histamine. Upon addition of known basic histamine liberators such as 1,10-diaminodecane (DA10) and propamidine (*6*), or compound 48/80 (*7*), at final concentrations of 10 to 200 μg/ml, or of rat serum anaphylatoxin prepared according to Novy and DeKruif (*8*), histamine is progressively released from the particles. This liberation is temperature-, *p*H-, and concentration-dependent. The rate of release by 10 μg/ml 48/80 is usually sufficient to set free all the histamine within 60 min at *p*H 8 and 37°C. There is a much smaller, but measurable, rate of release in the controls. Doubling the liberator concentration approximately doubles the rate of release. The rate is 2 to 3 times less at *p*H 7, in the case of 48/80, and is even more markedly reduced at the lower *p*H in the case of DA10. The Q_{10} is approximately 2. These factors probably explain the failure by Hagen (*1*) and Copenhaver, Nagler, and Goth (*2*) to observe histamine release at room temperature by 48/80 and stilbamidine, respectively, in their unbuffered preparations.

All the foregoing observations have been repeated on a similar liver particle suspension (*9*) prepared by replacing the sucrose solution with Tyrode buffer, in which the histamine is less stably bound. In addition, a sucrose suspension of particles from sheep-liver capsule behaves in a similar fashion to the sucrose homogenate of whole dog's liver. Riley and West (*10*) have shown that in this capsular tissue mast cells are the predominant cellular elements.

The evidence cited here strongly suggests that histamine is not bound to a tissue component by a primary chemical bond but is most likely enclosed in a diffusible form within a mitochondrionlike particle. If this is so, the final step, in any series of reactions leading to the release of histamine, would be a change in the properties of the particle-cytoplasm interface, permitting the histamine to diffuse out. This change can be thought of most simply as a rupture or increase in the permeability of a membrane enclosing the particle. Our conclusion is thus in accordance with that independently reached by Hagen: histamine can be released by procedures that might be expected to damage a surface membrane.

It should be noted that, in the case of histamine release by the simple basic histamine liberators, all the components necessary for this action are present, after addition of the liberator, in the washed granular material obtained from the histamine-rich tissue. In the tissues examined by us, and in many other tissues, histamine appears to be located mainly in the mast cells (*10*), and it is attractive to suppose that the characteristic granules of these cells contain histamine and have a membrane that can be lysed by physical and chemical agents. The idea that such intracellular bodies are enclosed by a membrane that serves as a diffusion barrier is supported by permeability (*11*) and electronmicrographic (*12*) studies of liver mitochondria. In intact tissue, as Riley (*13*) has shown, the mast cells themselves are disrupted by the action of a basic chemical histamine liberator or of anaphylatoxin, presumably by an extension to the cellular membrane of the lytic action responsible for distintegration of the intracellular granules.

A complete report of this and related investigations will be published elsewhere (*14*).

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In vivo Activity of Pantothenylcyst(e)ine for Rats and Chicks

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Pantothenylcystine has been reported by Brown and Snell (*1*) to exhibit less than 2 percent of the activity of pantothenate for *L. arabinosus, L. casei,* and *S. carlsbergensis.* King and Cheldelin (*2*) and Brown and Snell (*3*) in studies comparing the activity of pantothenic acid conjugates for *A. suboxydans* found that pantetheine and pantothenylcysteine were more active than pantothenic acid or pantoic acid but somewhat less active than 4′-phosphopantetheine or coenzyme A. From these studies it was concluded that those derivatives containing a —SH group are, in general, considerably more active than the corresponding disulfide forms.

In view of the published data concerning the requirement for cystine in the biosynthesis of coenzyme A (*4*) and the microbiological activity of pantothenylcyst(e)ine, it seemed of interest to determine whether this latter compound would show biological activity in pantothenic acid-depleted animals. Recently Hoagland and Novelli (*5*) have shown that pantothenylcystine can be converted to pantetheine by treatment with a rat-liver supernatant. These findings are of interest in view of the data presented here, which show that pantothenylcyst(e)ine has little if any significant pantothenic acid activity in rats or chicks.

One-day-old White Leghorn S.C. chicks of mixed sex were prepared for the pantothenic acid assay as described by Thompson, Bird, and Peterson (*6*). In the two chick experiments reported, the birds were

given a practical diet for 3 days, followed by a pantothenic acid-deficient diet for 12 and 7 days, respectively. Following this depletion period, 10 chicks were assigned to each experimental group, taking into consideration equal distribution of body weight.

In experiment 3, weanling albino rats averaging 40 g received a pantothenic acid-deficient diet of the following percentage composition: hot alcohol-extracted casein 24.5, cerelose 60.3, corn oil 10.0, Jones and Foster (*7*) salt mix 4.0, vitamin mix 0.5, and choline chloride 0.15. The vitamin mix, which was diluted with casein, supplied the following vitamins in milligrams per kilogram of finished diet: thiamine 8, riboflavin 8, pyridoxine 8, niacin 100 *p*-aminobenzoic acid 10, menadione 5, biotin 0.1, and vitamin B_{12} 0.03. The diet also contained 120 mg α-tocopheryl-acetate, 90,000 USP units vitamin A, and 9000 USP units vitamin D each per kilogram. At the end of a 27-day depletion period, the rats were uniformly distributed according to body weight among the respective groups. The negative control group contained 11 animals; the other two groups contained 10 animals each.

Table 1. Growth promoting activity of pantothenylcyst(e)ine.

Treatment	Pantothenic acid equivalent (mg)	Weight gain* 7 days (g)	Weight gain* 14 days (g)
Experiment 1 (chicks)			
None		9 (10)	4 (7)
Calcium pantothenate			
0.25 mg/100 g diet	0.23	17 (10)	29 (6)
0.50 mg/100 g diet	.46	36 (9)	64 (6)
0.75 mg/100 g diet	.69	41 (10)	81 (9)
1.0 mg/100 g diet	.91	34 (10)	74 (10)
Pantothenylcystine			
0.8 mg/100 g diet	.55	5 (10)	9 (4)
75 μg/day injected IM†	.051	11 (10)	20 (5)
75 μg/day injected IP	.051	10 (10)	17 (9)
Experiment 2 (chicks)			
None		4 (10)	11 (10)
Calcium pantothenate			
43.2 μg/day injected IP	0.04	27 (10)	54 (8)
87.4 μg/day injected IP	.08	49 (10)	98 (10)
Pantotheylcysteine			
Mercury mercaptide			
77 μg/day injected IP	.04	9 (10)	17 (9)
154 μg/day injected IP	.08	7 (10)	19 (9)
308 μg/day injected IP	.16	11 (9)	22 (7)
Experiment 3 (rats)			
None		4 (11)	9 (9)
Calcium pantothenate			
50 μg/day injected IP	0.046	28 (9)	61 (8)
Pantothenylcystine			
67.5 μg/day injected IP	.046	8 (10)	20 (9)

* Figures in parentheses indicate survival.
† IM means intramuscularly; IP means intraperitoneally.

Supplements of calcium pantothenate, pantothenylcystine, or the mercuric mercaptide of pantothenylcysteine were either incorporated in the pantothenic acid-deficient diet or injected intramuscularly or intraperitoneally as aqueous solutions (*8*). Those supplements given by injection were administered three times weekly. Supplementation in both the rat and chick experiments was continued for 2 wk. Growth data obtained from these experiments are presented in Table 1.

In experiment 1, rather severe mortality occurred, which probably resulted from an excessive depletion period. Although there was a marked response to calcium pantothenate, no response occurred with a supplement of pantothenylcystine that should have been sufficient to supply the chick with about half the amount of pantothenic acid required for maximum growth. Moreover, pantothenylcystine did not produce any significant response when injected either intramuscularly or intraperitoneally.

Since the reduced forms of pantethine derivatives show more activity than the corresponding disulfides in the tests employing microorganisms, it seemed worth while to test the mercury mercaptide of pantothenylcysteine in chicks. However, previous work (*6*) had shown that the mercuric mercaptide of pantetheine was no more active than pantethine in chicks. As is indicated in experiment 2, the mercury mercaptide of pantothenylcysteine was also inactive in promoting growth in pantothenic acid-depleted chicks.

Similarly, in experiment 3, pantothenylcystine exhibited little if any significant activity in pantothenic acid-depleted rats.

Under the conditions of the experiments reported, pantothenylcyst(e)ine does not promote the growth of pantothenic acid-depleted chicks or rats. Furthermore, the rat or chick apparently is not capable of cleaving this compound and utilizing the pantothenic acid moiety. In view of the data of Hoagland and Novelli (*5*) concerning the *in vitro* activity of pantothenylcystine, further investigation is indicated in order to clarify the discrepancy between the *in vivo* and *in vitro* activity of pantothenylcysteine.

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