ACETYL-COENZYME A CARBOXYLASE ACTIVITY IN WHOLE BLOOD AND ITS DEPENDENCY ON THE BIOTIN STATUS OF CHICKENS

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SUMMARY: Acetyl-coenzyme A carboxylase activity is shown to be present in the whole blood of chickens, pigs and humans. In chicken blood, the acetyl-coenzyme A-dependent carbon dioxide fixation depends on the biotin supply of the animals. This finding is considered as a basis for the development of an enzyme test system using blood for the assessment of the biotin status in animals and humans.

The enzyme acetyl-CoA carboxylase (EC 6.4.1.2) participates in the synthesis of long-chain fatty acids catalyzing the following reaction:

$$\text{acetyl-CoA} + CO_2 + \text{ATP} \longrightarrow \text{malonyl-CoA} + \text{ADP} + P_i$$

It is present in bacteria, yeast, plant and animal tissues. Although there have been indications of its presence in platelets (1) and reticulocytes (2), acetyl-CoA carboxylase activity, to our knowledge, has not yet been demonstrated in whole blood.

Biotin bound covalently to the enzyme protein is involved in the CO_2 fixation step (3). Acetyl-CoA carboxylase activity in rat and chicken liver and rat adipose tissue is known to be affected by the biotin status (4). The enzyme of rat adipose tissue appears to be more sensitive to the biotin supply than the liver enzyme (5).

MATERIALS AND METHODS

Animals: One-day-old chicks (Nichols, 36 ± 3 g, SD given) of either sex were kept in heated batteries and received a biotin-deficient ration (6) for 7 days. They were then divided at random into 2 groups, one group continuing to receive the biotin-deficient diet (deficient group), while for the control group, the diet was supplemented with 360 µg biotin/kg. The experiment was terminated when the animals were 28 days old, i.e. when all animals of the deficient group had developed severe lesions, e.g. feet lesions score 4 (6), and no deficiency symptoms were detected in the animals of the control group. The chicks were weighed regularly and blood was taken by heart puncture with a heparinized syringe.

Acetyl-CoA carboxylase assay: A method for the determination of acetyl-CoA carboxylase activity in rat adipose tissue (7) was adapted and applied to whole blood. ^{14}C radioactivity from $H^{14}CO_3^-$ added to an incubation mixture is transformed into an acid-stable form.

Heparinized blood was haemolyzed by dilution with H_2O and by freezing and thawing. The standard assay procedure for chickens is as follows: 1 ml of blood is diluted with 1 ml of H_2O and immediately frozen in dry ice or in a deepfreezer. At this stage, the frozen sample may be stored for weeks without considerable loss of enzyme activity. Haemolysis is accomplished after thawing the sample by the addition of 0.2 ml aqueous saponin solution (1 %). Cell debris are separated by centrifugation (20,000 x g, 15 min), the supernatant being used for the assay.

In a "pre-incubation" step, 0.6 ml of a mixture containing 100 mM Tris (Cl^-), 13.3 mM $MgCl_2$, 0.166 mM EDTA (Na^+), 8.3 mM citrate (K^+), 5 mM GSH and 0.6 mg bovine serum albumin (fraction V, Fluka), adjusted to pH 7.0 (20°C) with 7 N HCl, is added to 0.3 ml of the supernatant, mixed well and incubated at 37°C for 30 min in an Eppendorf centrifuge tube.

Then, 0.1 ml 20 mM ATP (Na^+, Boehringer, Mannheim) adjusted to pH 7.0 as above, 0.1 ml 2 mM acetyl-CoA (Li^+, Boehringer, Mannheim) and 0.1 ml 100 mM $NaH^{14}CO_3$ (specific activity 0.2 µCi/µmole, stock solution: 60 µCi/µmole distributed by The Radiochemical Centre, Amersham) are added and the mixture is incubated in the stoppered tube at 37°C for 30 min. The reaction is stopped by the addition of 0.2 ml 7 N HCl and, after mixing and centrifugation, 1 ml of the supernatant is placed in a liquid scintillation vial and brought to dryness at 85°C, using additional ventilation. The residue is dissolved in 0.2 ml H_2O. As scintillator solution, 15 ml of a mixture of toluene containing 8 g Butyl-PBD (Ciba-Geigy) per l and ethanol (1 : 1 by volume) is used. Radioactivity is measured in a Nuclear Chicago Mark II liquid scintillation spectrometer. Enzyme activity a is expressed as µmoles of added $NaH^{14}CO_3$ transformed into the acid-stable form within 1 min by a haemolysate equivalent to either 1,000 ml of blood or 1 g haemoglobin. The dilution of the $H^{14}CO_3^-$ added to the system by HCO_3^- already present, e.g. in blood, was neglected.

For the statistical evaluation of the data, the U-test (8) and rank correlations (9) were applied.

RESULTS

An acetyl-CoA-dependent CO_2-fixing activity could be demonstrated in haemolysates of chicken, pig and human blood. Under the conditions of the assay, the acetyl-CoA carboxylase activity was highest in chicken and lowest in man (Table 1). The time course of this reaction is demonstrated for chickens in Fig. 1. Linearity with regard to CO_2 fixation was obtained up to 30 min of incubation. Fig. 2 shows the influence of the amount of chicken haemolysate added to the assay system.

The calculated enzyme activities are almost duplicated by adding 50 µmoles $NaH^{14}CO_3$ instead of 10 µmoles, as indicated in Fig. 3. Activities expressed per g Hb tend to be lower in blood of biotin-deficient animals in comparison to controls, no matter whether the assay is performed with addition of 10 µmoles or 50 µmoles $NaH^{14}CO_3$.

Data on acetyl-CoA carboxylase activity in haemolyzed blood and final weight of biotin-deficient chickens and controls in two trials A and B are presented in Table 2. In both trials, the deficient animals showed significantly lower acetyl-CoA carboxylase activities compared with controls ac-

Table 1: Acetyl-CoA-dependent CO_2 fixation in man, pig and chicken

Complete assay system contained haemolysate, Tris buffer, $MgCl_2$, EDTA, citrate, GSH, bovine serum albumin, ATP, acetyl-CoA and $NaH^{14}CO_3$. Specific activity of the $NaH^{14}CO_3$ added: 1 µCi/µmole to supernatants of haemolyzed blood of humans and pigs and 0.2 µCi/µmole for the assay of haemolyzed chicken blood. Enzyme activity a is expressed as µmoles CO_2 fixed per g Hb and min.

Omitted in assay system	Incubation time (min)	Human		Pig		Chicken	
		dpm	a	dpm	a	dpm	a
None	0	91	0	139	0	96	0
None	30	380	41	861	101	692	418
Acetyl-CoA	30	159	9	179	6	127	22

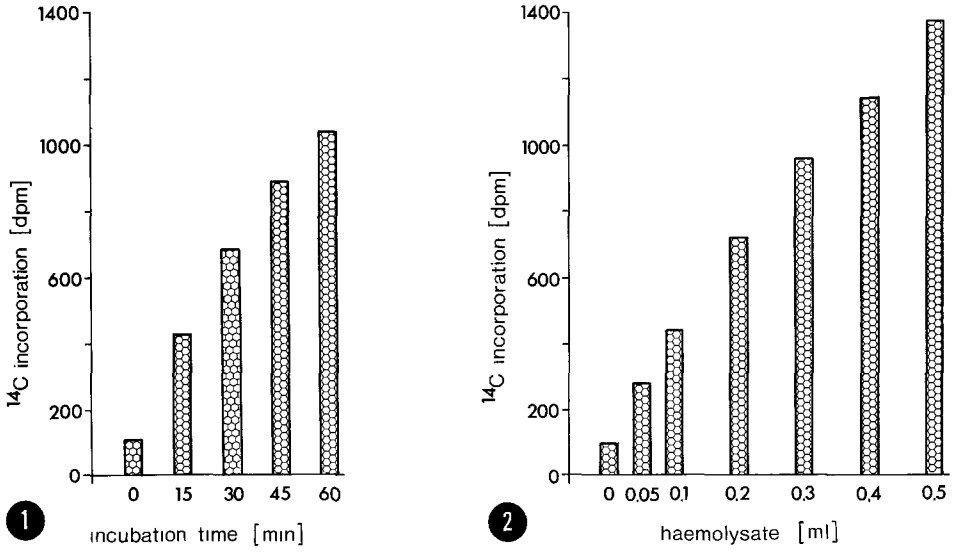

Fig. 1: Time course of $^{14}CO_2$ fixation in the acetyl-CoA carboxylase assay using 0.3 ml haemolyzed chicken blood. For experimental details see Table 1 and Materials and Methods.

Fig. 2: Effect of haemolysate concentration of chicken blood on the $^{14}CO_2$ fixed after incubating 30 min.

Table 2: Acetyl-CoA carboxylase activities and final weight of four-week-old chickens after feeding a biotin-deficient diet and the same diet supplemented with biotin.

	Deficient chickens			Supplemented chickens		
	Acetyl-CoA carboxylase		Weight	Acetyl-CoA carboxylase		Weight
	a (per l blood)	a (per g Hb)	(g)	a (per l blood)	a (per g Hb)	(g)
Trial A	363	3.9	274	370	4.8	610
n = 12	468	4.8	431	552	6.8	617
	388	3.8	403	629	7.0	676
	317	3.4	352	444	5.1	778
	315	3.6	358	498	6.0	630
	261	2.6	418	565	5.1	620
Mean	352.0	3.68	372.7	509.7	5.80	655.2
± SD	71.8	0.72	57.9	92.8	0.94	64.6
Trial B	391	3.6	345	775	8.0	685
n = 20	279	2.3	314	544	5.2	679
	178	1.8	384	568	5.6	666
	294	2.7	383	415	4.3	783
	363	3.0	487	560	5.8	591
	612	4.9	344	466	4.9	741
	396	3.8	315	495	5.4	575
	355	3.2	315	431	4.3	679
	491	4.7	443	444	4.7	693
	413	3.3	332	358	3.9	629
Mean	377.2	3.33	366.2	505.6	5.21	672.1
± SD	118.7	0.98	58.8	116.4	1.16	62.9
Trials A + B						
Mean	367.8	3.46	368.6	507.1	5.43	665.8
± SD	101.6	0.88	56.6	104.9	1.09	62.0

cording to the U-test (8). A statistically significant difference in the activities of deficient animals in comparison to controls was established in each trial A and B and also when evaluating both trials together. No statistically significant difference was found when comparing the activities of the controls from trials A and B as well as when comparing the activities of the deficient animals.

As indicated by the final weight, the weight gain was significantly depressed in biotin deficiency (6) (Table 2). There was a statistically significant correlation between weight and acetyl-CoA carboxylase activities a (per g Hb) and a (per l blood) in haemolyzed blood. The corresponding rank correlation coefficients τ of Kendall (9) were 0.42 and 0.34, respectively; the 2α values were below 0.01.

DISCUSSION

The assay system described for the determination of the acetyl-CoA carboxylase activity is, to our knowledge, the first enzymatic approach to evaluate the biotin status using blood. The method was shown to be suitable for distinguishing between controls and chickens which had developed pronounced symptoms of biotin deficiency. Its validity and reproducibility were demonstrated in two separate trials. Nevertheless, the system can still be improved. Increasing the concentration of $NaH^{14}CO_3$ seems to be favourable (Fig. 3); then, the dilution by bicarbonate already present in blood is less pronounced and the calculated enzyme activities better reflect the actual

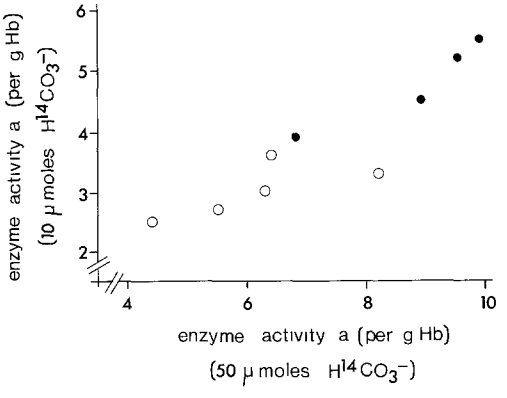

Fig. 3: Influence of bicarbonate addition on the calculated acetyl-CoA carboxylase activity a of haemolyzed blood of biotin-deficient chickens (o) and controls (•). Enzyme activity is expressed as µmoles $^{14}CO_2$ fixed per g Hb and min.

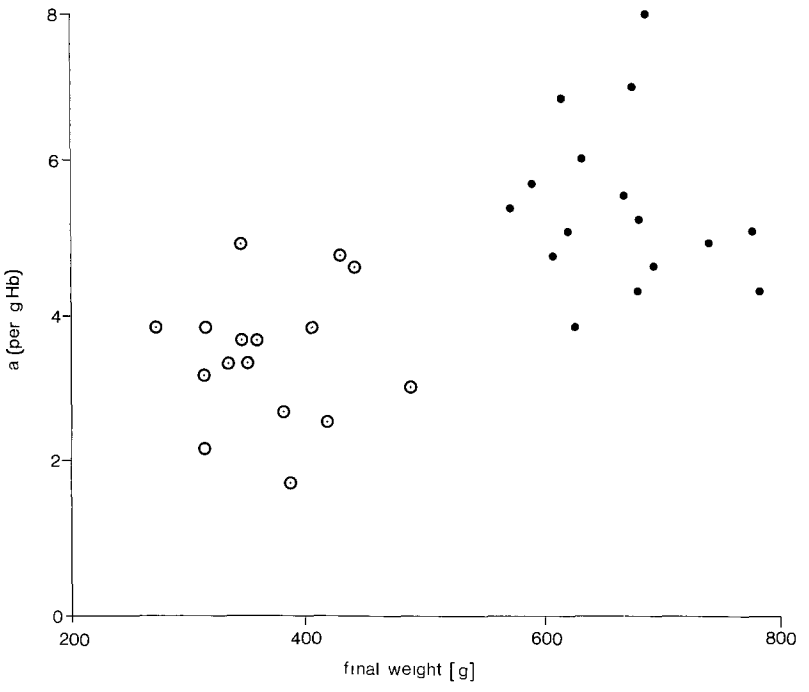

Fig. 4: Acetyl-CoA carboxylase activity a (per g Hb) and final weight of biotin-deficient chickens (o) and controls (•).

amount of bicarbonate transformed. Tris buffer at pH 7.0 has a very poor buffering capacity, but other buffer systems and/or hydrogen concentrations investigated did not improve the CO_2 fixation.

Acetyl-CoA carboxylase activity in animal tissues is known to be affected and regulated to a large extent by a variety of substances and physiological conditions (3). If other factors besides biotin should be involved in the regulation of this enzyme activity in blood, it might be at least doubtful whether acetyl-CoA carboxylase activity in blood as such could be taken as a specific parameter for the assessment of the biotin status. Possibly a determination of holoenzyme and apoenzyme could add further specificity to the test provided that acetyl-CoA carboxylase activity of blood turns out to be regulated by the ratio of holoenzyme to apoenzyme in biotin deficiency, as known for other tissues (5, 10).

There are available activation tests for the assessment of the thiamine (11), riboflavin (12) and pyridoxine (13) status using blood haemolysates. An attempt will be made to elaborate an activation test for acetyl-CoA carboxylase in haemolyzed blood, in which biotin will be linked to the apoenzyme,

if present in blood, in the presence of a ligase in order to form the active holoenzyme.

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