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.

[1] Aided by a grant from the Frederick Kolb Fund.

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

References

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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.

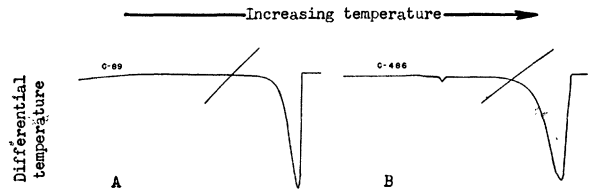

FIG. 1. (A) Calcite from Joplin, Missouri. (B) Aragonite from Chile.

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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calcium oxide and carbon dioxide. The peak temperature for this sample is 972° C. The aragonite (U.S.N.M.-R2554) is from Chile, exact locality unknown, and its thermal curve is shown in Fig. 1B. This curve shows a small peak at 447° C which represents the dimorphic transformation of aragonite to calcite. This transformation of a metastable material is irreversible, and hence does not take place at a reproducible temperature. Subsequently, the calcite, paramorphous after aragonite, undergoes decomposition at 897° C. The temperature of the dissociation of calcite is not a definite temperature in a nonequilibrium process. The presence of the low temperature peak, in this sample of aragonite at 447° C, representing the transformation of aragonite to calcite, serves to differentiate these two minerals. However, this peak requires a sensitive, continuous recording apparatus and can easily be overlooked.

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Comments and Communications

The Preparation of Graphs for Publication

In the preparation of graphs of many sorts, it is exceedingly convenient to use graph paper. Graphs so prepared are satisfactory for study but not for publication, because of the background of fine lines. If blue-lined paper has been used, these lines can be eliminated by photography; but this requires some experience and skill and, for some of us, is apt to mean more than one attempt. Moreover, blue-lined paper is not always available.

Recently I noticed that the lines on graph paper show through the paper well enough for the fixing of points on the back. The graph paper is placed upside down on a white surface, and the portion of the sheet to be used is outlined and certain reference points are indicated before the data points are marked. The finished graph, in ink on the plain white back of the paper, may be submitted directly or conveniently photographed.

This method may be helpful to many investigators where professional draftsmen are not available.

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Use of Omental Spread in Biological and Pathological Studies

The advantages of using the omentum of a small animal for making spread preparations instead of attempting to section the tissue have been pointed out by Cross (*Science*, 1949, **109**, 314). However, the technique described, of first taking up the desired portion of omentum on a bit of cigarette paper and then transferring it to a slide, is needlessly complicated. Instead, a piece of omentum may be removed and placed directly on a slide (Woodruff, C. E. *Amer. J. Path.*, 1934, **10**, 739). Then, making use of the property of this thin membrane of fixing itself to glass, one corner of the omentum may be teased out and allowed to dry on the slide. From this anchoring corner the balance of the membrane may be teased out to form a thin layer most of which will be only one cell thick. Certain stains such as the Ziehl-Neelson may be used on the omental spread without further fixation. Other stains may require the use of formalin or some other fixative prior to staining. The stained preparation is readily mounted in balsam and affords a three-dimensional picture of developing disease processes which one cannot obtain by the use of conventional histological sections.

Very satisfactory omental spreads may be obtained from mice and guinea pigs. The omenta of rabbits and dogs are of less value for this purpose, since the tissue fails to become fixed to the slide in a satisfactory manner.

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Calibration of Warburg Manometers

The paper by Loomis (*Science*, 1949, **109**, 491) concerning the calibration of Warburg manometers omits mention of the convenient method of Schales (*Arch. Biochem.*, 1944, **3**, 475), which also involves the principle of filling the manometers from below by means of a mercury reservoir. Schales' method possesses the further advantage of not requiring any spatial manipulation of the manometers in order to adjust the level of the mercury.

The disadvantage of Schales' method, in our experience, has been the difficulty of placing just the right amount of mercury in the flask in order that the fluid will rise to the point of junction of the side arm and the manometer when the flask is slipped on the ground joint. We have avoided this difficulty by placing a minimum amount of mercury in the flask (usually to the lower edge of the ground surface), and adding further amounts through the gas outlet tube with a capillary