THE METABOLISM OF SERINE IN BIOTIN DEFICIENCY

By G. B. Nadkarni and A. Sreenivasan, F.A.Sc.
(*Department of Chemical Technology, University of Bombay*)

Received May 10, 1957

Serine and ethanolamine occur in phosphatides (Folch and Schneider, 1941). When ethanolamine-1, 2-C^{14} is administered to rat, large amount of radioactivity could be traced in serine of body phosphatides (Pilgeram *et al.*, 1953). The conversion of ethanolamine to serine could be by fixation of carbon dioxide, under appropriate conditions. Such fixation has not been demonstrated, although fixation of $C^{14}O_2$ has been demonstrated in lysine, tyrosine and glutamic acid (Hanke and Siddiqui, 1950; Koppleman *et al.*, 1952) with their corresponding amines.

Biotin has been known to influence CO_2 metabolism (Burke and Winzler, 1943; Shive and Rogers, 1947; Lardy *et al.*, 1947, 1949 *a*, 1949 *b*; McLeod and Lardy, 1949; Feldott and Lardy, 1951; Plaut and Lardy, 1950; Wessman and Werkman, 1950; Ochoa *et al.*, 1947; Lardy and Adler, 1956) and has been shown to influence a number of CO_2 fixation reactions, such as pyruvate to oxalacetate (Shive and Rogers, 1947; Lardy *et al.*, 1947; Wessman and Werkman, 1950; Ochoa *et al.*, 1947), acetone to acetoacetate (Plaut and Lardy, 1950), ornithine to citrulline (Lardy *et al.*, 1949 *a*; Feldott and Lardy, 1951) and propionate to succinate (Lardy and Adler, 1956).

Biotin is also reported to influence the deamination of serine in bacteria (Lichstein and Umbreit, 1947; Lichstein and Christman, 1948). In biotin related deaminases, there is a requirement for biotin-AMP combination (Williams and Christman, 1953). No effect on the deamination rate by *Neurospora* enzyme (Yanofsky, 1953) was obtained with biotin, either alone or in combination with AMP. A function for biotin in the deamination, both oxidative and reductive, of aspartic acid by *Escherichia coli* is inferable from the observed stimulation in the activity of the system when cells of the organism are enriched by incubation with biotin in presence of glucose and inorganic phosphate, preferably after being rendered biotin-deficient by ageing (Gothoskar and Sreenivasan, 1953). The effect of biotin deficiency on deamination has not been studied in the animal organism.

The present work relates to a study of the *in vitro* synthesis of serine from ethanolamine and bicarbonate and of the influence of biotin deficiency on

this system as well as on serine deamination. Serine decarboxylase activity was also followed in view of the reported involvement of biotin in the decarboxylation of succinic (Delwiche, 1950) and oxalacetic (Lichstein and Umbreit, 1947) acids.

EXPERIMENTAL

Young rats (Wistar), initial average weight of 55 g., were kept on a purified biotin-deficient diet (based on Lardy *et al.*, 1949; McCoy *et al.*, 1948) consisting of (percentages): dried (Eakin *et al.*, 1941) raw egg white, 10; alcohol-extracted casein 8; starch 60; sucrose (vitaminised) 10; salt mixture (U.S.P. IV) 4; sesame oil 4; and shark liver oil 2. Vitamin supplements added to the diet were (mg./kg. diet): Thiamine HCl 4; riboflavin 5; niacin 10; calcium pantothenate 20; folic acid 5; pyridoxine HCl 5; vitamin B_{12} 0·2; vitamin K 3; and α-tocopherol 10.

In the diet of the biotin-fed group of rats, egg white was replaced by devitaminised casein and was supplemented with 1·0 mg. biotin per kg. of diet.

The first signs of deficiency such as loss in weight and dermatitis appeared in 8–10 weeks. Animals were then decaptitated and livers quickly removed, chilled and homogenised in cold distilled water to make a 20% suspension.

Ethanolamine to serine conversion.—This system was followed in Warburg flasks with two sidearms similar to the procedure of Lardy *et al.* (1949) and Utter and Kurahashi (1954). Incubations were at 37° C. for half an hour. The main compartment of each flask contained 1·0 ml. of 0·1 M phosphate buffer pH 6·4, 1·0 ml. of 0·2 M ethanolamine solution (pH previously adjusted to 7·0), 0·4 ml. of 0·05 M sodium citrate and 0·1 ml. of 0·05 M sodium fumarate. One sidearm contained 0·5 ml. of 0·1 M sodium bicarbonate and the other contained 0·5 ml. of the liver homogenate. The flasks were equilibrated for 10 minutes at 37° C. before tipping in the contents of both sidearms. Control flasks were kept with the ethanolamine solution replaced by 1·0 ml. of distilled water. The final pH of the reaction mixture was 8·3.

After 30 minutes reaction, 0·5 ml. of 1 N sulphuric acid was added to each flask to stop the reaction. After heating the contents of the flask in a boiling water-bath for 5 minutes, they were centrifuged and an aliquot of the supernatant used for determination of the serine formed microbiologically with either *L. mesenteroides* P-60 (Steele *et al.*, 1949) or *L. casei* (Alexander *et al.*, 1953).

In preliminary experiments, serine formed was detected and determined on paper chromatograms. The method was that of Levine and Chargaff

(1951) with some modifications. The chromatograms were run on paper sheets (Whatman No. 1) which were 45 cm. long, the width depending on the number of spots run. The solutions were spotted at least 3·0 cm. apart. Solutions of serine were spotted alongside by means of standard micropipette. Chromatograms were run by the descending technique, the solvents used being *n*-butanol-acetic acid-water (40 : 10 : 50) and phenol-water (80 : 20). After 18 hours' run, the sheets were dried and the spots developed with 0·4% solution of ninhydrin in acetone. Sometimes the solvent was run twice (Giri and Rao, 1952) and then developed with ninhydrin for clearer separation of the spots. The R_f values for serine with the two solvent systems were 0·18 and 0·40 respectively; those for ethanolamine were 0·33 and 0·75. The spots corresponding to serine were cut out and taken into test-tubes containing 4·0 ml. of 95% ethanol. The contents were carefully shaken for complete extraction. To the extract was added 0·2 ml. of 0·1% copper sulphate when the blue colour was changed to a stable pink. A further 0·8 ml. of ethanol was used to make the volume to 5·0 ml. The density of the colour was read on a Klett-Summerson photoelectric colorimeter using green filter (540 mμ.). The standards for serine were run between 0 and 20 μg. The spots for ethanolamine were fainter as compared to those for serine in equivalent amounts. The values obtained for serine were comparable to those estimated microbiologically.

Serine deamination, serine decarboxylation and liver nitrogen were all determined by methods reported by Nadkarni and Sreenivasan (1957).

Biotin.—Liver homogenate (equivalent to 1 g. of tissue) was hydrolysed with 3% sulphuric acid at 15 lb. pressure for 30 minutes. To the cooled hydrolysate was added 2·0 ml. of 2·5 M sodium acetate and the pH adjusted to neutral with dilute alkali. The volume was made up to 50 ml. Aliquots were used for biotin assay microbiologically with *Lactobacillus arabinosus* (Wright and Skeggs, 1944). The range of biotin concentrations for the standard curve was 0·00005 μg. to 0·00025 μg.

All the values reported are averages for at least quadruplicate independent samples.

Results

From preliminary experiments using paper chromatography for serine assay, it was ascertained that serine was being synthesised from ethanolamine with normal rat liver homogenates to the extent of $1·5 \pm 0·2$ μ moles per mg. N. The values were comparable to those obtained by microbiological assays. In later experiments the extent of serine synthesis from ethanolamine was followed by microbiological assays only.

Table I shows the effect of pH on the synthesis of serine from ethanolamine. Optimum synthesis could take place at pH 8·3.

TABLE I

Effect of pH on ethanolamine to serine conversion

pH of the buffer (0·1 M phosphate buffer)	Resultant pH (after adding bicarbonate)	Serine formed μ moles/mg. N
5·0	6·5	1·18
6·4	8·3	1·64
7·1	9·1	1·27

A decrease in serine formation in biotin deficiency is observable from the results in Table II.

TABLE II

Effect of biotin deficiency on ethanolamine to serine conversion

Group	Average weight gain (10 weeks) (g.)	μ moles of serine per mg. N
− Biotin	+16	0·88±0·04
+ Biotin	+90	1·74±0·04

(Average initial weight of rats was 55 g.)

To some of the biotin-deficient rats, 100 μg. of biotin was administered intraperitoneally, three hours prior to sacrifice. A definite increase in ethanolamine to serine synthesis was observable in these cases. Typical results are given in Table III which also includes data obtained when biotin (10 μg./flask) was added *in vitro* to the system and which shows no stimulation in serine synthesis.

Serine deamination is reduced as a result of biotin deficiency while serine decarboxylation is not affected (Table IV). The effects of biotin deficiency on liver nitrogen and liver biotin are summarised in Table V.

TABLE III

Effect of biotin on ethanolamine to serine conversion

Experiment	Biotin-fed	Biotin deficient (biotin injected)	Biotin deficient (biotin added *in vitro*)
	μ moles of serine per mg. N		
1	1·94	1·73	0·71
2	1·33	1·38	0·88

TABLE IV

Serine deaminase and serine decarboxylase activities

Group	Serine deamination		Serine decarboxylation μl. CO_2/mg.N/30 minutes
	Pyruvic acid	Ammonia	
	μ moles/mg. N		
— Biotin	3·54±0·20	3·47±0·24	3·93±0·20
+ Biotin	5·13±0·20	4·78±0·30	3·71±0·11

TABLE V

Liver analysis

Group	Liver weight per 100 g. body weight (g.)	Liver biotin μg./g. fresh weight	Liver nitrogen	
			Per cent. fresh weight	Per cent. dry weight
— Biotin	5·8±0·4	0·081±0·002	3·33±0·03	11·32±1·0
+ Biotin	2·8±0·3	0·242±0·004	3·89±0·02	13·56±0·8

DISCUSSION

Incorporation of metabolic CO_2 into proteins and amino acids is known (Abelson *et al.*, 1952 *a*, 1952 *b*; Davis *et al.*, 1956). In protein hydrolysates

of micro-organisms, label of $C^{14}O_2$ is contained in the carboxyl group of the amino acids (Abelson *et al.*, 1952 *a*) and this could virtually account for all of the incorporation of radioactivity into the proteins. Fixation of carbon dioxide into amines with formation of corresponding amino acids has also been indicated (Hanke and Siddiqui, 1950; Koppleman *et al.*, 1952).

The intimate relationship between serine and ethanolamine would suggest their interconvertibility, though alternative pathway of ethanolamine to serine is known (Weissbach and Sprinson, 1953). This interconversion is apparently influenced by physiological conditions. The decarboxylation of serine takes place at pH values below 7·0 and is inhibited at higher pH (Nadkarni and Sreenivasan, 1957). The formation of serine from ethanolamine and bicarbonate on the other hand is optimum at pH 8·3 and at lower pH it is less efficient.

The influence of biotin on serine synthesis from ethanolamine is another example of systems wherein CO_2 fixation has been shown to be mediated by the vitamin. Although further work seems necessary, doubts expressed on the coenzymatic role of biotin have recently been answered as due to defects in assay procedures for bound biotin in purified holoenzyme preparations (Lichstein, 1955).

The reduced rate of serine deamination in biotin-deficient animals may be related to the fact that pyruvate metabolism is influenced by this vitamin.

SUMMARY

Synthesis of serine from ethanolamine by carbon dioxide fixation is demonstrated to take place in presence of rat liver enzymes. The system is biotin-dependent.

Serine deaminase activity but not that of serine decarboxylase is also reduced in biotin deficiency.

REFERENCES

Abelson, P. H., Bolton, E. T. and Aldous, E. — *J. Biol. Chem.*, 1952 *a*, **198**, 165.

———— .. *Ibid.*, 1952 *b*, **198**, 173.

Alexander, J. C., Beckner, W. and Elvehjem, C. A. — *J. Nutr.*, 1953, **51**, 319.

Burke, D. and Winzler, R. J. .. *Science*, 1943, **97**, 59.

David, J. W., Cheldelin, V. H., Christensen, B. E., and Wang, C. H. — *Biochim. Biophys. Acta*, 1956, **21**, 101.

Delwiche, E. A. .. *J. Bact.*, 1950, **59**, 439.

Eakin, R. E., Snell, E. E. and Williams, R. J. *J. Biol. Chem.*, 1941, **140**, 535.

Feldott, G. and Lardy, H. A. .. *Ibid.*, 1951, **192**, 447.

Folch, J. and Schneider, H. A. .. *Ibid.*, 1941, **137**, 51.

Giri, K. V. and Rao, N. A. N. .. *J. Ind. Inst. Sci.*, 1952, **34**, 95.

Gothoskar, S. S. and Sreenivasan, A. *Ind. J. Med. Res.*, 1953, **41**, 69.

Hanke, M. E. and Siddiqui, M. S. H. *Fed. Proc.*, 1950, **9**, 181.

Koppleman, R., Mandeles, S. and Hanke, M. E. *Ibid.*, 1952, **11**, 242.

Lardy, H. A. and Adler, J. .. *J. Biol. Chem.*, 1956, **219**, 933.

———, McLeod, P. R., Grisolia, S. and Cohen, P. P. *Ibid.*, 1949 *a*, **180**, 1003.

————, Potter, R. L. and Burris, R. H. *Ibid.*, 1949 *b*, **179**, 721.

————, ———— and Elvehjem, C. A. *Ibid.*, 1947, **169**, 451.

Levine, C. and Chargraff, E. .. *Ibid.*, 1951, **192**, 465.

Lichstein, H. C. .. *Ibid.*, 1955, **212**, 217.

———— and Christman, J. F. .. *Ibid.*, 1948, **175**, 649.

———— and Umbreit, W. W. .. *Ibid.*, 1947, **170**, 423.

McCoy, R. H., McKibben, J. N., Axelrod, A. E. and Hofmann, K. *Ibid.*, 1948, **176**, 1319.

McLeod, P. R. and Lardy, H. A. .. *Ibid.*, 1949, **179**, 733.

Nadkarni, G. B. and Sreenivasan, A. *Proc. Ind. Acad. Sci.*, 1957, **46 B**, 138.

Ochoa, S., Mehler, A., Blanchard, M. L., Jukes, T. H. and Hoffmann, C. E. *J. Biol. Chem.*, 1947, **170**, 413.

Plaut, G. W. E. and Lardy, H. A. .. *Ibid.*, 1950, **186**, 705.

Pilgeram, L. O., Gal, E. M., Sassenrath, E. N. and Greenberg, D. M. *Ibid.*, 1953, **204**, 367.

Shive, W. and Rogers, L. L. .. *Ibid.*, 1947, **169**, 453.

Steele, B. F., Sauberlich, H. E., Reynolds, M. S. and Baumann, C. A. *Ibid.*, 1949, **177**, 533.

Utter, M. F. and Kurahashi, K. .. *Ibid.*, 1954, **207**, 821.

Weissbach, S. and Sprinson, D. B. .. *Ibid.*, 1953, **203**, 1031.

Wessman, C. E. and Werkman, C. H. *Arch. Biochem.*, 1950, **26**. 214.

Williams, V. R. and Christman, J. F. *J. Bact.*, 1953, **65**, 238.

Wright, L. D. and Skeggs, H. R. .. *Proc. Soc. Expt. Biol. Med.*, 1944, **56**, 95.

Yanofsky, C. .. *J. Biol. Chem.*, 1953, **202**, 567.