Technical Papers

Laboratory Preparation of $Ca^{45}CO_3$ and $Ca^{45}SO_4 \cdot 2H_2O$

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Calcium chloride labeled with calcium-45 can be purchased from certain suppliers under Atomic Energy Commission authorization. This is reasonably priced and is readily available. On occasion, however, salts such as calcium carbonate and gypsum labeled with calcium-45 may be required quickly for experimental work These labeled salts may be more conveniently referred to as $Ca^{45}CO_3$ and $Ca^{45}SO_4 \cdot 2H_2O$. Combining calcium-45 from a calcium chloride source with gypsum or lime to give a calcium-45 labeled product is not a novel procedure. However, considerable expense and time can be saved, and maximum yields of $Ca^{45}CO_3$ and $Ca^{45}SO_4 \cdot 2H_2O$ are possible using procedures outlined in this paper (*1*).

There are a few reports in the literature describing methods for these conversions. Where yield data are given, they indicate that calcium-45 is being inefficiently recovered owing to incomplete precipitation. This applies particularly to reports outlining the preparation of $Ca^{45}SO_4 \cdot 2H_2O$.

Blume and Hall (*2*) prepared $Ca^{45}CO_3$ by adding $Ca^{45}Cl_2$ to a solution of calcium nitrate and heating to 80°C. Precipitation was accomplished by adding a 2-percent solution of ammonium carbonate and 2 to 3 ml of ammonium hydroxide. The vessel was closed and the contents were digested for several hours. Harris *et al.* (*3*) prepared $Ca^{45}CO_3$ by precipitating a solution of radiocalcium and calcium chloride with carbon dioxide under pressure.

Robertson and Blume (*4*) labeled gypsum with radiocalcium by dissolving calcium sulfate in hot (1+3) nitric acid and adding $Ca^{45}Cl_2$ to this solution. The gypsum was crystallized out by cooling the solution. The yield was about 33 percent. Blume and Hall (*2*) prepared $Ca^{45}SO_4 \cdot 2H_2O$ by adding 10 percent sulfuric acid to a suspension of $Ca^{45}CO_3$ and heating and stirring this mixture for 6 to 8 hr. The product obtained was a mixture of anhydrite and gypsum.

For our study, calcium chloride tagged with calcium-45 was purchased (*5*) under Atomic Energy Commission authorization. Calculations were made to determine how much radioactive $Ca^{45}Cl_2$ was needed to supply the desired activity per unit weight of calcium in the finished product, whether $Ca^{45}CO_3$ or $Ca^{45}SO_4 \cdot 2H_2O$. Because this amount can be varied according to individual needs, the sequence of adding calcium-45 is indicated in the procedures rather than the actual amounts used.

Calcium carbonate. Labeled $Ca^{45}CO_3$ was prepared by dissolving a calculated weight of calcium chloride in (1+10) hydrochloric acid. For example, 8.8140 g of $CaCl_2 \cdot 2H_2O$ was dissolved in 40 ml of (1+10) hydrochloric acid and the solution was diluted to 300 ml. To this solution was added a previously determined amount of $Ca^{45}Cl_2$ and the solution was made alkaline with sodium hydroxide using phenolphthalein indicator. The $Ca^{45}CO_3$ was precipitated by adding 12 g of sodium carbonate to the solution. Experimentation showed that an excess of sodium carbonate was needed for complete precipitation. Twice the theoretical minimum amount necessary for precipitation was used. The resulting solution and precipitate was digested for 12 hr at 65°C and was allowed to stand overnight. The precipitate was filtered off on a No. 50 Whatman filter paper through a Buchner funnel and was dried at 70°C. The yield was 99.40 percent of the calculated weight that theoretically could be attained.

The purity of the product was determined by dissolving a weighed portion of the precipitate in dilute (3+100) HCl and titrating with versenate according to a modified method of Cheng and Bray (*6*). The purity of reagent calcium carbonate compared with that prepared in the laboratory is shown in Table 1. There was a high rate of activity recovery, as is shown in the table.

Calcium sulfate. Labeled $Ca^{45}SO_4 \cdot 2H_2O$ was prepared by dissolving analyzed calcium chloride in water. The amount of calcium chloride used depended on the weight of product required. Similarly the amount of $Ca^{45}Cl_2$ used depended on the specific activity—that is, millicuries per unit weight—desired. For example, 5.0 g of calcium chloride was dissolved in 50 ml of water containing the desired amount of $Ca^{45}Cl_2$, and 50 ml of water containing 10 ml of sulfuric acid was added to this solution. Experimentation showed that an excess of sulfuric acid was needed for complete precipitation. Four times the theoretical minimum necessary

Table 1. Purity of preparations and recovery of added calcium-45.

Source of $CaCO_3$ and $CaSO_4 \cdot 2H_2O$	Purity of product (%)	Recovery of Ca^{45} in product* (%)
$CaCO_3$ reagent	99.93	
	100.00	
$CaCO_3$ laboratory preparation	99.59	96.6
	99.72	97.3
	99.75	96.5
	99.51	98.1
$CaSO_4 \cdot 2H_2O$ reagent	99.82	
	99.50	
$CaSO_4 \cdot 2H_2O$ laboratory preparation	99.43	96.5
	99.20	97.8
	99.10	98.4
	99.22	98.8

* 0.2 μc Ca^{45} added to each sample.

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, $CaSO_4 \cdot 1/2H_2O$. 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

1. Published with the approval of the director of the Idaho Agricultural Experiment Station as Research Paper No. 369.
2. J. M. Blume and N. S. Hall, *Soil Sci.* **75**, 229 (1953).
3. H. C. Harris *et al.*, *Science* **113**, 328 (1951).
4. W. K. Robertson and J. M. Blume, Research Dept. No. 223, Division of Soil Management and Irrigation, U.S. Dept. of Agriculture.
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6. K. L. Cheng and R. H. Bray, *Soil Sci.* **72**, 449 (1951).

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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). Coenyzme 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 μM/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 trichloracetic acid to each tube. Sulfanilamide was determined by the method of Bratton and Marshall (*6*), by using a Rouy colorimeter with a 550-mμ 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)