Br. J. Nutr. (1978), **39**, 547

Blood pyruvate carboxylase (*EC* 6.4.1.1) activity as a criterion of biotin status in chickens and turkeys

BY C. C. WHITEHEAD AND D. W. BANNISTER

Agricultural Research Council's Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh EH9 3JS

(*Received* 28 *July* 1977 – *Accepted* 1 *September* 1977)

1. Blood pyruvate carboxylase (pyruvate-CO_2 ligase (ADP-forming); *EC* 6.4.1.1; PC) activities in young chickens and turkeys given low-biotin diets supplemented with biotin at graded levels were studied in three experiments.

2. In both species PC activity was related positively to the supplemental biotin level. The relationship was sigmoid and maximum activity was attained with supplemental levels above those required to give maximal growth response.

3. Enzyme activity decreased between 2 and 4 weeks of age but remained almost constant thereafter.

4. Activity in chicks was not affected by alterations in the fat or protein content of the diet.

5. Changing poults from high to low and from low to high supplemental biotin levels resulted in reversals in the levels of enzyme activity.

6. It is concluded that blood PC activity is a promising new criterion for assessing the biotin status of young chickens and turkeys.

Specific criteria are available to assess the nutritional status of poultry with respect to several of the B-vitamins, e.g. riboflavin (Glatzle, Weber & Wiss, 1968) and thiamin (Brin, Tai, Ostashever & Kalinsky, 1960). However, the study of biotin is hampered by the lack of a suitable functional test for this vitamin.

Biotin can be measured directly, and levels in both blood (Frigg, Weiser & Bollinger, 1973) and liver (Hood, Johnson, Fogerty & Pearson, 1976) of chickens have been found to be related to the biotin intake, on a group if not on an individual basis. However, the assay techniques, whether based on microbiological (Wright & Skeggs, 1944) or chemical (Dakshinamurti, Landman, Ramamurti & Constable, 1974; Hood, 1975) methods are difficult and laborious.

The metabolic role of biotin is that of cofactor for several enzymes involved in carboxylation reactions and the activities of these enzymes can be affected by the biotin status of the animal. For example, the activity of pyruvate carboxylase (pyruvate-CO_2 ligase (ADP-forming, *EC* 6.4.1.1; PC) in the chicken liver is related to the biotin level of the diet over a wide range (Atwal, Robblee & Milligan, 1971). However, enzyme activity can vary considerably with age and with dietary factors such as fat and protein level (Whitehead, Bannister & Cleland, 1978).

Activity of another biotin-dependent hepatic enzyme, acetyl-CoA carboxylase (acetyl-CoA-CO_2 ligase (ADP-forming), *EC* 6.4.1.2), is less sensitive to biotin intake than PC (Achuta Murthy & Mistry, 1972) perhaps because, as a cytoplasmic enzyme, it has freer access to available biotin than has the intramitochondrial PC. A further disadvantage is that activity can be affected by a variety of other factors, such as the presence of a natural hydrophobic inhibitor (Moss & Lane, 1971). This enzyme also occurs in chicken blood and its activity was found to be related to biotin status (Glatzle & Frigg, 1975). However, the difference in activity over a wide range of dietary biotin levels was not large and, if activity in blood is as susceptible to other factors as it is in tissues, it may not be a suitable criterion for the assessment of biotin status.

Table 1. *Composition (g/kg) of basal diets*

Ingredient	Diet no. 1	2	3	4	5	6	7
Wheat	700	570	570	620	620	580	720
Maize starch	45	150	—	150	—	100	—
Soya-bean meal	35	40	40	40	40	—	80
Herring meal	50	100	100	100	100	45	145
Low-vitamin casein	60	60	60	25	25	65	—
Gelatin	30	30	30	15	15	35	—
Promine-R*	—	—	—	—	—	100	—
Maize oil	40	10	70	10	70	30	30
Cellulose	—	—	90	—	90	—	—
Limestone flour	16	16	16	16	16	16	16
Dicalcium phosphate	15	15	15	15	15	15	15
Salt (sodium chloride)	3	3	3	3	3	2·5	2·5
Chlorine chloride	0·3	0·3	0·3	0·3	0·3	0·2	—
DL-methionine	2	2	2	2	2	—	—
Mineral mixture	2·5	2·5	2·5	2·5	2·5	2·5	2·5
Vitamin mixture 1†	2·5	2·5	2·5	2·5	2·5	—	—
Vitamin mixture 2‡	—	—	—	—	—	2·5	2·5
Santoquin§	+	+	+	+	+	+	+
Chemical analysis							
Crude protein (nitrogen × 6·25)	210	245	248	198	200	274	221
Diethyl ether extract	57	31	91	30	92	43	54
Calculated available biotin (mg/kg)	0·04	0·05	0·05	0·05	0·05	0·03	0·08

* Openheimer Casing Co., Edinburgh.

† Supplied (/kg diet): retinol 600 μg, cholecalciferol 15 μg, α-tocopherol 17 mg, menaphthone 1·3 mg, riboflavin 4 mg, nicotinic acid 28 mg, pantothenic acid 10 mg.

‡ Supplied (/kg diet): retinol 3600 μg, cholecalciferol 45 μg, α-tocopherol 24 mg, menaphthone 4·8 mg, thiamin 4·8 mg, riboflavin 12 mg, nicotinic acid 60 mg, pantothenic acid 20 mg, pyridoxine 6 mg, cyanocobalamin 0·03 mg, pteroylmonoglutamic acid 2·4 mg.

§ Monsanto Chemicals Ltd., London. Supplied by Leather Chemicals Ltd., Yeadon, Leeds.

More recently, a technique for the assay of PC in chicken blood cells has been published (Bannister & Whitehead, 1976) and the present paper describes subsequent experiments designed to assess this parameter as a criterion of biotin status in chickens and turkeys.

EXPERIMENTAL

Birds and husbandry

The chickens were Marshall's male broilers and the turkeys were mixed sex B.U.T. Triple 6. Up to 4 weeks of age they were housed in electrically-heated four-tier brooders. When birds were kept beyond 4 weeks, they were housed in single-bird cages, 400 × 300 mm, with wire floors and individual food and water troughs. Food and water were freely available at all times. Live weight and food consumption were measured weekly and birds were assessed periodically for clinical signs of biotin deficiency (Whitehead, 1977) and rated on an arbitrary scale from 0 (no lesions) to 5 (severe lesions).

Diets

The compositions of the basal experimental diets are given in Table 1. The diets were formulated to contain low levels of available biotin and were based mainly on wheat and purified proteins. Available biotin concentrations were calculated using the information

given by Anderson & Warnick (1970) and Frigg (1976). Other experimental diets were obtained by supplementing the basal diets with graded levels of D-biotin.

Enzyme assay

Blood samples (1 ml) were taken from the wing vein into heparinized syringes and PC activity was measured immediately by the method of Bannister & Whitehead (1976). Protein concentration was determined using an automated version of the method of Lowry, Rosebrough, Farr & Randall (1951) with haemoglobin as the standard; the enzyme activity was expressed as nmol $^{14}CO_2$ incorporated/g haemoglobin per h at 38°.

Procedure

Expt 1. Enzyme activities of the blood plasma of chicks fed on diets containing a range of biotin levels were measured. Basal diet 1 was supplemented with five levels of biotin: 0·01, 0·02, 0·05, 0·10 and 0·15 mg/kg diet. Each diet was fed to a group of twelve birds from 1-d-old to 4 weeks of age, when blood samples were taken and the experiment terminated.

Expt 2. In this experiment the effects of dietary biotin, fat levels and age on enzyme activity in chicks were studied. Up to 4 weeks of age basal diets 2 and 3 were used. There was 30 g fat/kg in diet 2 but in diet 3 this was increased to 90 g/kg by the addition of maize oil at the expense of an isoenergetic amount of starch, using cellulose as an inert filler. Both basal diets were supplemented with 0·05, 0·10 and 0·50 mg biotin/kg and each diet was fed to ten birds. From 4 to 8 weeks of age diets 2 and 3 were replaced by diets 4 and 5 respectively to accommodate the reduced protein requirements of the birds; the biotin levels, however, remained unchanged. Group size was reduced to five birds from week 4. Enzyme activities were measured at 2, 4, 6 and 8 weeks.

Expt 3. In this experiment the effect of dietary biotin level on enzyme activity in turkeys at different ages and also the speed of response of activity to changes in dietary biotin level were investigated. From 0 to 5 weeks, basal diet 6, supplemented with 0·05, 0·10, 0·15, 0·20, 0·30, 0·40 or 0·60 mg biotin/kg, was fed to groups of ten birds. Enzyme activities were measured at 2 and 4 weeks. Two other groups of four birds were given basal diet 6 or the basal diet supplemented with 0·60 mg biotin/kg until 3 weeks of age when the diets were changed. Enzyme activities were measured at intervals thereafter until 5 weeks of age.

At 5 weeks all birds were killed except five from each of the groups given supplemental levels of 0·05, 0·15, 0·30 and 0·60 mg biotin/kg. They were given diets based on basal diet 7, but since this was calculated to contain more biotin than diet 6, the levels of supplementation were reduced to 0, 0·10, 0·25 and 0·55 mg biotin/kg to ensure that biotin intake remained constant. The enzyme activities of these birds were measured at 6 and 12 weeks of age when the experiment ceased. All birds in this part of the experiment were dissected post mortem in order to determine their sex.

RESULTS

Expt 1. Results for this experiment are shown in Fig. 1. Birds given biotin at the two lowest levels showed dermal lesions characteristic of moderate biotin deficiency and signs of mild deficiency were apparent in the group given 0·02 mg supplemental biotin/kg. Growth was appreciably depressed in these groups. There was a direct relationship between level of supplemental biotin and blood PC activity over the range investigated and, although there was no significant increase in growth with supplemental biotin levels above 0·05 mg/kg, enzyme activity continued to increase over the whole range of biotin levels studied.

Expt 2. The results are given in Table 2. The performances of birds on the low- and high-fat diets were similar, growth was depressed by both basal diets and there were pro-

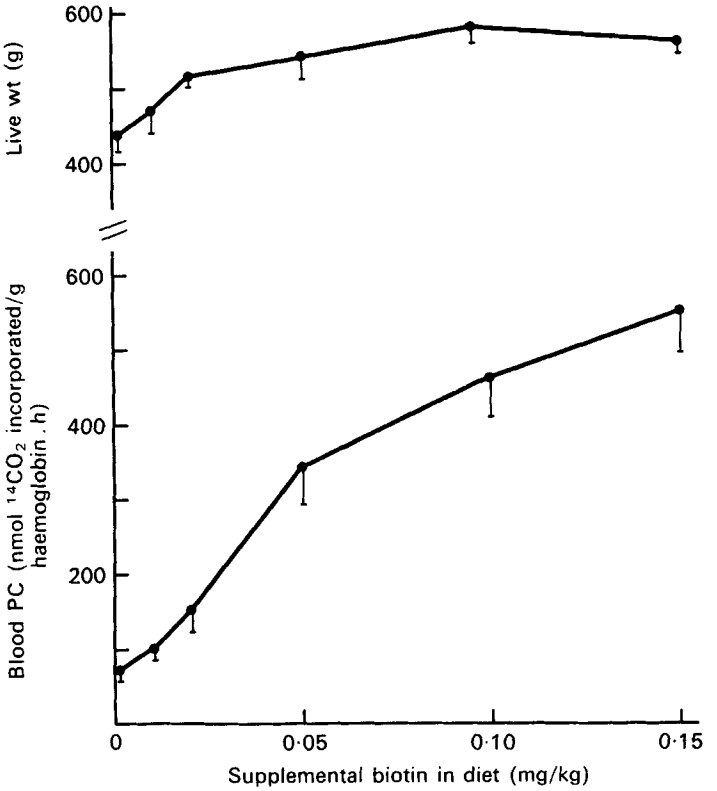

Fig. 1. Expt 1. Relationship between dietary supplemental biotin level (mg/kg) and blood pyruvate carboxylase (pyruvate-CO_2 ligase (ADP-forming), *EC* 6.4.1.1; PC) activity (nmol $^{14}CO_2$ incorporated/g haemoglobin per h) and live weight (g) at 4 weeks of age in chicks. Mean values with their standard errors represented by vertical bars. For details of diet, see Table 1.

nounced symptoms of biotin deficiency. However, no fatty liver and kidney syndrome occurred.

Blood PC activity was related to dietary biotin level, there being an approximately linear relationship between the two over the range 0–0·10 mg/kg. However, although at 4, 6 and 8 weeks enzyme activity showed little or no increase with more than 0·10 mg supplemental biotin/kg, at 2 weeks there was a significant response to a higher supplemental level.

There was a significant ($P < 0·05$) effect of age on enzyme activity. With all diets it decreased from week 2 to week 4 but thereafter remained comparatively steady although some of the groups given the lower levels of biotin did show an increase at 8 weeks.

There was no significant effect of dietary fat level on enzyme activity at 4, 6 or 8 weeks. However, at 2 weeks of age, chicks given the high-fat diets supplemented with the two highest levels of biotin had significantly ($P < 0·05$) higher enzyme activities.

Expt 3. The growth rate of turkey poults to 4 weeks was significantly ($P < 0·05$) depressed when the supplemental biotin level was 0·10 mg/kg or less (Fig. 2). Poults given a supplement of 0–0·05 mg/kg showed severe signs of biotin deficiency and those given 0·10 mg/kg exhibited mild signs.

Enzyme activity was related to dietary biotin up to a supplemental level of 0·30 mg/kg but above this level there was no further increase in activity at either 2 or 4 weeks of age (Fig. 2).

Table 2. *Expt 2. Blood pyruvate carboxylase (pyruvate-CO_2 ligase (ADP-forming), EC 6.4.1.1; PC) activity, live weight, food conversion efficiency (FCE) and severity of lesions of biotin deficiency in chickens fed on diets* containing different levels of biotin and fat*

(Mean values with their standard errors for ten birds/treatment at weeks 2 and 4 and for five birds/treatment at weeks 6 and 8. Activity was expressed as nmol $^{14}CO_2$ incorporated/g haemoglobin per h at 38° and lesions were assessed on the scale 0 to 5 (very severe))

Fat (g/kg)	Supplemental biotin (mg/kg)	Blood PC activity: 2 weeks Mean	2 weeks SE	4 weeks Mean	4 weeks SE	6 weeks Mean	6 weeks SE	8 weeks Mean	8 weeks SE	Live wt (g) at 4 weeks Mean	Live wt (g) at 4 weeks SE	FCE at 4 weeks	Severity of lesions at 4 weeks
30	0	208	32	101	15	98	30	119	30	619	23	1·95	1·6
	0·05	465	89	346	52	288	70	466	69	659	40	1·99	0·2
	0·10	631	81	579	33	565	64	600	80	690	27	1·88	0
	0·50	794	70	617	53	563	31	575	44	665	26	1·81	0
	Mean	524		410		378		440		658			
90	0	221	26	121	19	98	32	121	43	590	41	1·97	0·8
	0·05	431	56	296	39	389	23	560	49	694	25	1·95	0
	0·10	797	58	600	30	512	53	567	52	690	24	1·88	0
	0·50	1079	102	623	36	658	72	645	98	723	24	1·88	0
	Mean	632		410		414		473		674			

* For details of composition see Table 1.

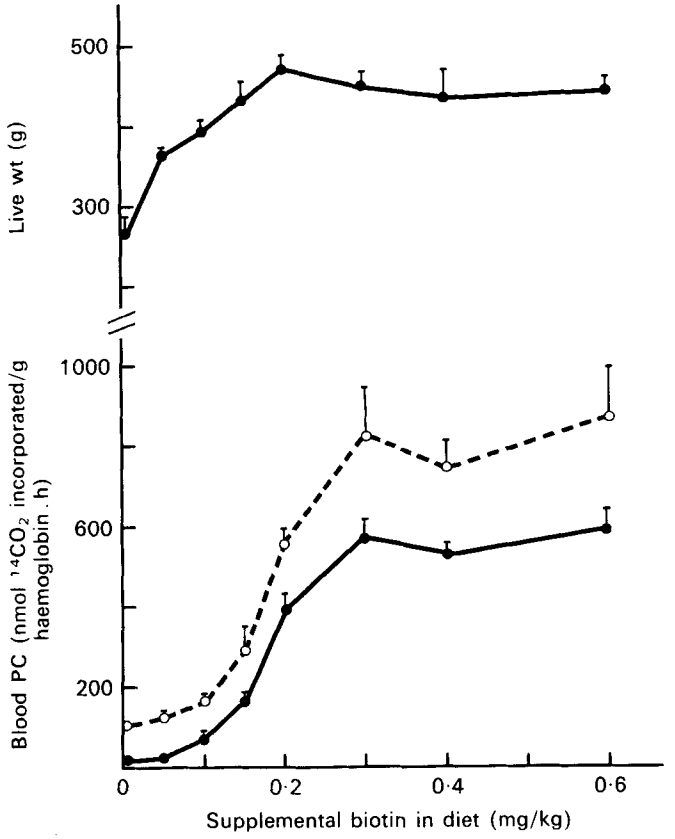

Fig. 2. Expt 3. Relationship between dietary supplemental biotin level (mg/kg) and blood pyruvate carboxylase (pyruvate-CO_2 ligase (ADP-forming), *EC* 6.4.1.1; PC) activity (nmol $^{14}CO_2$ incorporated/g haemoglobin per h) at 2 (○ - - - ○) and 4 (●—●) weeks of age and live weight (g) at 4 weeks of age in poults. For live weight, values are the means of the mean male and female weights, with their standard errors represented by vertical bars. For details of diet, see Table 1.

There was a decrease in enzyme activity between 2 and 4 weeks at all biotin levels (Fig. 3). Thereafter the PC activities of poults given the higher levels of biotin continued to decrease whereas those of poults given low levels increased, so that by 12 weeks the effect of dietary biotin level on enzyme activity was much less pronounced.

The speed of response of enzyme activity to changes in the dietary biotin level is shown in Fig. 4. After 2 d the activities responded to the new dietary level and after 11 d they were starting to level off at their new values. Even so, after 18 d the activities of both groups still had not reached the corresponding values of poults fed on the same diets continuously from 1 d of age (see Fig. 3).

The enzyme activities of male and female turkeys are given in Table 3. The small number of poults and the disparity in numbers between the sexes in some groups, resulted in only one of the differences (at 2 weeks between poults given the lowest level of biotin) attaining statistical significance ($P < 0{\cdot}05$). However, the mean activities of females were consistently higher at all but the highest biotin level, although the sex effect was less apparent at 4 weeks than at 2 weeks.

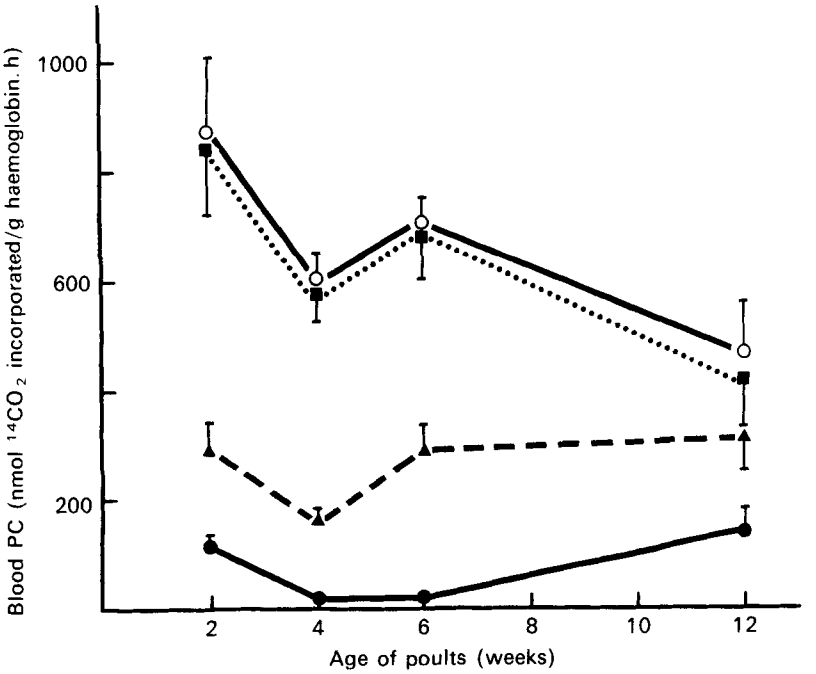

Fig. 3. Expt 3. Blood pyruvate carboxylase (pyruvate-CO_2 ligase (ADP-forming), *EC* 6.4.1.1; PC) activity (nmol $^{14}CO_2$ incorporated/g haemoglobin per h) at different ages (weeks) in poults fed on a basal diet supplemented with 0·05 (●—●), 0·15 (▲– – –▲), 0·30 (■...■) and 0·60 (○—○) mg biotin/kg. Mean values with their standard errors represented by vertical bars. For details of diet, see Table 1.

DISCUSSION

The studies demonstrate a positive relationship between blood PC activity and dietary biotin concentration over a wide range of supplementation in young chickens and turkeys. The shape of the relationship was sigmoid and in both species maximal activity was attained with dietary levels greater than those required to give the maximal growth response.

With chickens, enzyme activity showed good reproducibility between experiments. In Expts 1 and 2 similar increases in supplemental biotin level caused similar increases in activity at 4 weeks of age. Furthermore, enzyme activities of birds fed on diets containing approximately similar levels of total biotin were also similar despite the diets in the two experiments containing different protein levels. The latter was the probable cause of the differences in growth performances between birds on the two experiments.

The effect of another dietary factor, fat level, on enzyme activity was found to be minimal; only in the presence of high levels of biotin did high fat content increase enzyme activity in 2-week-old chicks. This relative independence of blood PC activity from nutrients other than biotin contrasts with the sensitivity of the hepatic enzyme to dietary fat and protein level (Whitehead *et al.*1978).

Enzyme activities and responses in poults closely resembled those in chicks. The upper levels were very similar in magnitude in the two species. However, at low dietary biotin levels, activity was much lower in poults.

Age of the chick influenced enzyme activity. Between 2 and 4 weeks there was a decrease of activity at all levels of biotin supplementation but beyond this age activity remained comparatively constant up to 8 weeks. The effect of age on poults was similar: there was a decrease in activity between 2 and 4 weeks, after which it remained relatively stable. However,

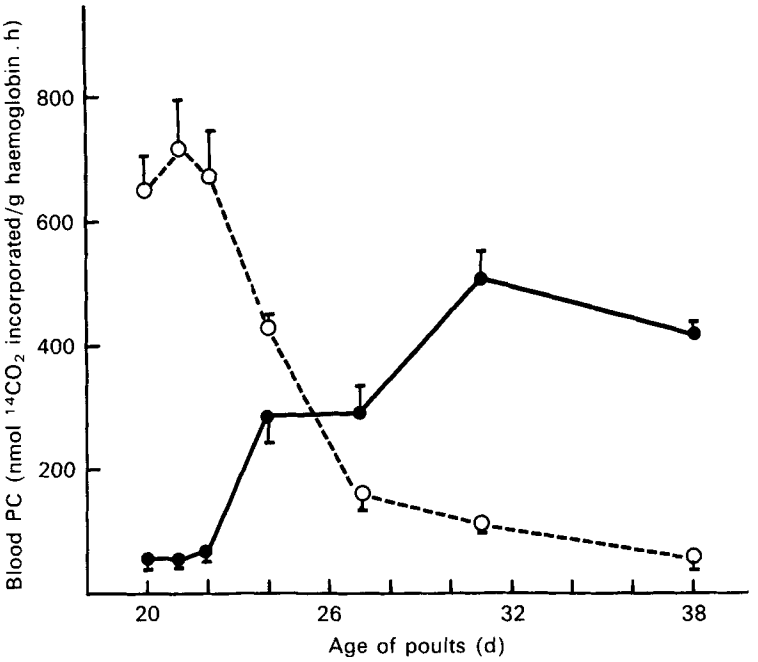

Fig. 4. Expt 3. Blood pyruvate carboxylase (pyruvate-CO_2 ligase (ADP-forming), *EC* 6.4.1.1; PC) activities (nmol $^{14}CO_2$ incorporated/g haemoglobin per h) in poults fed on diets in which the supplemental biotin level was changed from 0 to 0·60 mg/kg (●—●) and from 0·60 to 0 mg/kg (○ - - -○) at day 20. Mean values with their standard errors represented by vertical bars. For details of diet, see Table 1.

Table 3. *Expt 3. Blood pyruvate carboxylase (pyruvate-CO_2 ligase (ADP-forming)*, EC 6.4.1.1) *activities of male and female turkeys fed on diets* containing different levels of supplemental biotin at 2 and 4 weeks of age.*

(Mean values with their standard errors; no. of birds in parentheses. Activity was expressed as nmol $^{14}CO_2$ incorporated/g haemoglobin per h at 38°)

	Age of poults (weeks)							
	2				4			
	♂		♀		♂		♀	
Supplemental biotin (mg/kg)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	65 (6)	12	144 (4)	16	5 (6)	3	12 (4)	6
0·10	144 (5)	5	192 (5)	37	45 (5)	27	86 (5)	41
0·20	529 (3)	70	570 (6)	66	387 (3)	48	406 (6)	43
0·40	750 (7)	93	751 (3)	90	540 (7)	39	500 (3)	41

* For details of composition, see Table 1.

by 12 weeks the maximum and minimum activities of poults given the highest and lowest levels of biotin supplementation were tending to converge with the result that the effect of biotin, though still present, was less pronounced.

It is probable that female poults have higher PC activity in their blood than do males for a given level of intake, except at the highest levels of dietary biotin. The evidence, however,

is not conclusive because in one group only did the difference attain statistical significance. If the difference is real, it is probably a consequence of the poorer food conversion efficiencies associated with females.

Within a group of chicks or poults given the same diet, variability between individual enzyme activities was greater at 2 weeks of age than at subsequent ages and was also greatest in the groups with the lowest activities. Over all ages in the three experiments, the mean coefficient of variation of groups with activities greater than 100 nmol $^{14}CO_2$ incorporated/g haemoglobin per h was 30. Within groups showing clinical symptoms of biotin deficiency there was no correlation between enzyme activity and either severity of lesions or live weight. Hence enzyme activity is not an accurate criterion of the biotin intake or status of an individual bird, but the average of five to ten measurements would be sufficient to give a good estimate of flock biotin status.

Blood PC activity is thus suitable as a specific criterion for assessing the biotin status of a group of young birds. The level of enzyme activity varies considerably over a wide range of dietary biotin concentrations. It is steady during much of the growth period and during this time is unaffected by major dietary variables such as fat and protein level. It has the advantage over measurements of liver enzyme activity in that it does not entail the death of the bird and, on the basis of experience in our laboratory, is a much easier parameter to measure than blood biotin concentration. A disadvantage at present is that activity must be measured promptly after collection because of the instability of the enzyme (Bannister & Whitehead, 1976).

The enzyme assay should be particularly valuable in assessing the biotin status of birds in relation to their requirements for growth because there is an approximately linear relationship between activity and dietary biotin level in the region of the biotin requirement. Unfortunately the group sizes in these small-scale experiments were insufficient to establish with accuracy the minimum supplemental biotin levels required for maximum growth. Larger-scale experiments are required to determine more precisely enzyme activities associated with optimum dietary biotin requirement. However, on the basis of the present experiments, a blood PC activity greater than 400 nmol $^{14}CO_2$ incorporated/g haemoglobin per h would suggest that both chicks and poults were receiving an adequate supply of biotin.

In this paper, enzyme activities have been presented and discussed in relation to the supplemental biotin levels used, rather than in terms of the total biotin content of the diet. Theoretical available biotin contents of diets may be calculated, but these depend upon limited information on level and availability in feedstuffs. Until now, values for availability have depended upon growth response bioassays, but these do not always yield reliable results since growth can respond to many factors. With the demonstration that blood PC activity can be a specific criterion of the biotin status of a flock of birds, it may prove possible in the future to determine more readily the availability of biotin in foodstuffs and diets by using a bioassay based upon the measurement of this enzyme.

The authors wish to thank Mr J. Armstrong, Miss A. Campbell and Mrs C. E. McNeill for their technical assistance.

REFERENCES

Achuta Murthy, P. N. & Mistry, S. P. (1972). *J. scient. ind. Res.* **31**, 554.
Anderson, J. O. & Warnick, R. E. (1970). *Poult. Sci.* **49**, 569.
Atwal, A. S., Robblee, A. R. & Milligan, L. P. (1971). *J. Nutr.* **101**, 1555.
Bannister, D. W. & Whitehead, C. C. (1976). *Int. J. Biochem.* **7**, 619.
Brin, M., Tai, M., Ostashever, A. S. & Kalinsky, H. (1960). *J. Nutr.* **71**, 273.

Dakshinamurti, K., Landman, A. D., Ramamurti, L. & Constable, R. J. (1974). *Analyt. Biochem.* **61**, 225.
Frigg, M. (1976). *Poult. Sci.* **55**, 2310.
Frigg, M., Weiser, H. & Bollinger, A. (1973). *5th int. Congr. Wld Vet. Poult. Ass.*, Munich, **2**, 1286.
Glatzle, D. & Frigg, M. (1975). *Biochem. biophys. Res. Commun.* **66**, 368.
Glatzle, D., Weber, F. & Wiss, O. (1968). *Experientia*, **24**, 1122.
Hood, R. L. (1975). *J. Sci. Fd Agric.* **26**, 1847.
Hood, R. L., Johnson, A. R., Fogerty, A. C. & Pearson, J. A. (1976). *Aust. J. biol. Sci.* **29**, 429.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
Moss, J. & Lane, M. D. (1971). *Adv. Enzymol.* **35**, 321.
Whitehead, C. C. (1977). *Wld's Poult Sci. J.* **33**, 140.
Whitehead, C. C., Bannister, D. W. & Cleland, M. E. (1978). *Br. J. Nutr.* (in the Press).
Wright, L. D. & Skeggs, H. R. (1944). *Proc. Soc. exp. Biol. Med.* **56**, 95.

Printed in Great Britain