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THE ROLE OF VITAMINS IN METABOLIC PROCESSES

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The Metabolism and Function of Pantothenic Acid

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The identification of the 'filtrate factor' for chicks with the bacterial growth factor pantothenic acid was the result of the coming together of animal nutritional studies on the one hand and bacterial nutritional studies on the other. Co-operation between these two fields and studies in the field of intermediary metabolism with isolated enzyme systems has finally resulted in the identification of pantothenic acid as a precursor of coenzyme A (coA) and the establishment of the pivotal position of coA in reactions involving acyl transfer. Pantothenic acid thus falls in line with some other members of the B group of vitamins such as nicotinic acid and riboflavin as part of a coenzyme important both in the breakdown of foodstuffs and the synthesis of new cellular material.

The role of coA has been recently fully reviewed by Welch & Nichol (1952) and special aspects have been discussed by Lipmann, Jones & Black (1952), Ochoa (1952) and Barker (1951). In the present article it is proposed to discuss the metabolism of pantothenic acid with special reference to the synthesis of coA, and to attempt to relate the function of coA to some of the signs of pantothenic-acid deficiency.

Combined forms of pantothenic acid

The most satisfactory methods for the assay of pantothenic acid are still the microbiological methods which utilize the growth or metabolic response of exacting lactobacilli (cf. Pennington, Snell & Williams 1940; Barton-Wright 1946). In untreated extracts, values by these methods are low compared to values by less accurate and more tedious methods which use chicks (Jukes, 1942) or rats (Bacon & Jenkins, 1943). This is due to the fact that pantothenic acid exists in natural material almost entirely in combined forms which, although available to the chick and rat, do not support the growth of lactobacilli. In yeast (Farrer, 1951) and liver (see p.84) pantothenic acid is released from the combined forms by

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autolysis; in extracts of tissues, however, it is necessary to digest the material with enzymes. Takadiastase and papain (Harrison, 1949) used previously have been displaced by a mixture of intestinal phosphatase and an enzyme from chicken liver (Neilands & Strong, 1948; Novelli & Schmetz, 1951). Satisfactory agreement between the microbiological and chick assays has recently been reported (Coates, Ford, Harrison, Kon, Shepheard & Wilby, 1952) in material digested with these two enzymes.

Information about the nature of the combined form of pantothenic acid was yielded by the analysis (Williams, Hoff-Jørgensen & Snell, 1949) of a growth factor essential for *Lactobacillus bulgaricus* ('LBF factor'). This was found to contain pantothenic acid in combination with β-mercaptoethylamine (Brown, Craig & Snell, 1950). Synthetic material prepared from pantothenic acid and β-mercaptoethylamine was identical with natural 'LBF factor', and the name 'pantetheine' was given to it by Snell, Brown, Peters, Craig, Wittle, Moore, McGlohon & Bird (1950). At least six 'LBF factors' (McRorie & Williams, 1951) present in extracts of bacteria have been shown to be artifacts formed by the action of unidentified thiols on pantetheine (Brown & Snell, 1952).

The growth of *Acetobacter suboxydans* is stimulated by coA and some of its degradation products (Novelli, Flyn & Lipmann, 1949). Synthetic phosphates of pantothenic acid are inactive (Baddiley & Thain, 1951*a*) and it is likely that the effect is given only by compounds containing phosphates of pantetheine.

Combined pantothenate as the coenzyme for acetylation

Metabolic studies with bacteria (Dorfman, Berkman & Koser, 1942; Hills, 1943; McIlwain & Hughes, 1944, 1945) and yeast (Novelli & Lipmann, 1947) indicated that the combined form of pantothenic acid played a role in the oxidation of pyruvate and acetate and was essential for growth. The coenzyme role of bound pantothenate was however established by Lipmann and his workers in an enzyme system from pigeon liver, first described by Krebs, Sykes & Bartley (1947) and which acetylates aromatic amines such as the sulphonamides. The acetylating activity of liver preparation is lost upon incubation, owing to the destruction of a co-factor. The co-factor has been named coenzyme A (Lipmann, Kaplan, Novelli & Tuttle, 1947). Coenzyme A is also essential for the acetylation of choline in nervous tissue and in bacteria (Feldberg & Mann, 1946; Nachmanson & Bejtnan, 1946; Rowatt, 1948). Lipmann and his colleagues were struck by the similarity between coenzyme A and bound pantothenic acid in their behaviour towards intestinal phosphatase and the chicken-liver enzyme, and in fact animal and plant tissues and bacteria, analysed by microbiological assay and by the acetylation system, contained the bulk of their combined pantothenic acid in the form of coA (Novelli, Kaplan & Lipmann, 1949).

Coenzyme A

Estimation. Coenzyme A can be estimated by measuring the catalytic effect on the acetylation of sulphonamide (Kaplan & Lipmann, 1948) or 8-aminoazobenzene (Handschumacher, Mueller & Strong, 1951). Erroneously high results in the

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Table 1. *Degradation of coenzyme A*

Method of degradation	Reference	Products isolated	Biological activity of isolated products
None			Coenzyme for acetylation
Digestion with nucleotide pyrophosphatase at pH 4·5	Novelli & Schmetz (1951)	(a) Adenosine 5′ phosphate	
		(b) Inorganic phosphate (1 mol.)	
		(c) Pantetheine combined with phosphate (probably 4′ ester)	(c) Inactive as coenzyme for acetylation. Active as LBF factor and stimulates the growth of *Acetobacter suboxydans*
Digestion with phosphomonoesterase	Novelli, Gregory, Flyn & Schmetz (1951)	(a) Inorganic phosphate (1 mol.)	
		(b) Product containing pantetheine linked by phosphate to adenosine (Pantetheine-adenosinenucleotide, PAN)	(b) Inactive as coA but stimulates growth of *A. suboxydans* and behaves as LBF factor
Digestion with intestinal phosphatase	Novelli, Gregory, Flyn, & Schmetz (1951)	(a) Adenosine	
		(b) Inorganic phosphate (3 mol.)	
		(c) Pantetheine	(c) LBF factor, no coA activity. Does not stimulate growth of *A. suboxydans*
Digestion with liver enzyme and intestinal phosphatase	Novelli, & Schmetz (1951)	(a) As (a) and (b) above	CoA, LBF factor and acetobacter activity [destroyed
		(b) Pantothenic acid	
		(c) β-Mercaptoethylamine	
Digestion with barley 'b' nucleotide phosphatase	Wang, Shuster & Kaplan (1952)	(a) PAD and inorganic phosphate (1 mol.)	(a) Converted to coA by liver-enzyme fraction
Barium-hydroxide hydrolysis	Baddiley & Thain (1951*a*)	(a) Adenosine 5′ phosphate	
		(b) 4′ Phosphate ester acid pantothenic acid	(b) Stimulates growth of *A. suboxydans*

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presence of pantetheine and its phosphates may be overcome by fractionation of the enzyme system with acetone (Chou & Lipmann, 1952). The transacetylase system from *Bacterium coli* (Stadtman, Novelli & Lipmann, 1951) and systems in which acyl transfer is linked to the reduction of cozymase have also been used (Von Korff, 1952); they would appear to be more specific than the above methods but too few results on recovery of coA from crude extracts are available to enable assessment of their worth. CoA activity is generally expressed in Lipmann units, i.e. the amount needed to half saturate the liver enzyme under standard conditions. Each Lipmann unit is equivalent to 0·7 μg bound pantothenic acid or 1·9 μg coA.

Preparation and structure. One of the obstacles to the final formulation of coA has been difficulty of obtaining it free from other nucleotides and material containing sulphur (DeVries, Govier, Evans, Gregory, Novelli, Soodak & Lipmann, 1950). Satisfactory material containing 90% coA has been recently prepared from fermentation liquors of *Streptomyces fradei* (Gregory, Novelli & Lipmann, 1952). A method more applicable to the preparation from yeast has been used by Beinert, Von Korff, Green, Buyske, Handschumacher, Higgins & Strong (1952).

Elucidation of the structure of coA has largely resulted from the use of enzymic degradation combined with chemical and microbiological assays of the products and by the synthetic work of Baddiley (Baddiley & Thain, 1951*b*, *c*). From the evidence, summarized in Table 1, the most likely formulation would be as a dinucleotide containing pantetheine 4′-phosphate linked to 2′-phosphoadenosine by a pyrophosphate bridge (Fig. 1). Further support for this formulation is given by studies on the biosynthesis of the coenzyme.

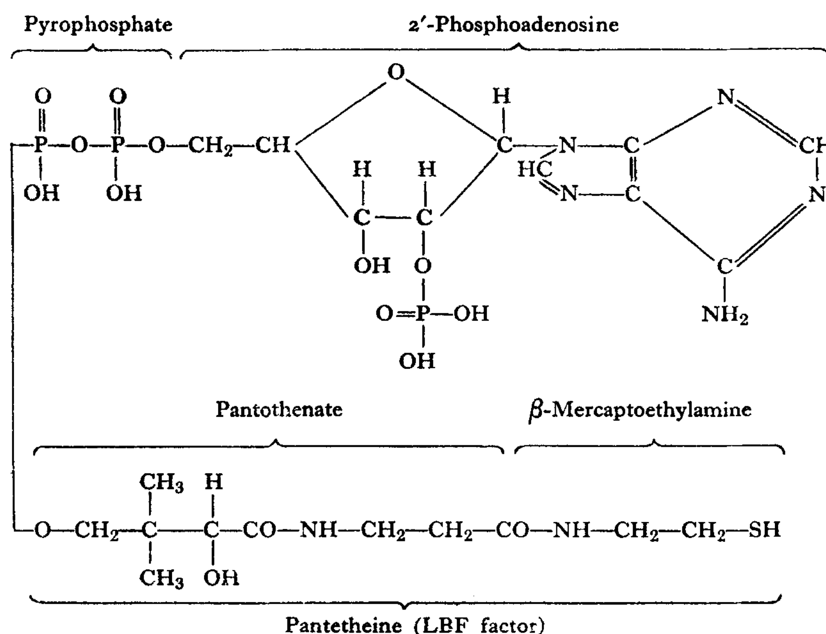

Fig. 1. Structure of coenzyme A.

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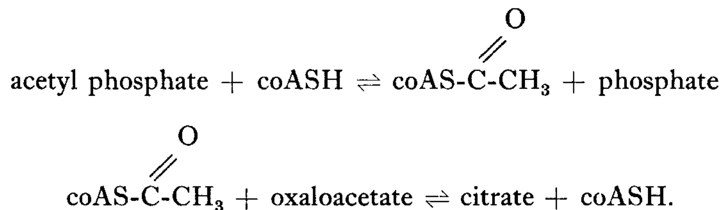
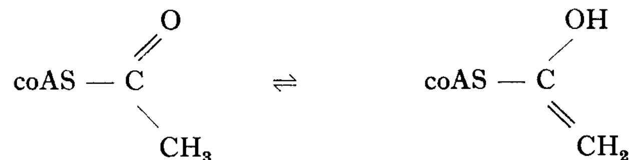
Mode of action. Evidence that the terminal thiol was the active group in coA was first provided by Lynen and his coworkers (Lynen, 1951; Lynen & Reichert, 1951; Lynen, Reichert & Rueff, 1951), who prepared from yeast a pantothenic-acid derivative containing a thiol-acyl group and which replaced both coA and acetate in the pigeon-liver acetylation system. Ochoa and his co-workers confirmed that Lynen's material was acetyl coA. Studying citrate synthesis they found that the synthesis of citrate from acetyl phosphate and oxaloacetate was dependent on the presence of coA. The reaction proceeded in two steps. The first step was catalysed by the transacetylase from *Bact. coli* and resulted in the formation of an 'active acetate'. The second step was catalysed by 'condensing enzyme' and citrate was formed by the condensation of 'active acetate' with oxaloacetate. Lynen's material substituted for the 'active acetate' and during the condensation there resulted the stoichiometric formation of a thiol group (Stern, Ochoa & Lynen, 1952). The reactions can thus be written:



Measurement of the equilibrium constant of this reaction confirms the suggestion of Lynen that the thiol-acyl bond is energy-rich, with a free energy of the order of 12,000-13,000 cal./mol. at pH 7·2 (Stern, Shapiro, Stadtman & Ochoa, 1951).

Activation of the methyl group in citrate synthesis and of the carboxyl group in acetylation could be brought about, if as suggested by Stern *et al.* (1951) acetyl coA existed as an equilibrium mixture of two tautomeric forms as follows:



CoA is present in tissues in catalytic amounts, and acetylation reactions can therefore only proceed if there are present both acetyl donors and acetyl acceptors. The functioning of coA is thus analogous to the action of the pyridine and flavine coenzymes in hydrogen transfer. Some of the most important acetyl donors and acceptors are shown schematically in Fig. 2. In addition to transferring acetyl groups, coA also transfers other acyl groups. This is shown most clearly by the work of Kaufman (1951) and Sanadi & Littlefield (1951) with the α-ketoglutarate oxidase of heart muscle. This system is dependent on coA and cozymase and when linked with the pigeon-liver system can give rise to succinylsulphanilamide by the following reactions:

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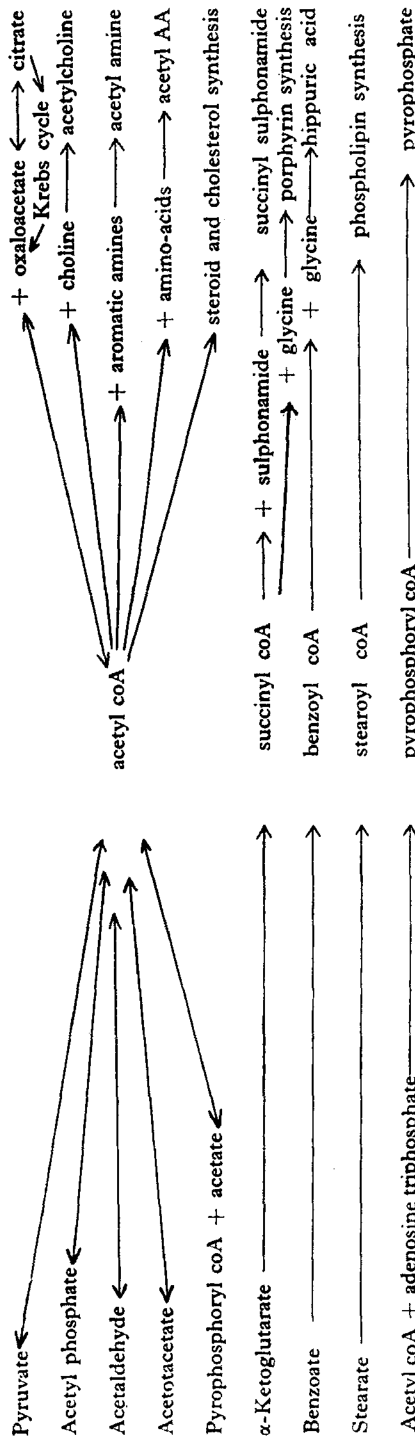

Fig. 2. Reactions in which coenzyme A serves as an acyl transfer agent.

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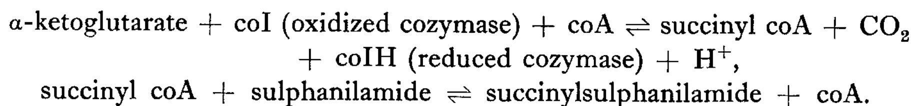
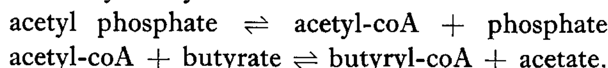

α-ketoglutarate + coI (oxidized cozymase) + coA ⇌ succinyl coA + CO_2 + coIH (reduced cozymase) + H^+,

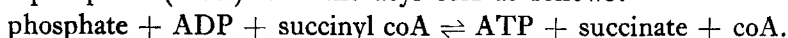
succinyl coA + sulphanilamide ⇌ succinylsulphanilamide + coA.

Succinyl coA formed by this system is probably a precursor of the porphyrin ring (Shemin & Wittenberg, 1951; Shemin & Kumin, 1952). The formation of acyl coA compounds from the C_2-C_8 fatty acids has been shown by Stadtman (1952) to be linked to transacetylase system in *Clostridium kluverii* as follows:


acetyl phosphate ⇌ acetyl-coA + phosphate

acetyl-coA + butyrate ⇌ butyryl-coA + acetate.

These reactions provide a general mechanism for the activation of one carboxylic acid by another as the first step in decomposition or synthesis of fatty acids (Barker, 1951). CoA also plays a role in oxidative phosphorylation. In the pyruvic-oxidase system (Korkes, del Campillo, Gunsalus & Ochoa, 1951) and the α-ketoglutarate system described above adenosinetriphosphate (ATP) is formed by reaction of adenosinediphosphate (ADP) with the acyl coA as follows:


phosphate + ADP + succinyl coA ⇌ ATP + succinate + coA.

Biosynthesis. Because it is probable that coA is the only functional form of pantothenic acid, the combined forms of the vitamin can best be considered as either intermediates in the synthesis of the coenzyme or products of its breakdown. Little is known about the origin of the pantoic-acid residue. The β-alanine residue can arise from aspartic acid by decarboxylation (Roper & McIlwain, 1948). Synthesis of pantothenic acid from pantoate and β-alanine has been studied in neurospora (Wagner, 1949) and in cell-free extracts of *Bact. coli* (Maas, 1952*a*). The synthesis is dependent on the presence of both Mn^{++} and K^+ or NH_4^+, adenosinetriphosphate is also needed as an energy source. Pantoic lactone does not replace pantoate in the extracted system but will replace it to some extent in washed cell suspensions.

The synthesis of coA from pantothenic acid in bacteria has been studied by the deficient-cell technique (Pierpoint & Hughes, 1952). Glycolysing washed suspensions of coA-deficient cells of *Lb. arabinosus* produced small amounts of coA from pantothenic acid but, if cystine or cysteine (10^{-4}M) were added, synthesis commenced (0·6 mμ mol./mg dry wt./h) and continued until the cells became saturated. The production of pantetheine appeared to be a limiting step in coA synthesis and cystine was essential. It is unlikely that, as suggested by Baddiley & Thain (1951*a*), the cysteine analogue of pantetheine is first produced and then decarboxylated since the analogue is inactive either for growth or synthesis. Further studies with analogues of pantetheine and compounds related to cystine emphasized the specificity of cystine but have not helped to decide its precise role.

The synthesis of coA from pantetheine in preparations of chick or pigeon liver proceeds in three steps as follows:

(1) pantetheine + ATP ⇌ pantetheine 4′-phosphate + ADP;

(2) pantetheine 4′-phosphate + ATP ⇌ pantetheine-adenosinenucleotide (PAD) + pyrophosphate;

(3) PAN + ATP ⇌ coenzyme A + ADP.

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The enzyme responsible for reaction 1 has been partly purified (Levintow & Novelli, 1952). Reaction 2 is similar to the action of nucleotide pyrophosphatase (Kornberg & Pricer, 1950); it is possible that the enzyme from liver is identical with Kornberg's enzyme, because the substrate, i.e. pantetheine 4′-phosphate, is produced by the action of potato or snake-venom pyrophosphatase on coA as well as by reaction 1. Reaction 3 is similar to the phosphorylation of coenzyme I to form coenzyme II (Kornberg, 1950). Kaplan & Wang (1952, personal communication) have been able to separate the enzyme responsible for PAD phosphorylation from the coI phosphorylase. This lends further support to the formulation of coA as a ' b ' phosphoadenosine derivative.

Inhibition of pantothenic-acid metabolism

Pantoyltaurine and pantethenol which inhibit growth and the utilization of pantothenic acid in bacteria (Snell, 1941; McIlwain & Hughes, 1944) are needed in much larger amounts when pantetheine replaces pantothenic acid (McRorie & Williams, 1951). This suggests that it is the synthesis of pantetheine that is blocked and not one of the later steps in coA synthesis. It is likely that the failure to produce consistently the signs of pantothenic-acid deficiency in animals by feeding analogues is due to the presence of pantetheine and its phosphates in the blood and tissues (see p. 000). A large number of other analogues of pantothenic acid also inhibit the growth of bacteria and in vitro growth of malarial parasites (cf. Johnson & Kupferberg, 1948), but it is by no means certain that they also inhibit the utilization of pantothenic acid in the synthesis of coA. For instance tolylpantothenone has been found to inhibit not the synthesis but the breakdown of pantothenic acid in extracts of *Bact. coli* (Maas, 1952*a*). The mechanism of the breakdown is not known in detail, but is not simply the reversal of the synthesis from pantoate and β-alanine. Enzymes that destroy essential metabolites are widely distributed in bacteria (cf. McIlwain & Hughes, 1948) and often complicate studies of the synthesis because they tend to be stable and more easily extracted than synthetic enzymes.

Experiments with mutant strains of *Bact. coli* (Maas, 1952*b*) which required β-alanine or pantoate as growth factors confirm the previous suggestion (McIlwain, 1943) that salycilate inhibits the synthesis of the pantoyl portion of pantothenic acid, but the precise point of inhibition is not known.

Metabolic changes caused by deficiency of pantothenic acid of higher animals

In general the pattern of signs in a vitamin deficiency is determined by the ability of the various organs to retain the vitamin and by their demands for its metabolically active form. This is well illustrated in the effect of pantothenic-acid deficiency upon the acetylation of sulphonamides and upon the function of the adrenal cortex. The acetylation takes place in the liver and is dependent on coA (see p. 84). Acetylation is reduced soon after young growing rats are placed on a deficient diet, whereas in older rats acetylation remains normal for 2 months (Shils,

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Chester & Sass, 1951 ; Riggs & Hegsted, 1951). In mice on a deficient diet, coA was retained by the liver for much longer than in the adrenal, muscles and brain (Melampy & Northrop, 1951), and a similar retention in ducks has also been reported (Olson & Kaplan, 1948): impairment of pyruvate metabolism in ducks was found only after 75% reduction in coA concentration in the tissue. It seems likely that, as suggested by Kaplan & Lipmann (1948), the liver serves as a main store of coA, and that this is broken down before transport in the blood and resynthesized in the tissues (Govier & Gibbons, 1951). Synthesis of coA from pantothenic acid in deficient animals is extremely rapid, the ability to acetylate is restored in 3 h after injection of the vitamin (Shils *et al.* 1951).

The coA content of the adrenal is almost as high as of the liver, but in contrast the adrenal quickly becomes depleted when animals are placed on a deficient diet (Melampy & Northrop, 1951). Changes in the anatomy and function of the adrenal occur early in pantothenic-acid deficiency and before other signs appear. For instance the appearance of the *zona fasciculata* suggests an increased secretory and synthetic activity in the gland (Deane & McKibbin, 1946). In the early stages of pantothenic-acid deficiency rats give a lethargic response to water intoxication (Gaunt, Liling & Mushett, 1946) and have an increased resistance to some bacterial infections (West, Bent, Rivera & Tisdale, 1943-4; Lichstein, Waisman, Elvehjem & Clark, 1944). Reactions to other ' stress states ', such as swimming and anoxia, suggest that the deficient animal is already in a ' stress state ' in which an increased secretion of cortical hormones is taking place (see Morgan, 1951). As the degree of deficiency increases and other signs, eg. achromotrichia ('grey hair'), appear, the adrenal cortex degenerates and becomes vasculated and necrotic. The adrenal medulla appears unchanged (Deane & McKibbin, 1946). The animals then react to stress states in a manner suggesting impairment of cortical hormone supply (Dumm & Ralli, 1950). Pantothenic acid administered before this stage develops will prevent its appearance, if given later the vitamin has no effect. Cortical hormones protect the animal to some extent against pantothenic-acid deficiency (Hurley & Morgan, 1952), whereas pantothenic acid will prolong the life of adrenalectomized rats (Dumm & Ralli, 1948). It seems feasible that the effect of pantothenic acid on adrenal function is twofold. Firstly, pantothenic-acid deficiency, in common with riboflavin deficiency, would appear to stimulate the function of the adrenal cortex and the animal responds as if to a stress state (Morgan, 1951). Secondly, the increased activity of the adrenal cortex involves sterol synthesis which is probably dependent on coA (Klein & Villee, 1951). Increased demand for coA thus occurs at a time of decreased availability and leads to the complete degeneration of the cortex (Cowgill, Winters, Schultz & Krehl, 1952).

The two other classical signs of pantothenic-acid deficiency can also be produced by interference with adrenal function. The secretion of porphyrin into the lachrymal glands, known as 'bloody whiskers', may be produced by reducing the water intake of rats (Figge & Atkinson, 1941), and there also appears to be a connexion between the development of achromotrichia and alopecia and adrenal function (Williams, Gardner & DeVita, 1946). Cystine together with pantothenic

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acid is more effective in curing ' grey hair ' in the rat than is pantothenic acid alone (Pavcek & Baum, 1941). This suggests either an impairment in cystine production during pantothenic-acid deficiency or an increased demand for cystine during the synthesis of new hair protein. It is unlikely to be associated with pantetheine synthesis as in bacteria.

Other changes of metabolic activity during pantothenic-acid deficiency are those expected from consideration of the function of coA. Oxidation of fatty acids such as caproate is impaired (Cheldelin, Nygaard, Hale & King, 1951). Interference generally with fat metabolism occurs but differs in different species. In dogs an increased tendency towards fatty liver is found (Schaefer, McKibbin & Elvehjem, 1942), whereas in rats pantothenic-acid deficiency protects against fatty-liver production (Guehring, Hurley & Morgan, 1952).

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