Table 1. Effects of chloretone on direct acetylation. All reaction tubes contained solutions as described by Kaplan and Lipmann (*5*) in addition to coenzyme A and/or narcotic.

Reaction mixtures	Blank No coA or chloretone	Control I 1 unit coA	Experimental I 1 unit coA, 3 mM chloretone	Control 2 2 units coA	Experimental 2 2 units coA, 3 mM chloretone	Control 3 4 units coA	Experimental 3 4 units coA, 3 mM chloretone
Sulfanilamide (γ)	57	27.8	28.2	10.3	10.3	9.3	9.3
Acetylated sulfanilamide (γ)		29.2	28.8	46.7	46.7	47.7	47.7

Table 2. Effects of narcotics on direct acetylation. Control values obtained for each experiment were the same and did not very significantly. All reaction tubes contained solutions as described by Kaplan and Lipmann (*5*) in addition to coA and narotic.

Solution Reaction mixtures	Blank No coA or narcotic	Control 1 unit coA	Pentobarbital 1 unit coA, 3 mM pentobarbital	Secobarbital 1 unit coA, 3 mM secobarbital	Chloral hydrate 1 unit coA, 3 mM chloral hydrate	Phenobarbital 1 unit coA, 3 mM phenobarbital	Paraldehyde 1 unit coA, 3 mM paraldehyde
Sulfanilamide (γ)	56	28	28	27	27	28	28
Acetylated sulfanilamide (γ)		28	28	29	29	28	28

the source of coA (*8*); and/or (ii) the presence of split products in the reaction mixture. In the light of the Kaplan-Lipmann (*5*) curve of coA activity per unit, it is difficult to understand why Govier and Gibbons did not obtain a much more marked difference in their system (with 3 mM of pentobarbital), following addition of 5 units of coA.

Consequently, our findings prevent agreement with the postulate of McLennan and Elliott (*3*) that narcotic drugs act by inhibiting direct acetylating systems. Thus far, our results using a *"pure" system with added coenzyme A* are in accord with the observations of Johnson and Quastel (*1*) using *cell-free extracts.* However, a pure system is necessary to determine whether the narcotic action is on the ATP-coA acetate reaction (*9*). Comparison with the studies of Johnson and Quastel reveal that their preparations of cell-free extracts contained *unknown* amounts of ATP and coA. These values must be known in order to separate possible narcotic action on ATP utilization and coA inhibition from interference with high-energy phosphate bond synthesis or production. If ATP synthesis is decreased in a narcotized system, quantitative measurements of ATP should be made, and such studies are in progress. The initial results have been reported recently (*10*).

A system is described for the effects of narcotics on a "pure" *in vitro,* direct acetylating system containing known amounts of adenosinetriphosphate and coenzyme A. No interference with the acetylation—that is, no disturbance of either coenzyme-A activity or ATP utilization—was observed following addition to the system of chloretone, phenobarbital, pentobarbital, secobarbital, chloral hydrate, and paraldehyde. These findings are discussed in relation to those reported in other *in vitro* systems.

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Folic Acid Deficiency in the Dog

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It has been stated (*1*) that dogs do not need folic acid preformed in the diet, and the synthetic diets currently in use do not contain this vitamin as supplement. Krehl and Elvehjem (*2*) found that a "folic acid" concentrate derived from liver extract enhanced the response to niacin in niacin-deficient dogs. Similarly, Krehl *et al.* (*3*) observed that synthetic *L. casei* factor improved the response to standard doses of niacin in niacin-deficient dogs, and that this factor also seemed to play a role in maintaining a more adequate blood picture in these animals. However, Ruegamer *et al.* (*4*) found that, although folic acid was effective in bringing about a more consistent response

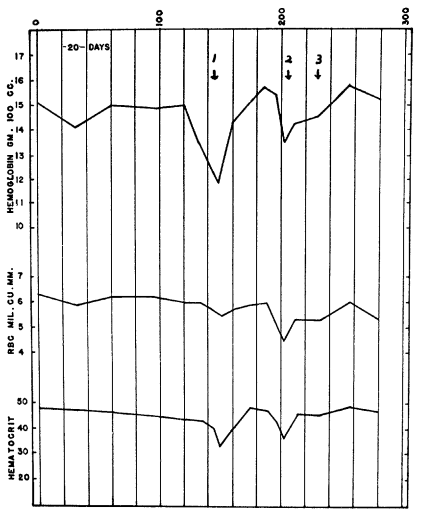

Fig. 1. Blood changes in folic acid deficiency: *1*, 30 mg folic acid; *2* and *3*, 15 mg of folic acid.

in weight in niacin-deficient dogs, it had no apparent effect on the anemia that developed progressively.

Folic acid deficiency was observed in this laboratory (*5*) in one of the control dogs used in nutritional studies that was consuming the following diet: basal ration consisting of vitamin test casein (GBI) 18.0, sucrose 68.0, vegetable oil (hydrogenated) 10.0, salt mixture USP XIV 4.0, and bone ash 4.0 g; vitamin supplements (GBI) per kilogram of body weight per day consisting of thiamine HCl 0.05, riboflavin 0.10, nicotinic acid 0.50, ca-pantothenate 0.22, pyridoxine HCl 0.04, choline chloride 0.20, inositol 0.50, p-aminobenzoic acid 0.50 mg, vitamin A 200 IU, vitamin D 10 IU, alpha tocopherol 1.0, and 2-methyl-naphthoquinone 1.0 mg.

The control animal, a 2-yr old mongrel female dog, started to lose weight soon after being placed on the synthetic ration, and by the end of the experimental period (278 days) had lost 12.5 percent of the initial weight (1.9 kg). At no time did it develop diarrhea or loose stools. The feces remained dark brown and hard in contrast to the grey and rather soft feces of the control dogs receiving similar diet. The hematocrit values showed a progressive and steady decrease (Fig. 1). The possibilities of iron, choline, vitamin B_{12}, or folic acid deficiencies were considered. The following curative measures were instituted: iron and ammonium citrate in doses of 250 mg, later increased to 500 mg, was given orally daily for 3 wk (45th to 60th day); choline chloride, 50 mg/kg body weight per day, was given by mouth for 2 wk (65th to 80th day); a single dose of 30 μg of vitamin B_{12} (*6*) was administered subcutaneously (84th day). Finally, the dosage of B vitamin supplements was doubled (102nd day).

By the 152nd day, when the last procedure was still in progress, the hemoglobin concentration decreased 39 percent, and the hematocrit value dropped from 49 to 33 percent (Table 1). The blood smears revealed hypochromia, moderate anisocytosis, and a few basophilic erythrocytes. At this time 30 mg of folic acid (*7*) was administered subcutaneously into the shoulder region. The response was prompt, and the blood values returned to normal within 14 days. However, they soon started to decline again.

The second attack, which occurred on the 200th day, was similar to the first. During both attacks the amount of folic acid required to bring about the maximum response was 0.015 mg/kg body weight per day. During the first attack the regeneration of hemoglobin amounted to 0.24 g/kg body weight per day, and during the second attack it was 0.22 g/kg.

During the attacks the animal did not appear sick but lacked the usual vivacity. The feces remained normal. The tongue assumed a bright red color, and small atrophic areas appeared on the dorsum (*8*). The plasma volume remained constant throughout the experimental period. The cell volume exhibited fluctuations corresponding to the variations in the hematocrit values (Table 1). The white cell counts decreased from 10.0 ± 2.9 to 8.1 ± 2.1 thousand.

During the preliminary observation period (Purina chow with 50 percent horse meat added) the total acidity of the histamine stimulated gastric juice was 99.0 ± 16.4 milliequivalents, and during the experimental period it was 92 ± 15.4 milliequivalents. In the control dogs using the same diet it was 96 ± 9.5 and 111.0 ± 5.0 milliequivalents, respectively. There was no change in the volume (40.5 ± 9.5 ml) or in the *p*H range (1.2–1.4) of the juice.

Table 1. Blood findings in a folic acid-deficient dog and response to folic acid administration.

	Days	Weight (kg)	Hemoglobin (g/100 ml)	Hemoglobin (g/kg body weight)	Red cells (mm^3)	Hematocrit (%)	Mean corpuscular volume (μ^3)	Plasma (% body weight)	Cells (% body weight)
Before	0	15.2	15.1	13.0	6.0	49.0	82	4.3	4.3
Attack	153	13.8	11.8	7.9	5.5	33.0	60	4.5	2.2
Response*	174	14.1	15.8	12.8	5.8	49.0	84	4.3	4.0
Attack	200	13.7	13.4	8.8	4.3	36.0	83	4.2	2.4
Response*	210	13.9	14.5	12.0	5.2	46.0	88	4.4	3.8

* 0.015 mg of folic acid per kilogram of body weight per day.

The autopsy revealed hypoplasia of the bone marrow and lingual lesions characterized by epithelial proliferation associated with papillary atrophy and nerve degeneration (*8*). The liver was normal.

On the basis of the foregoing observations, folic acid was administered to niacin-, riboflavin-, pyridoxine-, and pantothenic acid-deficient dogs. Given alone, it failed to bring about any observable effect; nor did it enhance the response to the administration of niacin, riboflavin, pyridoxine, and pantothenic acid in the deficient dogs. Similarly, it did not have any effect when it was given to the control animals.

It appears that some dogs may require folic acid preformed in the diet. The deficiency syndrome is characterized by hypoplasia of the bone marrow, hypochromic anemia with a tendency to microcytosis, and glossitis.

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Application of Mercury-Intrusion Method for Determination of Pore-Size Distribution to Membrane Filters

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The mercury-intrusion method for determining pore-size distribution is a dilatometric method (*1*) based on the employment of external pressure to overcome the forces of surface tension which prevent the entrance of the nonwetting liquid mercury into the pores of an immersed sample (*2*). The equation

$$pr = -2\sigma \cos \theta \qquad (1)$$

relates the applied pressure p to the radius r of a circular aperture through the coefficient of surface tension σ of mercury and its contact angle θ with respect to the material under test. Recent reports from this laboratory (*3*) have presented the results of the application of this method to cotton textile materials (*4–6*), assuming values for σ and θ that reduce Eq. 1 to

$$pr = 105.6, \text{ lb } \mu/\text{in.}^2, \qquad (2)$$

where p is the absolute pressure (lb/in.^{2}) and r is in microns. In this report, these assumptions are examined in the light of the data obtained on hydrosol-type membrane filters whose pore-radius dimensions presumably lie within a limited range.

These filters have been described as highly porous cellulose ester structures containing numerous uniform and submicroscopic channels, whose apertures on the upper surface of the filters are smaller than those on the lower surface, and whose pore size can be controlled in the manufacturing process (*7–11*). The particular variety used for this experiment was Millipore Filters, Type HA (hydrosol assay) (*12*), which have been shown to retain particles as small as 0.3 to 0.5 μ in diameter dispersed in water (*13*). Thus, the limiting radius of the smaller or upper aperture is set at 0.15 to 0.25 μ. From the reported frequency of pores per unit surface (*8, 11*), the limiting radius of the larger and lower aperture can be estimated to be 0.7 μ or less. Both dimensions are based on the assumption that the pores are approximately circular.

The specimens were taken from two different lots representing slightly different ranges with respect to manufacturing control, so that one (sample A) showed a greater resistance to flow than the other (sample B).

The pore-size distribution measurements were made with a porometer (*14*), which permits the estimation of the total void volume within the boundaries of the sample as well as the density of its structural material. The procedure was essentially the same as described previously (*6*) in that a sample (two or more filter leaves)was placed within the porometer chamber, immersed in mercury, and pressure was applied to cover the range from 1.32 to 1015 lb/in.^{2}. Assuming that all the void spaces were filled with mercury at 1015 lb/in.^{2}, the measured bulk volume per leaf at that pressure is equal to the volume of the filter substance (Table 1). From the weight of the leaf, the calculated density of the filter substance was 1.51 g/cm^3 for both samples A and B. The porosity of the original filter leaf—the percentage of void within its boundaries—was estimated from the bulk densities measured at 1.32 and 1015 lb/in.^{2} and had a value of 79 percent in both cases. This value is in agreement with the 75 to 90 percent values reported in the literature (*8, 10, 11, 13*).

The distribution curves for the two samples practically coincide between 80 and 0.7 μ effective radius,

Table 1. Porometer data for millipore filters (average for single leaf).

	Sample A	Sample B
Bulk volume at 1.32 lb/in.^{2} and 80 μ (cm^3)	0.2655	0.2628
Bulk volume at 1015 lb/in.^{2} and 0.1 μ (cm^3)	.0564	.0547
Total void volume, by difference (cm^3)	.2091	.2081
Percentage void volume (porosity)	79	79
Weight of leaf (g)	.0850	.0826
Density of leaf at 1015 lb/in.^{2} (g/cm^3)	1.51	1.51
Pore-radius position of peak of distribution curve (μ)	0.48	0.58