

for precipitation was used. Precipitation was completed by adding 110 ml of 95-percent ethanol. The mixture was digested on a steam bath for 34 hr, cooled, and filtered on a No. 50 Whatman filter using a Buchner funnel. The precipitate was washed free of chlorides with 95-percent ethanol and was then allowed to stand in 95-percent ethanol for 1 hr. The ethanol was filtered off and the precipitate was dried in a vacuum oven (25 in.-of-water) for 1 hr at 80°C. Yields of 99 percent or better are possible by this technique, as is shown in Table 1. The rate of activity recovered was also high.

Drying procedure and time appear to be critical factors in this procedure. In trials where the filtered and washed product was dried for 48 hr at 70°C, analyses showed that the product was dehydrated gypsum, $\text{CaSO}_4 \cdot 1/2\text{H}_2\text{O}$. This product was then converted to gypsum by digestion with water on the steam bath for 3 hr and drying in a vacuum oven (25 in.) overnight at 52°C.

References and Notes

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Effects of Narcotics on Coenzyme-A Activity in Acetylation

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There is, as yet, no definitive evidence relative to the primary site of action of narcotics in biological systems. Whether the effective mechanisms are related to the physiological membrane, to intracellular biochemical processes, or to both remains to be determined. A number of investigations have indicated the possibility, once the narcotic is inside the cell, of interference with certain events in the respiratory cycle. The most recent concepts suggest a relationship to the synthesis and utilization of adenosinetriphosphate (ATP) (1). The utilization of both ATP and coenzyme A (coA) is essential in the synthesis of acetylcholine. Since acetylcholine formation has been shown to be inhibited in narcotized systems (2, 3), the present investigation (4) was undertaken in order to examine the possible effects of narcotics on coA and ATP utilization in a "pure," direct acetylating system.

The acetylation system selected for use in this study is that described by Kaplan and Lipmann (5). This system was specifically chosen because of the high degree of quantitation of its elements and the purity of assay it allows. Pigeon liver extract was prepared and aged as described by Kaplan and Lipmann. It is

of particular importance that this extract contains extremely low concentrations of adenosinetriphosphatase (ATPase). Coenzyme A was obtained from the Nutritional Biochemical Co. This material is approximately 75-percent pure, containing (by enzyme assay) more than 20 percent of pantothenic acid and only 0.03 $\mu\text{M}/\text{mg}$ of inorganic phosphate. It was used in three forms: freshly prepared from the desiccated powder; freshly prepared from desiccated powder kept at room temperature for 2 mo; and after having been kept frozen in aqueous solution at -20°C for periods of 1 to 4 wk.

The concentrations of coA are expressed in terms of a unit defined as the amount of the coenzyme per milliliter of solution that will activate the system to one-half of the maximum activity. One unit is equal to 0.025 mg of coA per milliliter of solution. The concentration-activity curve for our system paralleled that of Kaplan and Lipmann, except for a moderately higher degree of acetylation per unit. The coA assay reaction mixture and the contents of the control reaction tubes and blanks were identical with those of Kaplan and Lipmann.

To each of the experimental tubes the narcotic of choice was added in the concentrations shown in Tables 1 and 2. All reactions were carried out in air, in tubes of 1.1-cm outside diameter. Reaction time was 2 hr at 37°C . The reaction was stopped by the addition of 4 ml of 5 percent trichloroacetic acid to each tube. Sulfanilamide was determined by the method of Bratton and Marshall (6), by using a Rouy colorimeter with a 550-m μ filter. The difference in sulfanilamide between the blank and the coenzyme-containing tubes represents the amount of sulfanilamide acetylated.

In this "pure" direct acetylating system, the degree of acetylation of sulfanilamide is a function of the number of units of coenzyme A added up to concentrations that give maximal activity. The initial quantities of adenosinetriphosphate and acetate are known, and the utilization of both is required in order that the reaction proceed. It can be seen from Table 1 that the percentage of sulfanilamide acetylated increases with increasing amounts of the coenzyme but is *not* affected by the addition of chloretone (3 mM). The data presented in Table 2 show a similar lack of effect on the acetylation mechanism by a series of other narcotics. In other words, the narcotics have no effect on the rate of sulfanilamide acetylation measurable with these techniques, under these conditions. It is of interest that the concentrations of narcotic used here are high enough to affect both the rate of oxygen consumption and the response to stimulation of nervous tissue in sympathetic ganglion and brain.

It can be concluded from the present experiments that, in this system, coenzyme A is not blocked by the narcotic. The lack of interference with acetylation leads to the inference that ATP utilization is not disturbed.

The reported pentobarbital inhibition of Govier and Gibbons (7) was not observed in the present series of experiments. It is suggested that two possible factors may have been responsible for their observation: (i)

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	Experi- mental I 1 unit coA, 3 mM chloretone	Control 2 2 units coA	Experi- mental 2 2 units coA, 3 mM chloretone	Control 3 4 units coA	Experi- mental 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 vary significantly. All reaction tubes contained solutions as described by Kaplan and Lipmann (5) in addition to coA and narcotic.

Solution Reaction mixtures	Blank No coA or narcotic	Control 1 unit coA	Pento- barbital 1 unit coA, 3 mM pento- barbital	Seco- barbital 1 unit coA, 3 mM seco- barbital	Chloral hydrate 1 unit coA, 3 mM chloral hydrate	Pheno- barbital 1 unit coA, 3 mM pheno- barbital	Paral- dehyde 1 unit coA, 3 mM paral- dehyde
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, Ruegger *et al.* (4) found that, although folic acid was effective in bringing about a more consistent response