Effect of Long-Term Panthenol Administration on the Pantothenate and Coenzyme A Contents of Rat Tissues.* (17659)

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Numerous studies(1-8) have shown that panthenol, the alcohol analog of pantothenic acid, exhibits quantitatively the known biological effects of pantothenic acid, and, moreover, has the advantage of greater stability *in vitro*(9) as well as better availability in mammals at therapeutic dosages(2-5). On the other hand, panthenol was reported by Snell and Shive(10) to have an *in vitro* antipantothenate effect on certain microorganisms, *e.g.*, antibacterial indices of 300, 1000 and 5000 against *Leuconostoc mesenteroides, Streptococcus lactis* 125, and *Lactobacillus arabinosus*, respectively. Panthenol had no effect, however, against microorganisms such as *Escherichia coli* and *Staphylococcus aureus*, which are capable of synthesizing pantothenic acid. In all cases where panthenol competitively inhibited the microbiological growth effects of pantothenic acid, the inhibition was overcome by the presence of sufficient pantothenate. It is clear therefore that this antipantothenate effect stems from the circumstance that these microorganisms are incapable of oxidizing panthenol to the acid, a function which mammals perform efficiently.

The present experiments were designed to test whether any anti-pantothenate effect might be detected when high doses of panthenol are administered to rats, mice, dogs and rabbits receiving a stock diet adequate with regard to pantothenic acid content. As a measure of possible anti-pantothenate action, consideration has been given to (1) acute toxicity, (2) chronic toxicity, as evidenced by the production of histological or hematological changes and alteration of the normal rate of growth, and (3) influence of long term feeding upon tissue stores of pantothenic acid. In regard to the last Novelli *et al.*(11) have established that practically all the pantothenic acid found in mammalian tissues may be accounted for as the coenzyme of acetylation, coenzyme A. For this reason, both coenzyme A and free pantothenate as well as total tissue stores were determined in testing for panthenol-induced changes in the storage of pantothenic acid by the rat.

Experimental. (1) *Acute Toxicity.* In acute toxicity studies of panthenol, the intravenous LD_{50} for mice was 7 g per kilo and for rabbits 4 g per kilo. All the rabbits survived 3 g per kilo intravenously and the mice 6.25 g per kilo.

(2) *Chronic Toxicity.* The rate of growth of 24 rats receiving 2 mg of panthenol daily for 6 months was compared to that of 12 control rats receiving the same stock diet, which supplied an adequate amount of pantothenic acid.† As the curves (Fig. 1) relating weight gains to duration of supplementation were practically superimposable over the entire period, it is evident that long term administration of panthenol to growing rats had no

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† This dog chow contains 10 μg of pantothenic acid per gram, *i.e.*, sufficient to provide a daily intake of 100 to 150 μg per rat per day.

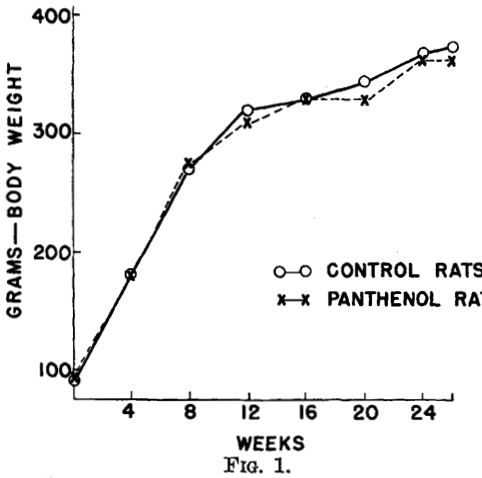

FIG. 1.

Effect of feeding 2 mg of panthenol per day upon the rate of growth of normal rats.

deleterious effect. In a similar study lasting 3 months, 20 mg of panthenol fed daily to rats and 500 mg to dogs produced no histopathological changes. White and red blood cell counts, hemoglobin determinations, nonprotein nitrogen and blood sugar levels showed no deviation from normal.

(3) *Influence of Long Term Feeding upon Tissue Stores of Pantothenic Acid.* Daily doses of 2 mg of panthenol or an equivalent amount (2.3 mg) of calcium pantothenate were fed to rats maintained on the above stock ration for test periods of 24 and 45 days and for 5 to 6 months. Undosed control rats were given the same diet. In the case of the 24 and 45 day test animals, the panthenol or calcium pantothenate was administered by stomach tube and all groups were parallel. For the 5 to 6 months tests, the rats received the same supplements as a part of their ration. Although in these longer experiments, the 5-month test with the calcium pantothenate group was not run simultaneously with the 6-month test of panthenol and control groups, conditions were otherwise controlled so that all three groups are comparable.

(a) *Total pantothenate.* The total pantothenate content of liver, heart, kidney, and spleen tissues from these rats was estimated (Table I) after complete liberation of the pantothenic acid from its naturally occurring bound form as coenzyme A by means of the liver enzyme, alkaline phosphatase procedure described by Novelli *et al.*(11). The liberated vitamin was assayed microbiologically with *L. arabinosus (17-5)* by the method of Skeggs and Wright(12). In the early experiments, the assays were read turbidimetrically with color blanks, while in the later tests, titrimetric measurements were employed. Assays of the same samples by both procedures showed close agreement.

Pooled livers from each of the 3 groups in the 24- and 45-day tests were analyzed simultaneously as soon as the rats were sacrificed. It is evident (Table I) that in these tests neither panthenol nor pantothenate administration appreciably affects the total pantothenic acid content of rat livers. Similarly, the data obtained after 5 to 6 months show no significant increase of liver stores in the dosed rats. Parallel analyses of pools of kidneys from the control, panthenol and pantothenate groups after 24 and 45 days and hearts from the 5 to 6 months series indicate only slightly increased stores of pantothenate after both substances. In the 5 to 6 months series, the 42% increase in kidney content after panthenol compared to only 2% after calcium pantothenate cannot be explained at this time. In contrast, splenic storage was unaffected under these conditions.

(b) *Test for unconverted panthenol.* It was also of interest to determine whether panthenol *per se* is stored in the tissues of these rats. To test this possibility, the microbiological assay procedure of De Ritter and Rubin(13), using *Acetobacter suboxydans* (A.T.C.C. 621 H), was applied to an enzyme-treated suspension of 3 livers from the panthenol-dosed rats of the 6 months test. Following complete alkaline hydrolysis of an aliquot of this suspension, the pantoic acid found was equivalent to 90 γ of pantothenic acid per gram of liver. Direct determination of pantothenic acid before hydrolysis on the same day and with the same organism indicated a content of 79 γ/g. The pantothenic acid content by *L. arabinosus* assay was 93 γ/g. From the agreement of those 3 values it is evident that, under these

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13. De Ritter, E., and Rubin, S. H., *Anal. Chem.*, 1949, v21, 823.

TABLE I.
Rat Tissue Storage of Pantothenic Acid.

Organ	Test period	Total pantothenic acid content (μg/g fresh tissue)					
		Control		Panthenol (2 mg/day)		Ca Pantothenate (2.3 mg/day)	
		No. of organs*	Avg content	No. of organs	Avg content	% incr.	No. of organs	Avg content	% incr.
Liver	24 da.	2 (1)	67	2 (1)	68	2	2 (1)	67	0
	45 "	3 (1)	74	3 (1)	83	12	2 (1)	79	7
	5-6 mo.	7 (5)	100	9 (6)	94	—6	5 (3)	75	—25
Kidney	24 da.	10 (2)	51	10 (2)	55	9	10 (2)	57	13
	45 "	10 (2)	48	10 (2)	61	27	10 (2)	54	12
	5-6 mo.	12 (4)	60	16 (4)	85	42	8 (2)	61	2
Heart	5-6 "	10 (2)	47	14 (2)	56	20	12 (2)	62	32
Spleen	5-6 "	12 (2)	27	14 (2)	27	0	14 (2)	27	0

* The numbers in parentheses indicate the number of analyses performed on separate pools of organs.

conditions, no significant amounts of panthenol or pantoic acid are stored in the liver.

(c) *Relative coenzyme A content of livers.* To test whether panthenol or pantothenate administration affects the normal coenzyme A content of liver tissue, organs from each of the various groups in the 24 and 45 tests were pooled and extracts prepared according to the procedure described by Kaplan and Lipman(14). Such extracts were reported to contain intact the coenzyme A, the integrity of which these authors proved by its activity in the enzymatic acetylation of sulfanilamide. The activity of our extracts was determined with *Acetobacter suboxydans,* which we find in agreement with Novelli *et al.*(15), utilizes the intact coenzyme. The response of this organism, grown on the medium of De Ritter and Rubin(13), was measured with reference to a pantothenic acid standard to provide an estimate of relative rather than absolute coenzyme A potencies of the livers from the various groups. A summary of the comparative data is given in Table II, all potencies being expressed in terms of "apparent pantothenate." In both the 24 and 45 day tests, panthenol and pantothenate cause a slight and equal increase in the relative activity of about 20% over the controls. In the longer test period of 5 to 6 months, neither substance affects the activity.

To demonstrate that this activity is due primarily to coenzyme A rather than to free pantothenate, which also has activity in this assay, it was necessary to determine the amount of free pantothenate present in each case.

(d) *Free pantothenate in livers.* Novelli *et al.*(11) have found that normally no more than 3% of the total pantothenate of rat heart, liver, and kidney tissue is unbound. To compare the amounts of free and bound pantothenic acid in livers from the present control and dosed groups, the coenzyme A extracts were analyzed with *L. arabinosus* both directly and after enzymatic liberation of the bound

14. Kaplan, N. O., and Lipmann, F., *J. Biol Chem.,* 1948, v174, 37.

15. Novelli, G. D., Flynn, R. M., and Lipmann F., *J. Biol. Chem.,* 1949, v177, 493.

TABLE II.
Coenzyme A Activity of Liver.

Test period	Average Coenzyme A activity* (μg apparent pantothenic acid/g of fresh tissue)		
	Control	Panthenol (2 mg/day)	Ca pantothenate (2.3 mg/day)
24 days	134	165	162
45 "	161	190	190
5-6 mo.	199	193	183

* Determined with *Acetobacter suboxydans*.

TABLE III.
Free Pantothenate in Coenzyme A Extracts of Rat Liver.

Test period, days	Pantothenic acid content* (μg/g fresh tissue)								
	Control			Panthenol (2 mg/day)			Ca pantothenate (2.3 mg/day)		
	Free	Bound	% Free	Free	Bound	% Free	Free	Bound	% Free
24	1.4	40	3.5	2.8	67	4.2	1.7	41	4.2
45	1.6	50	3.2	2.5	55	4.5	2.8	55	5.1

* Assay with *L. arabinosus* to measure free and total pantothenate, respectively, *before* and *after* enzymatic liberation; difference equals bound pantothenate.

vitamin. As this organism is insensitive to the intact coenzyme but responds quantitatively to the free vitamin, the concentration of free pantothenic acid in the extracts is measured by the direct assays. It is evident (Table III) that practically all of the pantothenic acid present in livers of rats chronically administered either panthenol or calcium pantothenate is also in the bound form.

Discussion. In view of the anti-pantothenate activity exhibited by panthenol towards certain microorganisms(10), the present experiments have tested for such effects in mammals. Previously reported panthenol availability studies(1,2,4) have demonstrated that the rat readily converts panthenol to pantothenic acid as shown by the urinary excretion of pantothenate. As a consequence, for panthenol to exert an *in vivo* anti-pantothenate effect, it must do so before its conversion by displacing and thereby decreasing normal tissue stores of pantothenate. Comparison of the effect of chronic administration of panthenol or pantothenate upon total tissue stores have tested this possibility. In no case do we find any appreciable decrease attributable to panthenol. In fact, the total kidney and heart stores are increased slightly by both panthenol and pantothenate, whereas splenic and liver stores remain unaffected. On this basis, it is concluded that panthenol does not displace normal tissue stores of pantothenic acid.

In determining the mode of storage of pantothenic acid, the microbiological procedures employed yield only a relative estimate of the coenzyme A content of the livers from the various groups. However, these values (Table II) in terms of apparent pantothenic acid activity per gram of tissue are in direct proportion to the metabolically active coenzyme A present in the extracts(15). Preparation of our extracts by procedures reported by Kaplan and Lipmann(14) to protect the integrity of the coenzyme A molecule and to preserve its acetylation activity precludes the presence of degradation products of coenzyme A such as the pantothenic acid conjugase, reported by King *et al.*(16) to support the growth of *Acetobacter suboxydans*. The small percentages of free pantothenate and the lack of significant amounts of pantoic acid indicate that the *Acetobacter suboxydans* assay measured chiefly coenzyme A.

It is clear that panthenol and pantothenate have practically identical effects upon the

16. King, T. E., Locher, L. M., and Cheldelin, V. H., *Arch. Biochem.*, 1948, v17, 483.

levels of both pantothenate and coenzyme A in rat tissues. There was no appreciable accumulation of free pantothenate or panthenol during these tests. The lack of inhibitory effect of panthenol upon the rate of growth, the histopathological and hematological picture, as well as the high intravenous LD_{50} found for mice and rabbits provide further evidence that panthenol has no *in vivo* antipantothenate effect. Furthermore, in clinical tests of panthenol(6,7) no untoward or toxic effects were observed. For these reasons, it must be concluded that panthenol is generally non-toxic to mammals at dosage levels in the therapeutic range.

Summary. Toxicity tests of panthenol show that this biologically active, hydroxy analogue of pantothenic acid has no *in vivo* antivitamin effect in mammals. In acute toxicity tests, mice tolerated up to 6.25 g per kilo and rabbits up to 3 g per kilo. In chronic feeding tests, 2 mg per day of panthenol had no inhibitory effect upon the growth rate of normal rats on an adequate stock diet and 20 or 500 mg daily produced no histopathological or hematological changes in rats or dogs, respectively. When fed as above at the 2 mg per day level for periods up to 6 months, panthenol did not differ significantly from an equivalent supplement of calcium pantothenate in its effect on rat tissue storage of pantothenate. Total pantothenate in the liver, spleen, kidney and heart, and coenzyme A in the liver were equal or slightly greater in the dosed groups than in the controls. Liver storage in all groups was almost entirely as coenzyme A, and no appreciable amounts of panthenol or free pantothenate accumulated.

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Effect of Testosterone Propionate on Permanent Canine Tooth Eruption in the Monkey (*Macaca mulatta*). (17660)

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An obvious dimorphic character in the common rhesus monkey is the tusklike permanent canine tooth of the male. In the female the degree of modification of the canine is very much like that in human dentition. In fact, the teeth of the *Macaca mulatta* show many similarities to the teeth of man(1) and the dental formulae for both the deciduous and permanent teeth of man and this monkey are the same. In the normal male monkey of the rhesus colony† where these observations were made, the permanent canines begin to erupt during the latter half of the fourth year and all of them are present by the end of the fifth year. When recording the appearance of the deciduous teeth, it is necessary to make daily examinations during the first 3 months of infancy and then weekly observations are made, but after the second year the monkey's permanent teeth are not checked against his chart more often than once a month. There is, therefore, a plus or minus error of around 30 days in the data given here. However, in the normal male monkey all the permanent canines do not ap-

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† The Obstetric Monkey Colony, Yale School of Medicine, New Haven, established in 1931, now consists of 5 generations of this rhesus monkey.