Hydrolysis of Adenosine Triphosphate by Trichloracetic Acid[1]

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In the determination of adenosine triphosphatase activity, the usual procedure utilizes addition of trichloracetic acid for precipitation of the protein and stopping of the enzyme action. The estimation of inorganic phosphorus is then done on the filtrate. In determining dephosphorylation of an adenosine triphosphate substrate by human serum (*2*), we had occasion to leave the trichloracetic acid filtrates overnight in the icebox. Subsequent estimations of these same filtrates for inorganic phosphorus revealed values which were much higher than those previously obtained. Our interest in this casual observation led us to undertake an investigation of this phenomenon.

Experimentally, a solution of sodium adenosine triphosphate[2] in veronal-HCl buffer at pH 8.9, in a concentration of 1 mg in 1 ml of buffer was used. Filtrates were prepared in the following manner: to each 2.5-ml portion of the buffered Na-ATP solution 0.5 ml of water (instead of serum) and 5 ml of 8% trichloracetic acid was added, and the resulting solution was well mixed and then filtered. The inorganic phosphorus was determined on 4 ml of the filtrate by the method of Fiske and Subbarow (*1*). Sufficient filtrate was made to insure duplicate estimations of inorganic phosphorus at the end of the various time periods. The results are recorded in Table 1.

TABLE 1

HYDROLYSIS OF NA-ATP BY TRICHLORACETIC ACID

Sample	Preparation	Control	Time in hr		
			2	4	24
		μg P/ml			
1	Na-ATP 1 mg in 1 ml of veronal-HCl buffer, pH 8.9, room temperature	4.1	6.3	8.1	27.3
2	Do., in icebox	4.1	5.1	5.8	10.3
3	Na-ATP 1 mg in 1 ml H_2O, room temperature	4.4	5.5	7.9	
4	Do.	2.4	6.8	8.4	

In order to obviate the possible effect of the buffer solution employed, an aqueous solution of Na-ATP in the same concentration was prepared and treated in the same manner as described above. These results are also shown in Table 1.

It is evident that there is a definite increase in inorganic phosphorus when adenosine triphosphate in trichloracetic acid is allowed to stand at either room or icebox temperature. No such change takes place when solutions of adenosine triphosphate without addition of trichloracetic acid are allowed to remain for the same periods of time. Trichloracetic acid filtrates prepared from serum alone do not hydrolyze under similar conditions of time and temperature.

The results indicate that spontaneous hydrolysis of adenosine triphosphate by trichloracetic acid does occur. It is essential that determinations of inorganic phosphorus be made under identical conditions in order to avoid errors due to such hydrolysis.

References

1. FISKE, C. H. and SUBBAROW, Y. *J. biol. Chem.*, 1925, **66**, 375.
2. MEISTER, A. *J. clin. Invest.*, 1948, **27**, 263

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[2] The tetra sodium salt of adenosine triphosphate · $3H_2O$ was obtained from Rohm & Haas, Philadelphia, Pennsylvania.

Differentiation of Aragonite from Calcite by Differential Thermal Analysis

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I have been making differential thermal analysis studies of carbonate minerals since 1942. Some results of the application of these studies to problems in petrography and mineralogy have been published (*2, 4*). The detailed thermal analysis studies on these minerals are now being prepared for publication. In view of the current interest in differential thermal analysis studies, it seems desirable to put on record an observation, made several years ago, on the identification of aragonite by this method.

The differential thermal analysis apparatus used in these studies is almost identical with the one designed by Hendricks, Alexander, and Nelson (*1, 5*), and with a sensitivity dependent on resistance in series with the galvanometer of 999.9 ohms.

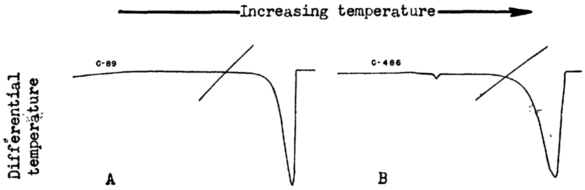

FIG. 1. (A) Calcite from Joplin, Missouri. (B) Aragonite from Chile.

Typical curves for aragonite and calcite are given in Fig. 1. The short diagonal lines are portions of the curve used to determine the temperature of the thermal reactions recorded (*3*). The principal curve shows the course of the thermal reactions.

The calcite sample, illustrated in Fig. 1A, is from Joplin, Missouri, and is part of the tube sample analyzed by R. C. Wells (*6*). The curve shows one large peak representing the dissociation of calcium carbonate into

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