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Activation of Mitochondrial Propionyl-CoA Carboxylase

HALINA Y. NEUJAHR* and S. P. MISTRY

Division of Animal Nutrition, University of Illinois, Urbana, Illinois, U.S.A.

1. The 35–55 % $(NH_4)_2SO_4$ fraction of the 105 000 × *g* supernatant of rat, beef or pork liver was found to exhibit considerable propionyl-CoA carboxylase activity in spite of the fact that the supernatant exhibited only negligible activity before fractionation.

2. Treatment of the $(NH_4)_2SO_4$ fraction with dilute acid gave a soluble preparation (S3) which increased two- to five-fold the propionyl-CoA carboxylase activity of the extract of acetone powder of rat liver mitochondria but was without effect on the same enzyme obtained from the liver supernatant.

3. The activation was observed with the mitochondrial enzyme preparation from a normal as well as from a biotin-deficient rat, provided the latter exhibited some initial activity.

4. S3 preparations from severely biotin-deficient rats also exhibited activation effects which were comparable to those of S3 preparations from normal animals.

5. The activation was inhibited by avidin and the inhibition could be reversed by an excess of biotin.

6. A similar activating effect, its inhibition by avidin, and the reversal by biotin, was observed with K^+.

7. The potassium content of S3 preparation from normal rat or beef liver was approximately 10 μmole per ml. 0.1 ml of such a preparation activated the mitochondrial propionyl-CoA carboxylase to the same extent as 1 μmole of K^+ per incubation mixture.

Propionyl-CoA carboxylase has been isolated from mitochondria by Ochoa and his associates [1,2] and by Lane and coworkers [3,4]. This enzyme catalyzes the Mg^{2+}-dependent reversible carboxylation of propionyl-CoA to methylmalonyl-CoA [5]. The crystalline enzyme was shown to contain biotin [2].

In our studies on the function of biotin, we found that the activity of the rat mitochondrial enzyme could be stimulated by the *in vitro* addition of a supernatant fraction from liver which had little or no enzyme activity *per se* [6]. During the initial study we found that propionyl-CoA carboxylase was present not only in the mitochondria but also in the liver supernatant. However, similar attempts to activate the supernatant enzyme were not

* Present address: Division of Food Chemistry, Royal Institute of Technology, Stockholm 70, Sweden.

successful. The possible presence of biotin in the preparations stimulating the carboxylase activity of mitochondria was examined by distribution studies with (^{14}C)biotin as well as by avidin inhibition. In the course of the purification of the activating preparations it was found that K^+ have a similar activating effect on the mitochondrial propionyl-CoA carboxylase. It seems, therefore, that this cation is responsible for the observed activation of the enzyme by the supernatant fraction from rat liver.

METHODS

Animals. Weanling male albino rats of the Sprague-Dawley strain, weighing 40 to 45 g were fed *ad libitum* a 20 % spray-dried raw egg-white diet, complete in all nutrients except biotin. The animals showed visible signs of biotin deficiency within 4 to 5 weeks. Normal rats received a standard commercial ration (Purina Laboratory Chow).

Beef and pig livers were secured from freshly slaughtered normal healthy animals.

Enzyme preparations and assay. A 10 % liver homogenate was prepared in 0.25 M sucrose with 0.01 M EDTA and 0.001 M L-cysteine using the Elvehjem-Potter homogenizer for a small preparation and the Waring blendor for a large-scale preparation. After the removal of nuclei and unbroken cells by centrifuging the homogenate for 10 min at 600 × *g*, the mitochondria were sedimented at 8500 × *g* for 10 min. The 18 000 × *g* fraction as well as the microsomes contained no propionyl-CoA carboxylase activity and were discarded.

The mitochondria were washed once with sucrose-EDTA buffer, resuspended in a small volume of 0.1 M tris buffer pH 7.6, with 0.001 M L-cysteine and converted to an acetone powder essentially as described by Halenz and Lane [3]. The preparation was stored in a refrigerator for several months without noticeable loss of the carboxylase activity. For the enzyme assay a 2, 5 or 10 % extract of acetone powder was prepared in 0.1 M tris buffer, pH 7.6. In general, a 2 % extract proved satisfactory. The extraction was carried out for 30 to 60 min in cold with occasional stirring, the suspension was centrifuged and the clear supernatant was used as the source of the mitochondrial enzyme.

The 105 000 × *g* liver supernatant, obtained after centrifuging the homogenate for 1 h, was fractionated in cold with solid $(NH_4)_2SO_4$, added slowly in very small amounts with continuous stirring. The 35—55 % fraction containing most of the propionyl-CoA carboxylase activity was redissolved in 0.1 M tris buffer, pH 7.6, and the amount of protein adjusted to 20 mg/ml as determined by the method of Lowry *et al.*[7]

The enzyme assay was performed essentially according to Halenz and Lane [3] as described in Table 1. Every determination, blank as well as the system with the enzyme, was carried out in duplicate.

Activating preparations. The 35—55 % $(NH_4)_2SO_4$ fraction was gradually acidified with ice-cold 0.1 M HCl with continuous stirring to pH 3 and kept for 1 hour at 0°. The suspension was centrifuged, the precipitate was discarded and the clear supernatant was neutralized with 0.2 M NaOH. Henceforth, such preparations will be referred to as "S3". They contained 2 to 3 mg of protein per ml but had no carboxylase activity. In enzyme activation experiments, 0.1 ml of S3 per incubation mixture was used. Treatment of the 35—55 % $(NH_4)_2SO_4$ fraction with 0.1 M acetic acid at pH 4.6 gave similar activating preparations which, however, contained considerable carboxylase activity and therefore were less convenient for use in activation experiments.

Materials. Glutathione, ATP and coenzyme A were secured from Sigma Chemical Co. and (^{14}C)$NaHCO_3$ was purchased from New England Nuclear Corporation. Propionyl-CoA was prepared by the method of Simon and Shemin [8]. (Carboxy-^{14}C)biotin (100 μC/mg) was a gift from Professor O. Wiss and rat livers containing labeled biotin were given by Dr. K. Dakshinamurti. These livers were from biotin-deficient animals which received in the sixth week a daily incjection of 10 μg of labeled biotin per animal.

Avidin and avidin-biotin complex. The avidin used in the present study was prepared by Dr. K. Dakshinamurti in our laboratory essentially according to the method of Woolley and Longsworth [9] and contained 1.5 antibiotin units per mg (1 unit binds 1 μg of biotin).

Fresh solutions of avidin in 0.1 M tris buffer were prepared for every experiment since the biotin binding activity of the solutions deteriorated on freezing or storage at 0°.

In exchange studies with avidin-bound labeled biotin, the avidin-biotin complex was precipitated from a volume of 2 ml, either by saturated $(NH_4)_2SO_4$, by the addition of 20 volumes of ice-cold acetone or by ultra filtration through dialysis bags as described by Gregory [10].

Measurement of radioactivity. Usually the radioactivity was determined in a 0.05 ml sample with a Packard liquid scintillation spectrometer. When samples were plated the radioactivity was measured at infinite thinness in a Nuclear-Chicago windowless gas flow counter. Except where indicated, all the results are given for a 0.05 ml sample.

RESULTS AND DISCUSSION

Occurrence of propionyl-CoA carboxylase in the soluble cell fraction. The 105 000 × *g* supernatant of rat liver contains considerable amounts of propionyl-CoA carboxylase. The enzyme activity is very low in the original homo-

Table 1. Propionyl-CoA carboxylase in rat liver supernatant and mitochondria. Standard mixture contained (in μmoles): Glutatione, 2.5; ATP, 2; $MgCl_2$, 2; (^{14}C)$NaHCO_3$, 7.5 (1.175 μC); propionyl-CoA, 0.5; tris buffer pH 8.4, 50; and enzyme 0.1 ml. Total volume was 0.75 ml. The reaction was started following the addition of propionyl-CoA and after incubation at 37° for 20 min was stopped with 0.1 ml of 20 % trichloroacetic acid. The mixture was then heated for 5 min at 60° under the hood to remove the excess of (^{14}C)$NaHCO_3$, centrifuged at low speed and the clear supernatant, containing methylmalonate and succinate, was counted for radioactivity.

Cell fraction	Enzyme activity, Counts/min (^{14}C)CO_2 fixed	
	Per mg protein	Total
Supernatant (105 000 × *g*)		
in tris, pH 7.5	2 500	83 100
in sucrose-EDTA, pH 7.8	240	48 450
in sucrose-EDTA, pH 7.8, with 50 × Mg^{2+}	180	38 500
in sucrose-EDTA, pH 7.8, fractionated with $(NH_4)_2SO_4$, 35–55 %	16 600	265 000
Mitochondria (extract of acetone powder)	12 000	192 000

Table 2. Propionyl-CoA carboxylase in beef and pork liver supernatants. Enzyme assay is given in Table 1.

Fraction	Enzyme activity, Counts/min (^{14}C)CO_2 fixed per mg protein
Beef liver supernatant	
$(NH_4)_2SO_4$ 0–55 % (liver 1)	7 105
$(NH_4)_2SO_4$ 35–55 % (liver 1)	7 735
$(NH_4)_2SO_4$ 35–55 % (liver 2)	7 650
$(NH_4)_2SO_4$ 35–55 % (liver 3)	11 616
Pork liver supernatant	
$(NH_4)_2SO_4$ 35–55 %	5 610

genizing medium consisting of sucrose-EDTA-cysteine, but increases upon fractionation with $(NH_4)_2SO_4$. It can be seen from Table 1 that the supernatant enzyme is approximately as active as the mitochondrial enzyme. Furthermore, it appears that the suppression of the enzyme activity is probably not due to the binding of Mg^{2+} by EDTA, since a fiftyfold excess of Mg^{2+} did not relieve the inhibition.

Although the occurrence of propionyl-CoA carboxylase in the liver supernatant has not been reported by other workers, it is evident from Tables 1 and 2 that not only rat but also beef and pork liver supernatants contain considerable carboxylase activity. This activity is subject to rather large variations from one preparation to another.

Since propionyl-CoA carboxylase is a biotin enzyme and earlier study in this laboratory showed that 97 % of labeled biotin in rat liver supernatant was found in the pH 5.2 precipitate,[11] a purification of the enzyme was attempted by this means. It will be seen from Fig. 1 that, contrary to expectation, the major part of the activity of the supernatant did not precipitate even at pH 4.6.

Activation of mitochondrial propionyl-CoA carboxylase by a preparation obtained from the 105 000 × g supernatant. Treatment of the $(NH_4)_2SO_4$ fraction of the 105 000 × *g* supernatant with dilute acid at pH 3, as described in the METHODS, gave S3 preparations which had no carboxylase activity *per se*. However, when mitochondrial acetone powder extracts from normal as well as from biotin-deficient rats were incubated with S3 preparations from rat, beef or pork liver a two- to five-fold activation of the mitochondrial enzyme was observed. Some typical activation experiments are shown in Table 3. The mitochondrial enzyme from very deficient animals displayed essentially no activity, and S3 preparations had no activating effect, indicating that probably also the protein (*cf.* Ref.[11]) of the enzyme had been damaged. Similar S3 preparations from mitochondrial acetone powder instead of from the supernatant had no activating effect on the enzyme. Furthermore, only the mitochondrial, but not the supernatant enzyme could be activated by S3.

Table 3. Activation of rat liver mitochondrial propionyl-CoA carboxylase by S3 preparations * from normal rat, beef and pork liver supernatants. Enzyme assay is given in Table 1.

Source of enzyme	Addition	Enzyme activity, Counts/min $(^{14}C)CO_2$ fixed
Normal mitochondria	None	144
	Rat S3	706
	Beef S3	415
Biotin deficient ** mitochondria	None	84
	Rat S3	320
	Beef S3	251
	Pork S3	305

* Supernatant from the 35–55 % $(NH_4)_2SO_4$ fraction acidified to pH 3; see text for details.
** The animals were on the raw egg-white diet for 4 weeks.

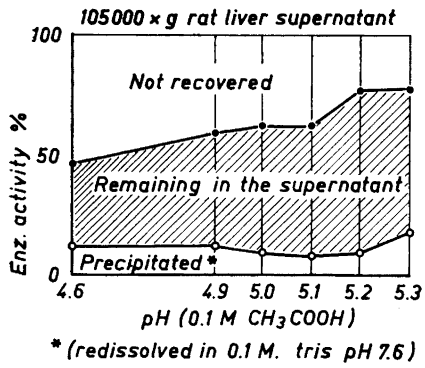

Table 1. pH fractionation of propionyl-CoA carboxylase from rat liver supernatant. Enzyme assay is given in Table 1.

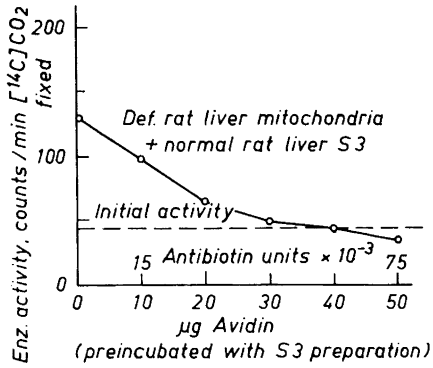

Fig. 2. Effect of avidin on propionyl-CoA carboxylase activation. 2 % acetone powder extract of biotin-deficient rat liver mitochondria in 0.1 M tris, pH 7.6, was incubated with S3 from normal rat liver. Enzyme assay is given in Table 1.

Attempts to activate the supernatant enzyme by combining the neutralized pH 3 or pH 4.6 supernatant with the corresponding precipitate (resuspended in 0.1 M tris buffer, pH 7.6) were also unsuccessful.

Effect of avidin on the enzyme activation by S3. The effect of preincubation of S3 with increasing amounts of avidin on the activation of mitochondrial propionyl-CoA carboxylase can be seen from Fig. 2. In the case of the S3 preparation tested, about 36 µg of avidin were required to abolish the activating effect and restore the enzyme to its initial activity. Higher amounts of avidin seemed to bind the enzyme itself. The specificity of the avidin inhibition can be seen from Table 4, where under similar conditions bovine serum albumin did not inhibit the activation effect of S3. Furthermore, the effect of avidin on the activation could be reversed by an excess of free biotin, as shown in Table 5. A twenty-fold excess of biotin (µg of biotin per unit of antibiotin) completely released the activation effect of S3. This was taken as an indica-

Table 4. Specificity of avidin inhibition of propionyl-CoA carboxylase activation. Enzyme assay is given in Table 1. Avidin or albumin was preincubated with the S3 preparation for 1 h at 25°.

Addition to the mitochondrial enzyme from normal rat liver	Enzyme activity, Counts/min (^{14}C)CO_2 fixed
None	93
S3	536
S3 + avidin 20 µg	136
S3 + albumin 20 µg	509
S3 + avidin 100 µg	2
S3 + albumin 100 µg	437

Table 5. Activation of propionyl-CoA carboxylase, inhibition of the activation by avidin and the reversal by biotin. Enzyme assay is given in Table 1.

Pretreatment of S3		Biotin/antibiotin	Enzyme activity in presence of S3 *
1 h with avidin, μg	Followed by 0.5 h with biotin, μg		Counts/min $(^{14}C)CO_2$ fixed
0	0		530
100	0	0	3
100	0.3	2	10
100	3	20	510
100	30	200	536
100	300	2000	520

* Enzyme activity without the S3 preparation was 90 counts/min $(^{14}C)CO_2$ fixed.

Table 6. Release of avidin-bound (^{14}C)biotin by an excess of free biotin. Where avidin was used, it was incubated with labeled biotin for 1 h at 25° and then for another hour with excess biotin where indicated.

Addition to 3 μg of (^{14}C)biotin	Counts/min remaining in solution after the removal of avidin-biotin complex by the method *		
	A	B	C
None	5276	545	5315
Avidin, 2 mg	142	19	125
Avidin, 2 mg + biotin, 30 μg	268	35	226
Avidin, 2 mg + biotin, 300 μg	388	64	324

* The complex was removed by precipitation with $(NH_4)_2SO_4$ (A) or with acetone (B) or removed by ultrafiltration (C).

tion of a possible exchange at the binding sites of avidin between free biotin and the vitamin presumably present in the activating agent.

Launer and Fraenkel-Conrat [12] have demonstrated an exchange of avidin-bound (^{14}C)biotin with free biotin. As will be seen from Table 6, under our experimental conditions such an exchange is also possible. However, it is rather small compared to the very marked release of the presumably avidin-bound activating material by a relatively small excess of free biotin (Table 5) unless the active S3 preparation contains an extremely small amount of biotin.

In another approach to the problem of the possible presence of biotin in the activating material, we made S3 preparations from livers of rats which received large doses of (^{14}C)biotin. The S3 preparation, *i.e.*, the pH 3 soluble activating material, from the 35—55 % $(NH_4)_2SO_4$ fraction of the 105 000 × *g* liver supernatant, contained only 5 % of the radioactivity of the $(NH_4)_2SO_4$ fraction, whereas 95 % of (^{14}C)biotin remained in the inactive pH 3 precipitate. On the other hand, the S3 preparation made from an extract of acetone powder of liver mitochondria contained as much as 40 % of (^{14}C)biotin. However, as mentioned before, the S3 from mitochondria had no activating effect on the enzyme.

Table 7. Activation of propionyl-CoA carboxylase by S3 preparation from biotin-deficient rat liver. Enzyme assay is given in Table 1.

Source of enzyme *	Addition	Enzyme activity, Counts/min $(^{14}C)CO_2$ fixed	
		Expt. 1	Expt. 2
Deficient mitochondria	none	23	1
Deficient mitochondria	D S3 **	163	34
Normal mitochondria	none	93	277
Normal mitochondria	D S3 **	460	1166

* 2 % acetone powder extract in 0.1 M tris, pH 7.6.
** The preparation had no propionyl-CoA carboxylase activity *per se*.

Activation of the mitochondrial enzyme by S3 from biotin-deficient liver. Another surprising observation was made in an experiment in which the S3 preparation derived from a deficient rat liver supernatant was found to activate the mitochondrial enzyme from deficient as well as from normal rats. The results are shown in Table 7.

In an attempt to explain this observation, the activating effect of S3 preparations from normal and deficient livers was tested at various dilutions. It will be apparent from Fig. 3 that it is not just a question of different amounts of the activating material present in the two preparations, since the activating effect of both preparations decreased with increasing dilution in the same way over a very wide range of concentrations.

This led us to investigate the relationship between the activating effect and the stage of biotin deficiency. It can be seen from Table 8 that the activating capacity of the S3 preparation does not decrease as the animal becomes progressively deficient. Under the same conditions the ability of the mitochondrial enzyme to become activated by the normal S3 preparation decreases significantly. An explanation of these results had to wait until attempts to purify the activating material were made.

Table 8. Stage of biotin deficiency and propionyl-CoA carboxylase activation. Enzyme assay is given in Table 1. N S3 and D S3 are activating preparations from normal and biotin deficient rat liver supernatants, respectively.

Weeks on deficient diet	Addition to the enzyme from deficient mitochondria	$(^{14}C)CO_2$ fixed, counts/min	Addition to the enzyme from normal mitochondria	$(^{14}C)CO_2$ fixed, counts/min
			None	94
			N S3	474
4	None	47		
	N S3	214	D S3	503
6	None	14		
	N S3	72	D S3	432
8	None	11		
	N S3	21	D S3	475

Table 9. Effect of adsorbents on S3. HCl, pH 3, was used for elution. Enzyme assay is given in Table 1.

Addition to the mitochondrial enzyme from normal rat liver	Enzyme activity, Counts/min $(^{14}C)CO_2$ fixed
None	144
S3	533
S3 after charcoal treatment	408
S3 after Dowex 50 treatment	342
S3 after DEAE treatment	706
Charcoal eluate	544
Dowex 50 eluate	604
DEAE eluate	645

Attempts to purify the activating material. In a preliminary experiment, the affinity of the S3 preparation to certain adsorbents was tested in batch adsorption. It will be seen from Table 9 that the activating substance could be adsorbed on charcoal or on Dowex 50 and eluted by dilute HCl, pH 3. Surprisingly enough, treatment of the S3 preparation with DEAE-cellulose enhanced the activating effect instead of decreasing it. The elution of S3 from Dowex 50 seemed to result in a small increase in activity compared to the original preparation (Table 9).

In an experiment, 100 ml of the S3 preparation from beef liver was passed slowly through a column (2 × 20 cm) of Dowex 50 × 2. Not only was the adsorption very poor, but the activity of a number of HCl eluates exceeded the activity of the initial S3 preparation. Furthermore, the total activity recovered exceeded by far the amount put on the column. Hence, it was assumed that either some purification was achieved by the removal of certain inhibitors from the S3 preparation or that certain ions were eluted from the column which replaced S3 in the activation of mitochondrial propionyl-CoA carboxylase. Therefore, a number of salts were tested for their activating effect on the enzyme.

Activation of mitochondrial propionyl-CoA carboxylase by K^+. Of the salts tested, K_2HPO_4 or KCl had an activating effect comparable to that of S3. It can be seen from Table 10 that the participation of phosphate is excluded in view of the results obtained with Na_2HPO_4—NaH_2PO_4. The less

Table 10. Activation of propionyl-CoA carboxylase by K^+. Enzyme assay is given in Table 1.

Addition to the mitochondrial enzyme from normal rat liver		Enzyme activity, Counts/min $(^{14}C)CO_2$ fixed
None		371
S3		1035
K_2HPO_4—KH_2PO_4, 5 μmoles	K	896
Na_2HPO_4—NaH_2PO_4, 5 μmoles	Na	279
KCl, 5 μmoles		1240

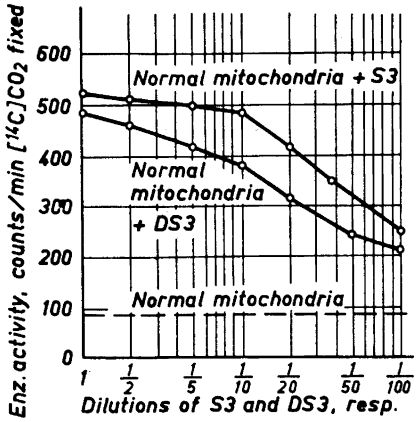

Fig. 3. Activation effect and dilution of S3 preparations. 2 % acetone powder extract of normal rat liver mitochondria in 0.1 M tris, pH 7.6, was incubated with S3 from normal or D S3 from biotin-deficient rat liver. Enzyme assay is given in Table 1.

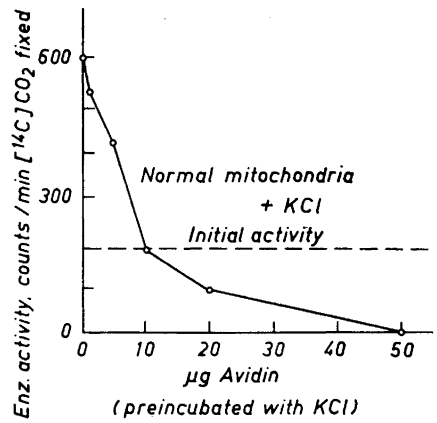

Fig. 4. Effect of avidin on propionyl-CoA carboxylase activation by KCl. 2 % acetone powder extract of normal rat liver mitochondria in 0.1 M tris, pH 7.6, was incubated with 5 µmoles of KCl. Enzyme assay is given in Table 1.

pronounced effect of K_2HPO_4—KH_2PO_4 as compared to that of KCl may be due to an incomplete dissociation of K_2HPO_4—KH_2PO_4 at the pH employed for the assay of the enzyme. Although 5 µmoles of K^+ per incubation mixture was used in this study, in later experiments 1 µmole proved equally effective. This amount produced an activating effect comparable to that given by the S3 preparation.

Higher concentration of Mg^{2+} did not replace the activating effect of K^+. With 10 µmoles instead of the usual 2 µmoles of Mg^{2+} per incubation mixture, which is the optimal concentration, K^+ still had the same activating effect on propionyl-CoA carboxylase.

The effect of avidin on KCl activation of the enzyme was then tested, and the results given in Fig. 4 show a striking similarity to the avidin inhibition

Table 11. K^+ Activation of propionyl-CoA carboxylase, inhibition of the activation by avidin and the reversal by biotin. Enzyme assay is given in Table 1.

Pretreatment of KCl		Biotin/antibiotin	Enzyme activity in presence of 5 µmoles of KCl *
1 h with avidin, µg	Followed by 0.5 h with biotin, µg		Counts/min $(^{14}C)CO_2$ fixed
0	0		932
100	0	0	0
100	0.3	2	12
100	3	20	626
100	30	200	558

* Enzyme activity without KCl was 322 counts/min $(^{14}C)CO_2$ fixed.

Table 12. Ineffectiveness of K^+ in the reversal of avidin inhibition. 2 % acetone powder extract of normal mitochondria was used as the source of enzyme. Enzyme assay is given in Table 1. Avidin was preincubated with KCl for 2 h at 25°.

KCl, μmoles	Enzyme activity, counts/min $(^{14}C)CO_2$ fixed			
	Avidin, μg			
	0	2	5	10
0	229	236	118	16
5	696	515	358	47
25				65
250		257	240	56

curve obtained earlier with the S3 preparation (*cf* Fig. 2). Again, as in the case of the S3 preparation, the avidin inhibition of the effect of K^+ could be reversed by a twenty-fold excess of biotin. This is shown in Table 11 (see Table 5 for comparison).

In order to explain these results, 1 to 250 μmoles of K^+, after preincubation with different levels of avidin, were tested with the mitochondrial enzyme. A complete inhibition of the carboxylase was obtained with 50 μg of avidin at all levels of K^+. The results given in Table 12 show that at lower concentrations of avidin the inhibition was partial. However, it appears that increasing concentrations of K^+ are unable to reverse the inhibition.

These results do not rule out the possibility that K^+ are bound by avidin. They indicate, however, that even if this were the case, K^+ would be unable to displace biotin or biotin-containing compounds from the binding sites of avidin.

A possible explanation of the avidin effect would be that increasing amounts of avidin progressively inhibit the initial activity of the mitochondrial enzyme, whereas that part of the enzyme which is not bound by avidin is activated by K^+ or S3 preparations to levels which, at lower avidin concentrations, exceed the initial activity of the enzyme. Hence, the reversal of this inhibition by biotin may simply be the result of binding the inhibitor with excess biotin that is added after the preincubation of the activating agent, thus making avidin unavailable for inhibition of the mitochondrial enzyme.

Potassium content of S3 preparations and liver mitochondria. Preliminary determinations of K^+ in certain propionyl-CoA carboxylase and activating preparations indicate that the K^+ content of S3 from normal rat or beef liver corresponds approximately to 1 μmole per incubation mixture or about 10 μmoles per ml of S3. This is in agreement with the activation effects observed with S3 or KCl solutions (*cf*. Tables 3 and 10). S3 preparations from deficient animals were found to contain only half as much K^+.

It seems, therefore, that the activating effect of S3 preparations on the mitochondrial propionyl-CoA carboxylase is due to their K^+ content. Since K^+ are the main cations of the intracellular fluid, it can be understood why activating preparations could be obtained only from the 105 000 × *g* supernatant and not from mitochondria. The results of this investigation substantiate the conclusion that propionyl-CoA carboxylase is activated by K^+.

A number of enzymes, as cited by Richards and Rutter [13] have been reported to be activated by K^+. These authors have recently added yeast aldolase to the list of such enzymes. Very recently, another effect of K^+ also has been reported [14]. The requirement of Mg^{2+} for propionyl-CoA carboxylase, as is the case with kinases, is consistent with the observations of other workers that the K^+-activated enzymes usually contain or require the addition of divalent metal ions.

A relationship between biotin and potassium metabolism has been observed in calf and dog. When these two species are maintained on a potassium deficient diet, they show a progressive paralysis which can be cured by the administration of an excess of potassium or of biotin [15].

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