enzymes give better information.

*Biotin-dependent enzymes.* In birds, the most important of the biotin-dependent enzymes are PC and ACC. Activities of both are related to biotin status but whereas activity of ACC in deficient birds is reduced to 60% of normal, activity of PC is reduced to 20% (Arinze & Mistry, 1971). Thus the much greater variation in activity of PC makes this enzyme more suitable as an indicator. In tissues, PC activities in liver and kidney are most sensitive to changes in biotin intake. In chicks activity in the liver is proportional to biotin intake over a wide range of dietary biotin levels (Atwal *et al.* 1971). Unfortunately, PC activities in liver change markedly with age and can also be affected by other aspects of the diet such as protein or fat level (Whitehead & Bannister, 1978).

Since measurement of tissue enzymes invariably necessitates the death of the animal, a more suitable criterion of biotin status is the activity of an enzyme present in blood. In avian species, red blood cells are nucleated and the remnants of mitochondria that they are believed to contain, account for the comparatively high levels of activity of PC that have been found in the blood of young chickens and turkeys (Bannister & Whitehead, 1976; Whitehead & Bannister, 1978). Moreover, activity of this enzyme has been found to give a good indication of biotin status in these species over a wide range of dietary biotin intakes. Activities decrease over the first few weeks of life, but are comparatively constant in broilers from 4 to 8 weeks of age. In poults, activity is a satisfactory criterion up to at least 12 weeks of age. Activity can be affected by dietary fat and protein levels, though to a much lesser extent than hepatic activity (Whitehead & Bannister, 1978). Activity of PC can be preserved in a glycerol-based medium, thus extending its usefulness to field samples (Glatzle *et al.* 1980a,b).

The relationship between enzyme activity and biotin intake is sigmoid in nature but over a wide range of dietary levels the enzyme response is linear. At the higher levels, there is a close relationship between enzyme activity and growth rate in broilers, as is shown in Fig. 2, with the result that the activity of PC is a good criterion of biotin requirement as well as status in broilers (Whitehead & Bannister, 1980). Unfortunately, as a result of changes in activity with age, this enzyme does not give a satisfactory indication of biotin status in adult birds.

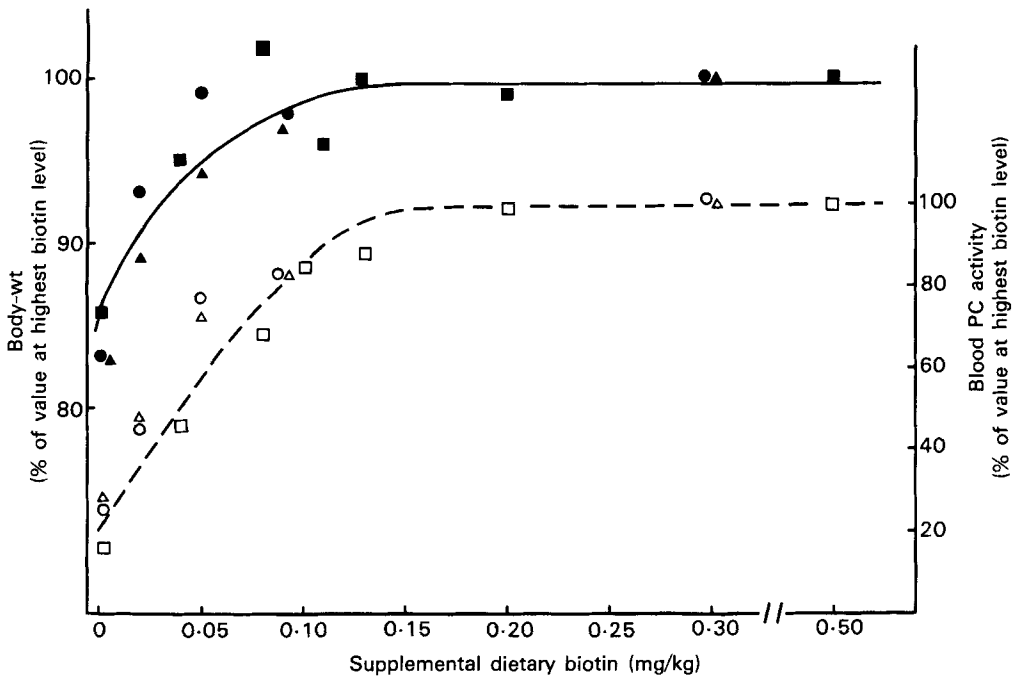


Fig. 2. Responses of body-weight (—) and blood pyruvate carboxylase (PC) activity (---) at 5 weeks in three experiments in male (●○, ■□) and female (▲△) broilers fed on diets containing different levels of supplemental biotin (Whitehead & Bannister, 1980).

ACC also occurs in blood but, as in liver, does not show a large response to changes in dietary biotin intake (Glatzle & Frigg, 1975).

Since the reduction in enzyme activity arising from a biotin deficiency is caused by a lack of biotin rather than apoenzyme, the extent to which enzyme specific activity can be enhanced by incubation *in vitro* with biotin can be used to give information on vitamin status. Thus an activation coefficient greater than unity implies that there was insufficient vitamin present originally to permit maximum enzyme specific activity. In chickens, Glatzle *et al.* (1980a,b) have shown that the activation coefficients of both ACC and PC give a good indication of biotin status. These authors have also demonstrated activation of PC, but not ACC, in blood. It is an advantage of this technique that it provides a better indication of the status of an individual than does measurement of enzyme specific activity.

**Fatty acid composition.** Among the indirect biochemical effects of biotin deficiency, changes in tissue fatty acid composition can be the most obvious. In deficient chicks, the proportion of palmitoleic acid is increased, mainly at the expense of stearic acid (Balnave, 1966). Unfortunately, these changes in fatty acid composition can be affected by other dietary constituents such as protein or fat (Whitehead *et al.* 1976). Nevertheless, in some situations, the palmitoleic acid:stearic acid value can be used to give some indication of biotin status (Edwards, 1974) as shown in Table 1.

Table 1. *Palmitoleic acid:stearic acid values in tissues of chicks fed low biotin diets containing maize oil (20 g/kg diet) or deficient in essential fatty acids (EFA) and supplemented with graded levels of biotin\**

(Mean values with their standard errors)

Tissue	Supplemental dietary biotin ( $\mu\text{g}/\text{kg}$ )												
	20		40		60		100		180		340		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
EFA-deficient	Toe	14.2	1.0	11.5	1.4	9.9	1.9	9.8	1.9	5.5	0.2	6.3	1.0
	Liver	1.4	0.2	1.9	1.3	1.4	0.5	1.2	0.5	0.5		0.4	
Maize oil (20 g/kg diet)	Toe	9.7	0.7	17.1	2.6	16.6	2.0	10.6	1.6	4.5	0.2	4.3	
	Liver	1.0	0.2	1.2	0.6	2.9	1.1	0.3		0.1		0.2	

\*Edwards, 1974.

*Fatty liver and kidney syndrome (FLKS)*. FLKS is a nutritional disorder involving an almost total failure of PC in the liver of young chicks (Bannister, 1977) and can occur even when the diets are only very marginally deficient in biotin. It is worse when other aspects of the diet, such as fat or protein levels, accentuate the metabolic need for lipogenesis since it appears that when biotin supply in the liver is limited, the requirement of ACC is met preferentially at the expense of PC (Whitehead *et al.* 1978). Thus hepatic gluconeogenesis via pyruvate becomes minimal and if the bird is subject to a stress or starvation that inhibits or depletes other metabolic sources of glucose, a severe hypoglycaemia develops rapidly and leads to death (Bannister *et al.* 1979). In affected birds, the loss of PC activity is confined to the liver; activity in the blood or kidney is not depressed (Bannister & Clelland, 1978). It is therefore not possible to identify birds that are most at risk by measurement of any variable directly related to biotin metabolism. However, it has been found that low liver PC activities are closely correlated with high plasma activities of lactate dehydrogenase and high palmitoleic acid:stearic acid values in plasma triglycerides and it has proved possible to identify especially susceptible individuals in advance by measurement of these two plasma parameters (Whitehead *et al.* 1978).

#### *Other animals*

Recent evidence that treatment with biotin can heal foot lesions and improve reproductive performance in pigs (Brooks *et al.* 1977) has stimulated efforts to develop biochemical criteria of biotin status in these animals. Plasma biotin levels are related to dietary intake and have been used to support the diagnosis of deficiency in herds affected with lameness (Brooks *et al.* 1977). However, as with poultry, biotin levels can vary considerably between individuals and give no indication of status in relation to requirement.

The activities of biotin-dependent enzymes in tissues of mammals are related to biotin intake, but plasma measurements are obviously preferable for valuable

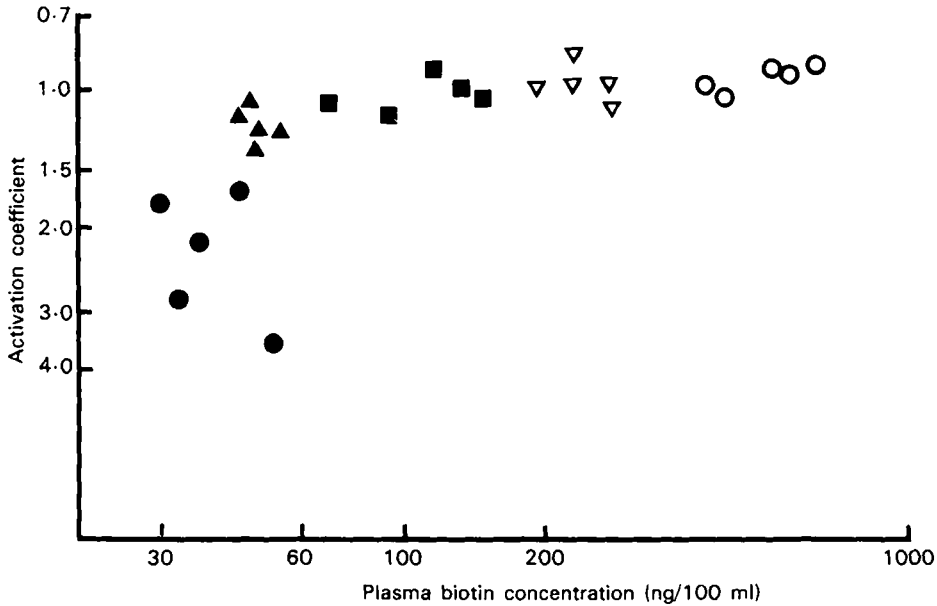


Fig. 3. Activation coefficients for pyruvate carboxylase in the blood of young pigs given diets supplemented with 0 (●), 33 (▲), 100 (■), 300 (▽) and 900 (○) µg biotin/kg (Glatzle, 1979).

animals. Unfortunately, the lack of nucleated red cells means that blood PC activity is much lower than in avian species. Nevertheless, activity can be measured and has been found to show a relationship with biotin intake in young pigs (Glatzle, 1979; Whitehead & Bannister, 1980). Moreover, this relationship may also give an indication of dietary biotin requirement.

Despite low PC specific activity in mammalian blood, activation coefficients have been measured in rats (Bitsch *et al.* 1977) and pigs (Glatzle, 1979) and in the latter have shown relationships with both dietary and plasma biotin levels (Fig. 3) which may be of use in determining status in relation to requirement.

### Humans

Uncertainties over biotin intakes and requirements in humans make assessment of status difficult. The two principal criteria of status that have been used are biotin levels in urine and blood. Blood levels can show extremely wide variation between individuals, so although differences have been observed between the means of groups, such as lower levels in the elderly, the significance of low plasma levels is uncertain, especially since instances of biotin deficiency appear to be rare (Bonjour, 1977). However, in children, urine and blood levels appear to give an indication of biotin status. Levels in these fluids are lower than normal in infants affected with seborrhoeic dermatitis and the related Leiner's disease (Berger, 1950; Svejcar & Homolka, 1950; Chiari, 1952), two conditions for which the standard treatment is injection of biotin.



There is little information on the activities of biotin-dependent enzymes in relation to biotin intake in humans. However, conditions brought about by a specific failure of biotin-dependent enzymes such as PCC and B-methylmalonyl-CoA carboxylase have been identified and treated with large doses of biotin (Gompertz, 1974; Keeton & Moosa, 1976).

Instances of unexpected sudden death can occur in infants in the condition known as Cot Death Syndrome or Sudden Infant Death Syndrome (SIDS). Although the cause is unknown, similarities in the condition to FLKS have led to suggestions that a marginal biotin deficiency coupled with a sudden stress may be involved (Johnson *et al.* 1978). Post-mortem analysis have shown that the liver biotin concentrations of SIDS victims are lower than those of infants in the same age group who died from known causes (Johnson *et al.* 1980). Although there is thus circumstantial evidence that biotin may be involved in the condition, adequate biochemical criteria are not available at present to make a more detailed assessment of biotin status in humans.

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