Meetings

Carbon Dioxide: Chemical, Biological, and Physiological Aspects

A symposium on Carbon Dioxide: Chemical, Biochemical, and Physiological Aspects was held at Haverford College, Haverford, Pennsylvania, 19–21 August 1968. It brought together researchers involved in the physiology and chemistry of CO_2, the physical chemistry of the reactions of CO_2 and bicarbonate, hemoglobin and peptide carbamates, carbonic anhydrase, and the movements of CO_2 in cells and tissues.

In the first session S. Epstein (California Institute of Technology) discussed the isotope ratio of C^{13} to C^{12} in nature and the reasons for its variation. For a given sample, the variation is described by the δ value:

$$\delta = [C^{13}:C^{12}(\text{sample})/C^{13}:C^{12}(\text{standard}) - 1] \times 1000$$

The standard is given by the CO_2 analysis from a certain fossil skeleton. The δ values in nature range widely, from about zero for marine limestones to about -25 parts per thousand for terrestrial plants to -30 for nonmarine petroleum. Fixation of CO_2 in photosynthesis involves substantial isotope fractionation which can give important information on the mechanism of the fixation process.

J. T. Edsall (Harvard) reviewed the structure and properties of CO_2, HCO_3^-, and H_2CO_3. The best available values for hydration and ionization constants still contain some puzzling discrepancies requiring further work. Carbon dioxide dissolves more readily in nonpolar media than in water and still more readily in solvents such as acetone which contain both hydrophobic and polar groups. This factor may be important for the interaction of CO_2 with enzymes.

The second session concerned carbamate reactions, which are now of particular interest because of the recent decisive work of Roughton and Rossi-Bernardi (1967). They found that carbamate binding to oxyhemoglobin is definitely less than to deoxyhemoglobin at a given CO_2 pressure and *p*H. This fact provides a convincing argument that carbamate is involved in CO_2 transport in blood.

M. Caplow (Yale) discussed the kinetics of carbamate formation. On the basis of his experimental data he proposed a mechanism involving hydrogen bonding of the H on the amine group, addition of CO_2 to the nitrogen with separation of charge on the molecule, and finally the formation of a carbamate ion with proton release.

R. E. Forster (University of Pennsylvania) reported kinetic data on the reaction of CO_2 with human hemoglobin in a rapid reaction apparatus with a CO_2 electrode. He found a bimolecular combination rate of 11,000 M^{-1} sec^{-1} and inferred a dissociation velocity constant of 500 sec^{-1} at 37°C.

J. V. Kilmartin (Cambridge University) and L. Rossi-Bernardi (Milan) reported the selective blocking of the α-amino groups of horse hemoglobin by treatment with cyanate. They obtained three derivatives. In the first, only the α-amino groups on the α chains are blocked; in the second only those on the β chains are blocked; and in a third all α-amino groups are blocked. Blocking the α-amino groups on the β chain did not alter the Bohr effect—the release of protons on oxygenation. However, blocking the groups on the α chains decreased the effect by about 30 percent. Blocking of all terminal amino groups essentially eliminated CO_2 binding by hemoglobin at physiological *p*H. Blocking of either the α or the β chains decreased CO_2 binding to a half. This reaction gives decisive experimental proof that the α-amino groups are responsible for the binding of CO_2 by horse hemoglobin. L. Rossi-Bernardi (Milan) described a new and much improved quenching method for measuring CO_2 bound to proteins as carbamate.

There were several sessions on the erythrocyte carbonic anhydrases. Three human isozymes—carbonic anhydrases A, B, and C—are known. Enzyme C has high specific activity; enzymes A and B have much lower activity. The red cells of mammals in general appear to contain at least one "high activity" enzyme (C) and generally also a "low activity" enzyme (B), probably genetically related to those found in man. All contain one essential zinc atom per molecule.

A. Liljas (Uppsala) reported on the x-ray work of the group at the University of Uppsala which is now obtaining high resolution data on crystals of human carbonic anhydrase C. As this group has shown earlier, the molecule is roughly ellipsoidal in shape with the essential zinc atom in a crevice large enough to permit the close approach of sulfonamide inhibitors. High resolution studies show that anionic inhibitors, sulfonamides, iodides, and negatively charged heavy metal complexes approach very close to the zinc atom. All these compounds, except for the iodide, approach within binding distance of the zinc.

J. H. Wang (Yale) proposed a detailed mechanism for the action of the enzyme. The CO_2 is loosely bound to a hydrophobic cavity near the zinc and reacts with an OH^- coordinated to the metal. Because the coordinated OH^- is a much weaker base than OH^- in free solution, the spontaneous rate of reaction of the coordinated OH^- with the nearby CO_2 should be about $1/10^{+8}$ of the experimentally determined rate for the enzyme-catalyzed reaction. Wang therefore concluded that an additional factor must facilitate the reaction and suggested that the OH^- is not only coordinated to the Zn, but is hydrogen bonded to a strategically located basic group on the protein, such as an imidazole.

P. O. Nyman (Göteborg) reported on the amino acid sequence of carbonic anhydrase B, which contains one SH group in a single chain of about 260 residues. Only one of the 11 histidines in the COOH-terminal quarter of the chain reacts specifically with bromoacetate. Another histidine in the NH_2-terminal third of the chain reacts specifically with *N*-chloroacetyl chlorothiazide. As shown earlier by P. L. Whitney *et al.*, the latter derivative has lost all enzymatic activity; the former has much lower activity than the native enzyme, and its *p*H dependence curve is shifted to high *p*H.

Mme. G. Laurent (Marseille) reported that treatment of human carbonic anhydrase B at high *p*H can split the enzyme into multiple electrophoretically different components of which the functional fragment is probably equivalent to enzyme A.

J. E. Coleman (Yale) studied the optical absorption and circular dichroism of complexes of carbonic anhydrase with a highly colored sulfonamide derivative. The sulfonamide itself has no optical activity, but on binding to the enzyme it induces large ellipticity bands not previously present. The replacement of zinc by cobalt in carbonic anhydrases gives rise to multiple visual absorption bands. In human carbonic anhydrase B, a "low activity" enzyme, these are optically inactive, although sulfonamides, metal-binding anions, bicarbonate, and acetate induce asymmetry so that strong ellipticity bands appear in the visible region. However, the cobalt derivatives of the "high activity" enzymes, bovine enzyme B and human C, give optically active bands, in the visible, even without the presence of the compounds that induce asymmetry in human B enzyme. This points to an important difference in the nature of the active center between the "high activity" and "low activity" enzymes. Likewise, in amino acid sequence (Nyman) the two high activity enzymes, although from different species, appear to be more closely related to each other than are the human B and C enzymes.

The next session was devoted to the role of CO_2 and bicarbonate in enzymatic carboxylation reactions. T. G. Cooper (Purdue University) presented evidence, obtained with T. T. Tchen, H. G. Wood, C. R. Benedict, and D. Q. Filmer, that CO_2 is the reactive species in the carboxylation catalyzed by P-enolpyruvate carboxykinase and P-enolpyruvate carboxytransphosphorylase. On the other hand, bicarbonate is the active species in the pyruvate carboxylase reaction as earlier work has shown it to be for propionyl-CoA carboxylase; both of these, unlike the other two enzymes mentioned above, are biotin enzymes.

M. Daniel Lane (New York University) reviewed the nature of enzymatic carboxylation mechanisms. The major groups of reactions include O-P bond cleavage in ATP and P-enolpyruvate in the reactions catalyzed by the biotin-dependent carboxylases and the P-enolpyruvate carboxylating enzymes, respectively; C-H bond cleavage in NADH and NADPH in the reductive carboxylations catalyzed by malic enzyme and isocitrate dehydrogenase; and C-C bond cleavage in the ribulose diphosphate carboxylase reaction. He presented a detailed mechanistic discussion of the various reactions. M. C. Scrutton (Rutgers Medical School) with A. S. Mildvan (University of Pennsyl-

vania) examined, largely by nuclear magnetic resonance studies, the vital role of the essential metallic ion (most commonly manganese) in CO_2 fixation by the biotin carboxylases; they proposed a detailed mechanism for this role in these reactions.

The final session primarily concerned aspects of the currently unexplained finding that the P_{CO_2} of alveolar gas in the lungs may become greater than arterial P_{CO_2}. E. J. M. Campbell (Royal Postgraduate Medical School, London) described studies in man in which this inequality was found to increase as transient CO_2 exchange increased. G. Laszlo (Johns Hopkins) found that in perfused dog lungs alveolar P_{CO_2} remained equal to arterial P_{CO_2} if there was no net CO_2 exchange. However, G. Gurtner (State University of New York at Buffalo) reported that alveolar P_{CO_2} was greater than arterial P_{CO_2} under conditions where CO_2 exchange would have been expected to be minimal. He suggested that there is a higher $[H^+]$ near the wall of the pulmonary capillary, which leads to a greater local $[H_2CO_3]$ and raises the P_{CO_2} in the alveoli. No completely satisfactory explanation for these apparent violations of the generally accepted belief that CO_2 equilibrates between alveolar gas and end-capillary blood was presented.

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Courses

Tropical Geography, 15 April–15 June. This course will be conducted in Costa Rica and will hinge on the use of geographical methods in a tropical context. Included in the course will be "Geography of Costa Rica" and "Forests and related resources in the tropics," which will place greatest stress on forest management and utilization. Following an orientation program in San Jose, students will be given increasing opportunities for individual work in any of the major areas of geographic inquiry. They will thus work in rotating pairs during the first 6 weeks and individually in the last 2 weeks, when they will undertake independent research on a topic of their choosing. The Organi-