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**26. Masao Shimizu and Yasushi Abiko : Investigations on
Pantothenic Acid and its Related Compounds. II.*¹
Biochemical Studies. (1). Biosynthesis of Coenzy-
me A from Pantothenate, Pantethine and from
S-Benzoylpantetheine *in vitro* and *in vivo*.*²**

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The *Lactobacillus bulgaricus* factor (LBF) which had been found by Williams, *et al.*¹⁾ was characterized as N-pantothenoyl-2-aminoethanethiol (pantetheine : PTSH) or the disulfide (pantethine : PTSS) and was chemically synthesized by Snell, *et al.*²⁾

Novelli, *et al.*^{3,4)} demonstrated that PTSH was an intermediate in the biosynthesis of CoA from pantothenate (PaA) in the rat. Later, Brown⁵⁾ reported an alternate pathway of CoA biosynthesis in *Proteus morganii* and rats in which 4'-phosphopantothenate was an intermediate, and it was postulated that the 4'-phosphopantothenate pathway was the main route in many microorganisms and higher animals.

Although the growth-promoting properties of PTSS and PaA were investigated for a number of microorganisms, few reports have appeared concerning the biological activity of PTSS and PTSH in animals comparing them with that of PaA. Rasmussen, *et al.*⁶⁾ reported that vitamin B₁₂-free LBF enhanced the growth of chicks and McRorie, *et al.*,⁷⁾ comparing the growth obtained by supplementing PaA-deficient rats with PaA and LBF, demonstrated that LBF could replace PaA in the nutrition of rats. Lih, *et al.*⁸⁾ found that CoA and LBF were equal to PaA in promoting growth in rats. Thompson, *et al.*⁹⁾ reported that PTSS was at least 90% as active in promoting chick growth as PaA when administered by mouth although PTSS possessed the same activity as PaA after intraperitoneal injection. Garattini and Paoletti¹⁰⁾ demonstrated that PTSS improved the weight curve of growing rats and also acted as a substitute in PaA-deficiency. Mascitelli-Coriandoli and Citterio¹¹⁾ reported that PaA was effective in maintaining CoA-level in testicles of aged male mice and that PTSS was much more effective.

Present report deals with the effectiveness of PTSS and S-benzoylpantetheine (PTS-Bz) as a precursor of CoA in comparison with PaA *in vitro* and *in vivo* in rats.

*¹ Part I. This Bulletin, 13, 180 (1965).

*² A part of this paper was read at the 36th Annual Meeting of the Japanese Biochemical Society, November, 1963, Tokyo, and the other part was read at the Kanto Branch Meeting of Pharmaceutical Society of Japan, June, 1964, Tokyo.

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1) W. L. Williams, E. Hoff-Gørgensen, E. E. Snell : J. Biol. Chem., 177, 933 (1949).

2) E. E. Snell, G. M. Brown, V. J. Peters, J. A. Craig, E. L. Wittle, J. A. Moore, V. M. McGlohon, O. D. Bird : J. Am. Chem. Soc., 72, 5349 (1950).

3) L. Levintow, G. D. Novelli : J. Biol. Chem., 207, 761 (1954).

4) M. B. Hoagland, G. D. Novelli : *Ibid.*, 207, 767 (1954).

5) G. M. Brown : J. Biol. Chem., 234, 370 (1959).

6) R. A. Rasmussen, K. L. Smiley, J. G. Anderson, J. M. Van Lanen, W. L. Williams, E. E. Snell : Proc. Soc. Exptl. Biol. Med., 73, 685 (1950).

7) R. A. McRorie, F. W. Sherwood, W. L. Williams : *Ibid.*, 75, 392 (1950).

8) H. Lih, T. E. King, H. Higgins, C. A. Baumann, F. M. Strong : J. Nutr., 44, 361 (1951).

9) R. Q. Thompson, O. D. Bird, F. E. Peterson : J. Nutr., 53, 365 (1954).

10) S. Garattini, R. Paoletti : Atti soc. lombarda sci. med. e biol., 9, 207 (1954) (C. A., 49, 4822 (1955)).

11) E. Mascitelli-Coriandoli, C. Citterio : Naturwissenschaften, 46, 429 (1959).

Materials and Methods

Substrates and Reagents—Pantethine and S-benzoylpantetheine were chemically synthesized as described in the preceding paper.^{*1} Calcium pantothenate (PaA-Ca) and disodium adenosine-5'-triphosphate (ATP-Na₂) were obtained from Daiichi Pure Chemicals Co., Ltd. CoA was purchased from Sigma Chemical Company (Missouri, U. S. A.) and Nutritional Biochemicals Corporation (Ohio, U. S. A.). Sodium cytidine-5'-triphosphate and reduced glutathione were obtained from Sigma Chemical Company and Nutritional Biochemicals Corporation, respectively. Protamine sulfate from salmon loe was a preparation of Nutritional Biochemicals Corporation.

Animal—Male Donryu rats from Central Laboratories for Experimental Animals (Tokyo) were used in this study. Rats used for *in vitro* experiments weighed approximately 200 g. and were fed "CLEA CA-1" diet, obtained from Central Laboratories for Experimental Animals. Some of the rats used for *in vivo* experiments were 23~24 days old and weighed an average 45 g. and others were 28~29 days old and weighed an average of 58 g; these animals were fed *ad libitum* the synthetic diets described as follows.

Synthetic Diets for *in vivo* Experiments—The basal diet consisted of 34% sugar (granule), 30% purified wheat starch, 24% purified milk casein, 8% cotton-seed oil, and 4% Phillips and Hart's salt mixture IV.¹²⁾ The following vitamins were added to each Kg. of the basal diet to prepare PaA-deficient diet: 2 mg. thiamine hydrochloride, 10 mg. riboflavin, 2 mg. pyridoxine hydrochloride, 5 mg. *p*-aminobenzoic acid, 10 mg. nicotinic acid, 200 mg. inositol, 500 mg. choline chloride, 23 mg. α -tocopherol, 2.1 mg. menadion, 12510 I. U. vitamin A, and 1772 I. U. vitamin D. Three diets containing PaA, PTSS and PTS-Bz respectively, were also prepared by adding to the PaA-deficient diet 30 mg. (about 63 μ moles) of PaA-Ca, 34.9 mg. (about 63 μ moles) of PTSS and 48.1 mg. (about 126 μ moles) of PTS-Bz, accordingly.

Rat Liver Extract—Fresh liver from an albino rat was homogenized with 3 volumes of ice-cold 0.02M phosphate buffer (pH 7.2) and the homogenate was centrifuged at 16,000 r.p.m. for 20 min. The supernatant solution was spun at 40,000 r.p.m. for an additional 1 hr. The soluble fraction was, then, dialyzed overnight against 200 volumes of 0.02M phosphate buffer (pH 7.2) at 2°. The dialyzed solution was used for CoA synthesis.

The CoA-synthesizing activity of the liver extract decreased considerably under storage at -5°. It was impossible to investigate CoA synthesis by using rat liver homogenate, since the homogenate contained activities which led to the rapid degradation of CoA. The destructive principals in liver homogenate were not removed by centrifugation at 16,000 r.p.m. for 40 min., and the formation curve of CoA by the supernatant fell rapidly after reaching a maximum as shown in Fig. 1 (Curve A). The CoA-degrading activities were almost completely removed by a second centrifugation of the supernatant at 40,000 r.p.m. for 1 hr. (Fig. 1, curve B) whereby the undesirable activity was concentrated in the pellet.

Pigeon Liver Extract—Pigeon liver extract used for the determination of CoA was prepared by the method of Kaplan and Lipmann¹³⁾ with a minor modification as follows. Fresh livers from pigeons were homogenized with 20 volumes of cold acetone (-5~-10°) in a Waring blender for 1 min. The insoluble material was collected on a Büchner funnel and was washed successively with cold acetone, acetone-ether (1:1, v/v) and ether. The solid material was rapidly air-dried at room temperature and then dried *in vacuo* over P₂O₅. The acetone-dried powder was kept in a desiccator at -3°, without any loss of activity for at least 6 months. Two grams of the acetone powder was homogenized with 20 ml. of cold 0.02M potassium bicarbonate and centrifuged at 16,000 p.p.m. at 0° for 30 min. The supernatant solution was kept frozen overnight.

After thawing it at 25° and removing the insoluble material by centrifugation, the resultant solution was dialyzed against 2 L. of 0.02M potassium bicarbonate under stirring at 2° overnight. The dialyzed solution

Fig. 1. Comparison of Time Courses of CoA Biosynthesis by the 16,000 r.p.m.-Supernatant and by the 40,000 r.p.m.-Supernatant of Rat Liver Homogenate

Incubation mixture contained 0.1 μ mole of PaA-Ca, 0.5 μ mole of cysteine, 10 μ moles of ATP-Na₂, 5 μ moles of MgCl₂, 50 μ moles of tris(hydroxymethyl)aminomethane and 0.4 ml. of rat liver extract in a total volume of 1.0 ml., pH 7.5. The mixture was incubated at 37°. Curves A and B represented the time courses of CoA formation by the 16,000 r.p.m.-supernatant and by the 40,000 r.p.m.-supernatant, respectively.

12) P. H. Phillips, E. B. Hart: J. Biol. Chem., **109**, 657 (1935).

13) N. O. Kaplan, F. Lipmann: *Ibid.*, **174**, 37 (1948).

was then treated with 2 ml. of Dowex 1×4 (chloride form, 200~400 mesh) and the resin was removed by centrifugation or filtration. The filtrate was treated with protamine sulfate, according to Levintow and Novelli,³⁾ to remove the enzyme activity which catalyses the phosphorylation of PTSH. To 10 ml. of the filtrate was added 0.5 ml. of 2% protamine sulfate solution and the resulting precipitate was centrifuged off. Additional 1 ml. of protamine sulfate solution was added to the supernatant and the precipitate was discarded by centrifugation. The supernatant fraction was found entirely inactive in converting PTSH to CoA but it contained the enzyme system for the acetylation of sulfanilamide and was used for the assay of CoA. In the latter part of this study, however, the aged extract of pigeon liver prepared according to the original procedure of Kaplan and Lipmann¹³⁾ was used because it was recognized that pantetheine kinase activity of the extract was completely inactivated by aging it at 25° for 6 hr.

Determination of CoA—CoA was determined by the method of Kaplan and Lipmann¹³⁾ which is based on the enzymatic acetylation of sulfanilamide by the pigeon liver extract. A test sample (0.3 ml.) was incubated at 37° for 2 hr. with 0.08 ml. of 1M potassium bicarbonate, 0.1 ml. of 0.1M cysteine, 0.25 ml. of the pigeon liver extract and 0.3 ml. of the CoA-assay medium, consisting of 10 ml. of 0.004M sulfanilamide, 10 ml. of 0.5M sodium citrate, 2.5 ml. of 1M sodium acetate and 8 ml. of 0.05M ATP-Na₂, pH 7. After incubation, 4 ml. of 5% trichloroacetic acid was added to the reaction mixture and the denatured protein precipitate was removed by centrifugation. To 0.5 ml. of the supernatant, 2.5 ml. of water, 0.5 ml. of 4N hydrochloric acid and 0.25 ml. of 0.2% sodium nitrite were introduced. After a 3 minutes standing, 0.3 ml. of 25% urea solution was added and allowed to stand for 10 min. Then 0.2 ml. of 0.2% Tsuda's reagent (N,N-diethyl-N'-naphthylethylene diamine oxalate) was added to develop a red color which was read at 540 mμ against a reagent blank. The rate of acetylation was calculated from the decrease in the amount of free sulfanilamide and the CoA present in the test sample was determined against a calibration curve of standard CoA. For the assay of CoA in tissues, animals were sacrificed by decapitation and the organs were quickly removed and chilled. The organs were homogenized at 0° with an appropriate volume (10~15 volumes for liver and kidney, 30 volumes for adrenal glands) of water and the homogenate was rapidly boiled. The boiled extracts were, then, centrifuged and the supernatants were assayed for the coenzyme.

Results and Discussion

Enzymatic Synthesis of CoA from PaA, PTSS and PTS-Bz *in vitro*

The time course of CoA synthesis from PaA by rat liver extract was demonstrated in Fig. 2 (Curve A). PaA-Ca (0.1 μmole) was added to the mixture of 0.5 μmole of cysteine, 10 μmoles of ATP-Na₂, 5 μmoles of magnesium chloride, 50 μmoles of tris(hydroxymethyl)aminomethane and 0.4 ml. of rat liver extract bringing the total volume to 1.0 ml. and the pH to 7.5 and the resultant mixture was incubated at 37°. At the various time intervals, the reaction mixture was heated in a boiling water bath for 2 minutes to stop the reaction. The supernatant solution, obtained by centrifugation, served as a sample for CoA assay. In this system, about 16 Lipmann units of CoA per ml. was produced during 2 hours' incubation.

In the over-all reaction of CoA formation from PaA, the maximal rate was observed at pH 7.0~7.5 with PaA concentration of more than $1.4 \times 10^{-4}M$ and with cysteine concentration of more than $2 \times 10^{-4}M$. No substrate inhibition was observed at the concentration of less than $6 \times 10^{-4}M$ PaA and of less than $1 \times 10^{-2}M$ cysteine.

The time course of CoA formation from PTSS was shown in Fig. 2 (Curve B). PTSS (0.1 μmole) was incubated at 37° with 10 μmoles of ATP-Na₂, 5 μmoles of magnesium chloride, 50 μmoles of tris(hydroxymethyl)aminomethane and 0.4 ml. of rat liver extract in a total volume of 1.0 ml. at pH 7.5. In this system, about 26 Lipmann units of CoA

Fig. 2. The Time Courses of CoA Biosynthesis from PaA and from PTSS

Incubation conditions were described in the text. Curves A and B represented the time courses of CoA formation from PaA and from PTSS, respectively.

per ml. was synthesized during a 2 hours' incubation period. The CoA formation from PTSS progressed linearly in the early stage of the reaction while there was a lag in the formation of the coenzyme from PaA, indicating that PTSS was a much more effective precursor of CoA than PaA *in vitro*.

Brown⁵⁾ reported that in the metabolism of PaA, the cysteine-coupling reaction of 4'-phosphopantothenate in *Proteus morganii* required cytidine-5'-triphosphate rather than adenosine-5'-triphosphate, but that in the mammalian system, CTP was not a requirement. The authors also observed that the addition of CTP to this system failed to stimulate the initial rate of CoA synthesis from PaA. According to Brown, PTSH is not an intermediate in the main route of the formation of CoA from PaA although it is a direct intermediate in the Novelli's pathway. It has been, however, confirmed by present results and even by Brown¹⁴⁾ himself that PTSS or PTSH can be an effective precursor of CoA. It seemed likely that the formation of CoA from PTSS would occur through the natural metabolic pathway by which animals reutilized the degraded products of CoA in the turn-over of the coenzyme but not through any indirect route or a special pathway such as an induced one.

The reductants, such as reduced glutathione or cysteine, did not exert an appreciable influence on the formation of CoA from PTSS. Fig. 3 shows a slightly higher yield of CoA in the presence of glutathione and a somewhat lower yield of CoA in the presence of cysteine than the CoA yield obtained in the absence of the reductant. These findings

Fig. 3. The Effect of the Reductants on CoA Synthesis from PTSS by Rat Liver Extract

The incubation mixture contained 0.1 μ mole of PTSS, 10 μ moles of ATP- Na_2 , 5 μ moles of MgCl_2 , 50 μ moles of tris-(hydroxymethyl)aminomethane and 0.4 ml. of rat liver extract with or without 0.5 μ mole of reduced glutathione or cysteine in a total volume of 1.0 ml., pH 7.5. The mixture was incubated at 37°. Curves A, B, and C represented the time courses of CoA formation from PTSS without any reductant, with reduced glutathione and with cysteine, respectively.

Fig. 4. Time Courses of CoA Formation by Rat Liver Extract from PTSS and from PTS-Bz

0.1 μ mole of PTSS or 0.2 μ mole of PTS-Bz was incubated with 10 μ moles of ATP- Na_2 , 5 μ moles of MgCl_2 , 50 μ moles of tris-(hydroxymethyl)aminomethane and 0.4 ml. of rat liver extract in a total volume of 1.0 ml., pH 7.5, at 37°. Curves A and B represented CoA formation from PTSS and from PTS-Bz, respectively.

suggested that PTSS could be synthesized to CoA without reducing it to PTSH and that the coenzyme so formed would be mainly in a disulfide form, or in the mixed disulfide form of CoA and PTSH. On the other hand, CoA which was synthesized in the presence of reductants seemed to be entirely in the reduced form. Further investigation in this respect will be reported elsewhere.

14) G. B. Ward, G. M. Brown, E. E. Snell: J. Biol. Chem., 213, 869 (1955).

Fig. 4 (Curve B) shows the time course of CoA synthesis from PTS-Bz by rat liver extract in comparison with that (Fig. 4, curve A) of CoA formation from PTSS by the same extract. A slightly lower yield of CoA was obtained when PTS-Bz was used as a substrate instead of PTSS, but the formation of CoA from PTS-Bz progressed linearly in the initial stage of the reaction just as observed in the case with PTSS.

Effect of PaA, PTSS, and PTS-Bz on the Levels of CoA in Tissues of Rats

Eighteen rats, averaging 58 g. in weight, were divided into 6 groups, each of which consisted of 3 rats. The first group was fed the PaA-deficient diet; the second group, the PaA-containing diet; the third group, the PTSS-containing diet; and the fourth group, the PTS-Bz-containing diet for 5 weeks. The fifth and the last groups served as controls and were fed "CLEA CA-1" diet *ad libitum* and with a limit of 7 g. of the food per rat daily, respectively.

Rats of each group, except that of "CLEA CA-1 limited" group, grew normally during the first 4 weeks. Growth of the "limited" group was impaired, and the body weight gained less than 40 g. during a 5-week period. After 4 weeks, growth of the PaA-deficient group was slowed whereas those of the other groups exhibited a normal growth.

At end of the fifth week, all animals were killed and the CoA content in liver, kidney and adrenal glands of each rat was determined. The results are shown in Table I. Average CoA level of liver of the normal control animals, which was fed "CLEA CA-1" *ad libitum*, was 157.7 Lipmann units per g. of the wet tissue, and that of the kidney, 57 units, and that of adrenals, 95.2 units. These values were virtually in agreement with the results obtained by other investigators.^{13,15-18} CoA levels

TABLE I. The Levels of CoA in the Organs of Rats of Each Group

Group	CoA units/g. of wet tissue		
	Liver	Kidney	Adrenal
PaA	149 (142)	63.1 (71.5)	94.7
	134	67.3	
	143	84	
PTSS	158 (149.7)	58.5 (66.5)	107.7
	150	67.5	
	142	73.5	
PTS-Bz	132 (143.5)	64.5 (67.5)	97.4
	147	64.5	
	151.5	73.5	
Deficient	83 (83.6)	41.1 (41.7)	52.6
	77	45	
	90.8	39.5	
CLEA <i>ad lib.</i>	133 (157.7)	60 (57)	95.2
	159	56	
	181	55	
CLEA limited	153 (158.3)	72 (67.6)	—
	161	69	
	151	52	

CoA was assayed by the method of Kaplan and Lipmann.¹³ Figures in the parentheses represented the average of the group. Adrenal glands from 3 rats of one group were assayed as a single batch for the coenzyme.

- 15) R.E. Olson, N.O. Kaplan: J. Biol. Chem., **175**, 515 (1948).
- 16) G.D. Novelli, N.O. Kaplan, F. Lipmann: J. Biol. Chem., **177**, 94 (1949).
- 17) G.E. Boxer, C.E. Shonk: Arch. Biochem. Biophys., **59**, 24 (1955).
- 18) K. Yamada, S. Sawaki, S. Hayami: Vitamins, **12**, 179 (1957).

of these organs of the PaA, PTSS, and PTS-Bz groups were all maintained in the normal range indicating that the effects of PaA, PTSS, and PTS-Bz on the CoA levels of tissues were the same in this condition and that these substances were utilized similarly as the precursor in the biosynthesis of CoA *in vivo*. On the other hand, in the PaA-deficient group, the CoA level was reduced by about a half in all organs tested, compared with those of the other groups. It was of interest to observe that the contents of the coenzyme of the "CLEA CA-1 limited" group were of the normal level while the growth of the animals of this group was strongly impaired.

Thompson, *et al.*⁹⁾ reported that as a growth-promoting substance in chicks, PTSS was somewhat inferior to PaA when given by mouth but it was just as active when injected intraperitoneally. In the present results with young rats, both of these substances and PTS-Bz were shown to be equally active in maintaining CoA levels in the normal range, and PTSS and PTS-Bz were found to be slightly superior to PaA in increasing the body weight of rats although these results were obtained from a single experiment conducted on a small scale.

Recovery of CoA-Level of the Liver of PaA-Deficient Rats by PaA, PTSS or PTS-Bz

Young rats of 23~24 days of age were fed the PaA-deficient diet for about 4 weeks. After reducing the level of CoA in their tissues, an attempt was made to follow periodically the recovery of CoA level of liver of these PaA-deficient rats by a treatment with PaA, PTSS or PTS-Bz. The procedure was as follows. A PaA-deficient rat was anesthetized by a subcutaneous injection of ethylurethane with a dose of 1.3 g. per kg. of body weight. According to the technique used in the experiment of regenerating liver, the left lobe of liver was removed and chilled. The lobe removed was quickly treated to prepare the boiled extract for CoA assay, as described above. After administration of PaA, PTSS or PTS-Bz by a stomach tube or by intravenous injection, the median lobe and then right lobe were removed successively at proper time intervals, and the boiled extracts were prepared for CoA assay in the same way.

CoA values which were obtained from each extract were believed to indicate the levels of the coenzyme in the liver before and after administration of the vitamin in the same rat. This procedure can be valid if the followings could be established: (1) an even distribution of CoA in the liver, (2) no effect of hepatectomy on the CoA levels of the residual liver lobes and (3) no effect of ethylurethane on the biosynthesis of CoA in the liver.

TABLE II. Distribution of CoA in the Liver of PaA-Deficient Rats

Rat No.	CoA units/g. of liver lobe		
	left lobe	median lobe	right lobe
1	121	128	124
2	107	105	107

TABLE III. Effect of Successive Partial Hepatectomies on the CoA Levels of the Liver of PaA-Deficient Rats

Time ^{a)} Rat No.	CoA units/g. of wet liver			
	0	30	60	120
3	102.2	111	111	—
4	92.7	90.3	90.3	—
5	80.6	85.9	—	78

a) Time in minute after the first hepatectomy.

The distribution of CoA in the liver of PaA-deficient rats was even in the same rat as shown in Table II. When the animals received no vitamin the successive partial hepatectomy afforded no influence on the CoA levels of the residual liver lobes of PaA-deficient rats at least during the 2 hours period after the first hepatectomy (Table III).

Table IV shows that ethylurethane did neither inhibit nor stimulate the rate of CoA synthesis from PaA *in vitro*. The amount of the drug added to the reaction system (3.3 mg./ml.) was calculated from an unphysiological presumption that all of the given drug would accumulate unchanged in the liver. Urea showed also no influence on the *in vitro* biosynthesis of the coenzyme. The amount of urea added to the reaction system (2.2 mg./ml.) was again calculated from a presumption that all of urethane given as an anesthetic would be converted to urea in the body and accumulated in the liver.

TABLE IV. Effect of Ethylurethane and Urea on the Biosynthesis of CoA from PaA by Rat Liver Extract

Time ^{a)} System	CoA units/ml. of reaction mixture		
	0	60	120
Control	3.4	15.7	20.8
with urethane	2.8	15.0	19.9
with urea	3.1	15.0	19.8

a) Incubation time in minute.

One milliliter of the incubation mixture contained 0.1 μ mole of PaA-Ca, 0.5 μ mole of cysteine, 10 μ moles of ATP-Na₂, 5 μ moles of MgCl₂, 50 μ moles of tris(hydroxymethyl)aminomethane and 0.4 ml. of rat liver extract, and with or without 3.3 mg. of ethylurethane or 2.2 mg. of urea. The mixture was incubated at pH 7.5 at 37°.

At a high concentration of ethylurethane, the following enzymes were reported to be inhibited: adenosine triphosphatase,¹⁹⁾ glycogenesis from glucose,²⁰⁾ oxidative phosphorylation in brain,²¹⁾ cholineesterase,²²⁾ pyruvate oxidase,²³⁾ lactic dehydrogenase,²³⁾ and electron-transport from flavin enzyme to cytochrom system.²⁴⁾ At a low concentration of the urethane, however, the following enzymes or enzyme systems were reported to be not inhibited: oxidative phosphorylation,²⁵⁾ hexokinase reaction,²⁶⁾ anaerobic glycolysis,²⁶⁾ succinic dehydrogenase,²³⁾ cytochrom oxidase²⁶⁾ and the enzyme systems cited above.^{19, 23)} Ethylurethane, therefore, would not interfere in the present experiment because the drug which was given as a narcotics was largely diluted in the body.

From these findings, "the successive partial hepatectomy method" was found to be applicable for the investigation on the recovery of CoA level in PaA-deficient rats to compare the *in vivo* activities of PaA, PTSS, and PTS-Bz as precursors of CoA.

The animals used in this series were fasted overnight just before the experiment. PaA-deficient rats were injected intravenously with the vitamins at the dose of 10 μ moles (equivalent to the amount of PaA) in 0.2 ml. of saline per 100 g. of body weight immediately after the first hepatectomy, and then the CoA levels of the liver lobes were successively assayed during the 2 hours period after injection. As shown

19) M. Gross: Arch. Biochem. Biophys., **53**, 119 (1954).

20) E. Kun, L.G. Abood: Arch. intern. Pharmacodynamie, **80**, 44 (1949).

21) I. Tyuma, A. Morikawa: J. Nara Med. Assoc., **6**, 23 (1955) (C. A., **50**, 3524 (1956)).

22) L. D'Agostino, N. Vicino, L. Salonna: Boll. soc. ital. biol. sper., **31**, 270 (1955) (C. A., **49**, 14840 (1955)).

23) K. Iwase: Folia pharmacol. japon., **52**, 245 (1956).

24) S. Kawashima: Nara Igaku Zasshi, **9**, 155 (1958) (C. A., **53**, 3338 (1959)).

25) E. Boyland, H.G. Williams-Ashman: Acta Unio Intern. contra Cancrum, **7**, 432 (1951) (C. A., **48**, 9556 (1954)).

26) S. Tanaka, T. Shimizu, Y. Ishioroshi, M. Koike, S. Hirako: Nagoya J. Med. Sci., **15**, 146 (1952) (C. A., **47**, 10117 (1953)).

in Table V, the injection of either PaA, PTSS, or PTS-Bz caused an almost complete recovery of CoA level in the liver of PaA-deficient rats within 2 hours after injection. Also in the case of oral administration, in which PaA-deficient rats received 50 μ moles of the vitamins (equivalent to the amount of PaA) in 1 ml. of 5% glucose solution per 100 g. of body weight, CoA contents of the liver nearly attained the normal level within 2 hours after administration (Table VI).

TABLE V. Recovery of Liver-CoA Levels of PaA-Deficient Rats by Intravenous Injection of PaA, PTSS or PTS-Bz

Vitamin	Rat No.	CoA units/g. of wet liver time (min.) after injection				
		0	30	60	90	120
PaA	24	78.6	89	116	—	—
	3	80.8	93.2	125	—	—
	10	86.4	—	—	94	—
	36	75.7	—	—	138.2	142
PTSS	7	68.9	106.4	125	—	—
	11	73.4	118	118	—	—
	25	64.5	97	125	—	—
	35	62.6	—	—	130.9	156
	12	84.5	—	—	143.1	141
PTS-Bz	1	68.4	103.2	125	—	—
	2	76.8	83.1	—	—	—
	6	76	—	—	132	139.6

Experimental conditions were described in the text.

TABLE VI. Recovery of Liver-CoA Levels of PaA-Deficient Rats by Oral Administration of PaA, PTSS or PTS-Bz

Vitamin	Rat No.	CoA units/g. of wet liver time (min.) after administration				
		0	30	60	90	120
PaA	4	76.8	70.5	111.8	—	—
	5	76	76.8	125.7	—	—
	31	62.9	—	—	127.1	145.9
	32	62.9	—	—	110.3	134.7
PTSS	13	92	99	119.7	—	—
	14	70.6	96.3	129	—	—
	17	78.5	—	—	87	118.9
	18	72.9	—	—	129	166
PTS-Bz	15	78.5	93.6	106	—	—
	16	84.9	92	119.7	—	—
	20	78.5	—	—	118.9	129

Experimental conditions were described in the text.

These results agreed with that of Olson and Kaplan.¹⁵⁾ They reported that the intraperitoneal injection of PaA into PaA-deficient ducks (28~78 units CoA/g. of liver) resulted in a complete recovery in CoA level in 2 hours after injection (118~141 units/g.).

Many studies on the effects of vitamins on the growth of animals have generally dealt with the recovery of body weight or with the disappearance of various deficiency symptoms in the vitamin-deficient animals after administration of the lacked vitamin. These indexes, however, would indicate some indirect and final outcomes of vitamin treatments and, in this way, it seems impossible to compare strictly the effects of some

analogous vitamins. The authors preferred dealing with the recovery of CoA level which was a more direct index and comparing the activities of PaA, PTSS, and PTS-Bz as precursors of CoA *in vivo*. Olson and Kaplan¹⁵⁾ also compared the CoA contents in the liver of PaA-deficient ducks before and after treatment with PaA using different individuals. As shown in Table I, however, there was a considerable variation in the tissue-CoA levels of each individual. "The successive partial hepatectomy method" presented here which can follow periodically the recovery of the coenzyme level in the same animal seems to be a useful tool in investigations of this kind.

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Summary

1. Coenzyme A was synthesized from pantothenic acid, pantethine or S-benzoylpantetheine by rat liver extract. Biosynthesis of CoA from pantethine progressed *in vitro* more rapidly than from pantothenate, and a slightly lower yield of CoA was obtained when S-benzoylpantetheine was used as a substrate than when pantethine was used.

2. Pantothenate, pantethine or S-benzoylpantetheine, incorporated into a pantothenic acid-deficient diet, supported the normal growth of young rats. Tissue-CoA levels of the rats fed these diets were maintained in the normal range while the levels of the other rats, which were fed the deficient diet, were reduced by about a half.

3. Intravenous injection or oral administration of these substances into pantothenic acid-deficient rats resulted in an almost complete recovery of CoA level in the liver within 2 hours after administration.

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