

The solubility of proteins in the presence of carbon dioxide

In a previous communication¹, we reported the fractionation of proteins with a cellulose-derived anion exchanger and a system of water and CO₂. We have now observed that CO₂ increases the rate of solution of proteins in water, and in some cases renders insoluble proteins soluble.

The increase in rate of solution is especially valuable for making concentrated solutions of bulky solids. In the preparation of a 30% solution of serum albumin, a round-bottomed flask was filled with CO₂, albumin powder (Cohn Fraction V) was added, then water, and the flask was stoppered and placed in the cold. Within 2 h solution was complete, whereas a control without CO₂ still contained most of its solid phase. No precipitate was obtained on removal of the CO₂. 2% solutions of haemoglobin, which under ordinary conditions require grinding and careful stirring, can be prepared simply by adding the freeze-dried powder to distilled water and covering with an atmosphere of CO₂. Dilute acetic acid could not duplicate the effect of the CO₂ on the solubilization of these soluble proteins.

CO₂-treatment is a useful general method for solubilizing some insoluble proteins under mild conditions. The technique has application in chromatographic procedures. In one experiment, 200 mg porcine serum γ -globulin (Cohn Fraction II) on a 5 g aminocellulose column (free-base form) was eluted with two hold-up volumes of distilled water saturated with CO₂ (1 atm.)¹. A voluminous precipitate which formed almost immediately as the protein fractions were being collected was attributed to the escape of CO₂. Confirmatory experiments demonstrated that water-clear, 10% solutions of bovine or porcine serum fraction II can be prepared in distilled water under an atmosphere of CO₂. Agitation, which may be a source of denaturation, is not required. Replacement of the CO₂ with air causes the reprecipitation of much of the protein. Mild denaturation renders porcine γ -globulin insoluble in CO₂-water. In one case withdrawal of CO₂ resulted in crystallization of a protein not crystallized heretofore in this laboratory. A kidney tissue preparation desorbed with CO₂ from the aminocellulose (fraction 43 in the previous communication¹), yielded crystals on standing unstoppered in the cold. The crystalline precipitate, amounting to almost one-half the total protein present, was easily separated. Upon exposure to CO₂ at one atmosphere the crystals quickly and completely redissolved. On withdrawal of the atmosphere of CO₂, the protein began to reprecipitate (requiring several days for completion). The crystals were also soluble in dilute salt (0.1 M) and did not reprecipitate on dilution with water. Crystalline zinc insulin was solubilized to a small but definite amount (0.1 mg/ml) by treatment with CO₂. Removal of the CO₂ atmosphere over the insulin solutions caused a precipitate to form starting at the liquid-gas interface. A euglobulin fraction made from bovine serum by the method of GREEN², or from pancreas by a similar procedure, was not solubilized. The latter material could be solubilized with dilute acetic acid.

Chemical changes due to interaction of CO₂ with proteins³ have not been observed by the usual physical methods. Release of the CO₂ from these treated solutions has no apparent effect on the soluble proteins but causes the precipitation of the less soluble. After treatment with CO₂ no alteration is noted in the solubility characteristics of crystalline egg lysozyme, in the electrophoretic behavior of serum albumin, or in the absorption spectrum of γ -globulin. These observations indicate that the CO₂ which hydrates and ionizes to a very small degree in distilled water has a very great and reversible effect on the solubility of proteins in water.

M. A. MITZ

Research Division, Armour and Company, Chicago, Ill. (U.S.A.)

¹ M. A. MITZ AND S. S. YANARI, *J. Am. Chem. Soc.*, 78 (1956) 2649.

² A. A. GREEN, *ibid.*, 60 (1938) 1108.

³ W. C. STADIE AND H. O'BRIEN, *J. Biol. Chem.*, 117 (1937) 439.

Received May 24th, 1957

Swelling of mitochondria isolated from different tissues

In a previous report¹, it was demonstrated that the swelling of mitochondria produced *in vitro* by thyroxine and its analogues varies according to the tissue from which the mitochondria are isolated. Thus thyroxine causes pronounced swelling of rat liver and kidney mitochondria, slight swelling of those from diaphragm and heart, and essentially none with mitochondria isolated from spleen, brain, or testis. This communication demonstrates that mitochondria from these tissues vary also in their response to other agents which have previously been shown to cause swelling of isolated liver mitochondria². The results serve to emphasize that data obtained with mitochondria isolated from any particular tissue must be interpreted in terms of that tissue only.

Mitochondria were isolated¹ from the organs of normal adult male Wistar rats, suspended