ON THE USE OF CARBONIC ANHYDRASE IN CARBONATE AND AMINE BUFFERS FOR CO_2 EXCHANGE IN MANOMETRIC VESSELS, ATOMIC SUBMARINES, AND INDUSTRIAL CO_2 SCRUBBERS

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There is a remarkable resemblance between the kinetics of carbon dioxide gas exchange in manometric vessels of 1 cu. inch volume (FIGURE 1)[1] and in atomic submarines of 100,000 cu. ft. volume (FIGURE 2),[2] and it is not the purpose of this paper to hide such a kinetic similarity. As will be indicated in some detail, the over-all mathematical treatment of CO_2 in the gas phase is much the same whether one is dealing with CO_2 absorption in (1) a manometric vessel central well (FIGURE 1) containing, for example, KOH, NaOH, LiOH, K_2CO_3-$KHCO_3$, Tris buffer (THAM), or mono- or di-ethanolamine; or in (2) an atomic submarine CO_2 scrubber containing monoethanolamine (FIGURES 3 and 4) or LiOH (FIGURE 5). The weaker alkaline CO_2 buffers mentioned (carbonate, Tris, and the ethanolamines) have all been found to undergo tremendous acceleration of CO_2 exchange when the enzyme carbonic anhydrase is added; in my hands accelerations in rate of 5- to 100-fold have been readily observed with all such buffers, depending upon conditions. Strong alkalis (pH > 11 to 12) destroy the enzyme rapidly, of course.

The manifold acceleration of CO_2 exchange by carbonic anhydrase may be observed with respect to either absorption or elimination of CO_2 gas, depending upon the side of the equilibrium point from which one is operating; this can be a variable function of, for example, the pH, base exchange, or temperature. In this connection it is worth recalling well-known physiological situations in which different pH-base equilibria are involved at constant (body) temperature. In the stomach mucosal wall and in kidney cells, carbonic anhydrase accelerates the removal of CO_2 from the blood, leading to increased acidity in the stomach lumen and urine; in the lungs and pancreas, on the other hand, carbonic anhydrase accelerates the removal of CO_2 gas from the blood, leading to more alkaline blood and pancreatic juice. Analogously, in a submarine CO_2 scrubber operated at two different temperatures, carbonic anhydrase could accelerate the absorption of CO_2 by the lean buffer mixture in the low-temperature absorber and, when this mixture has become enriched with CO_2 and raised to an appropriately higher temperature in the stripper, carbonic anhydrase could accelerate CO_2 stripping, provided that the absorbing and/or stripping operations were carried out somewhere between 0° and 60° C., the practical temperature limits for stable action by this enzyme. Parenthetically, it may be remarked that the submarine CO_2 scrubber provides an "extra breather" for the crew in addition to their lungs. Various engineering possibilities for feasibility studies in connection with CO_2 elimination from atomic submarine atmospheres will be considered in the latter part of this paper. Let us look at manometric situations first.

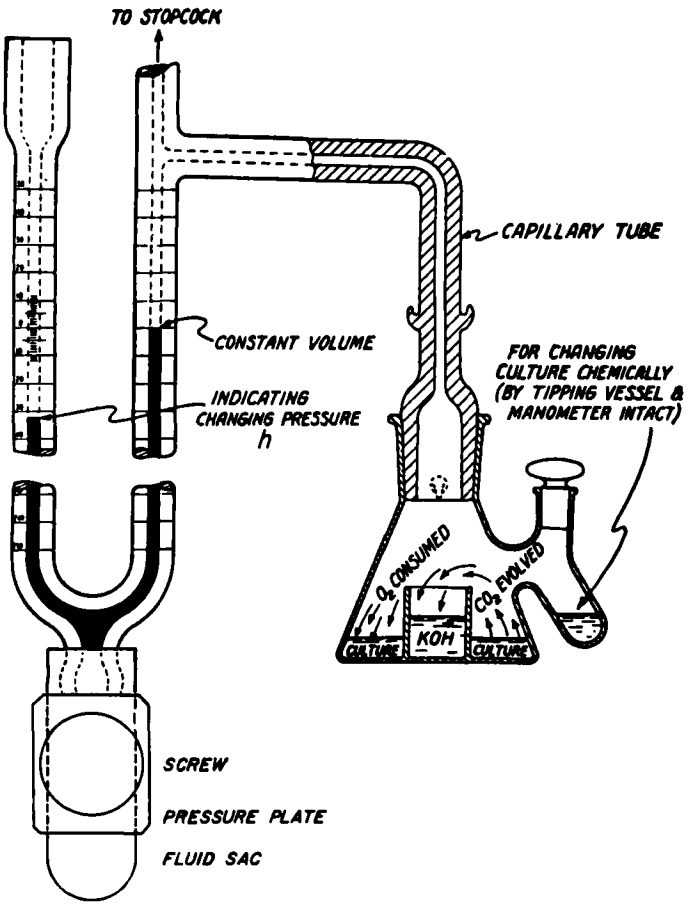

FIGURE 1. Typical Warburg manometer and illustrative vessel[1] for operation at constant volume of gas space; x (cu. mm. NTP) gas change $= hk$, where h = mm. pressure change (10,000 mm. Brodie fluid = 1 atmosphere), and $k = ([V_G \cdot 273/T] + V_L\alpha)/P_0$, V_G = volume of gas phase (cu. mm.), V_L = volume of liquid phase (cu. mm.), P_0 is a pressure of one atmosphere expressed in millimeters of confining fluid, T is the absolute experimental temperature, and α is the Bunsen absorption coefficient of the gas exchanged.

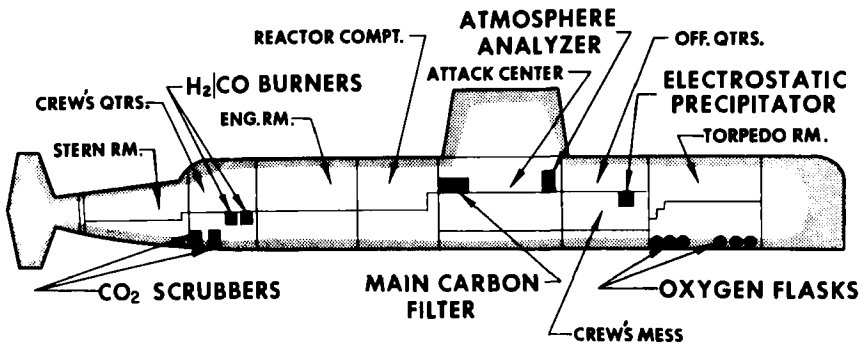

FIGURE 2. Atmosphere control equipment on a typical nuclear-powered submarine, with special reference to indicated size and location of CO_2 scrubbers.[2]

Manometric Measurements

The advantages and disadvantages of various ethanolamines as CO_2 buffers and absorbents in manometric measurements were pretty well outlined about 10 years ago by Pardee,[3] Krebs,[4,5] and Burk *et al.*[6] Among such amines for manometry, diethanolamine was found to be the most generally suitable with respect to desired K_b value, solubility, nonvolatility, CO_2 retention, and main-

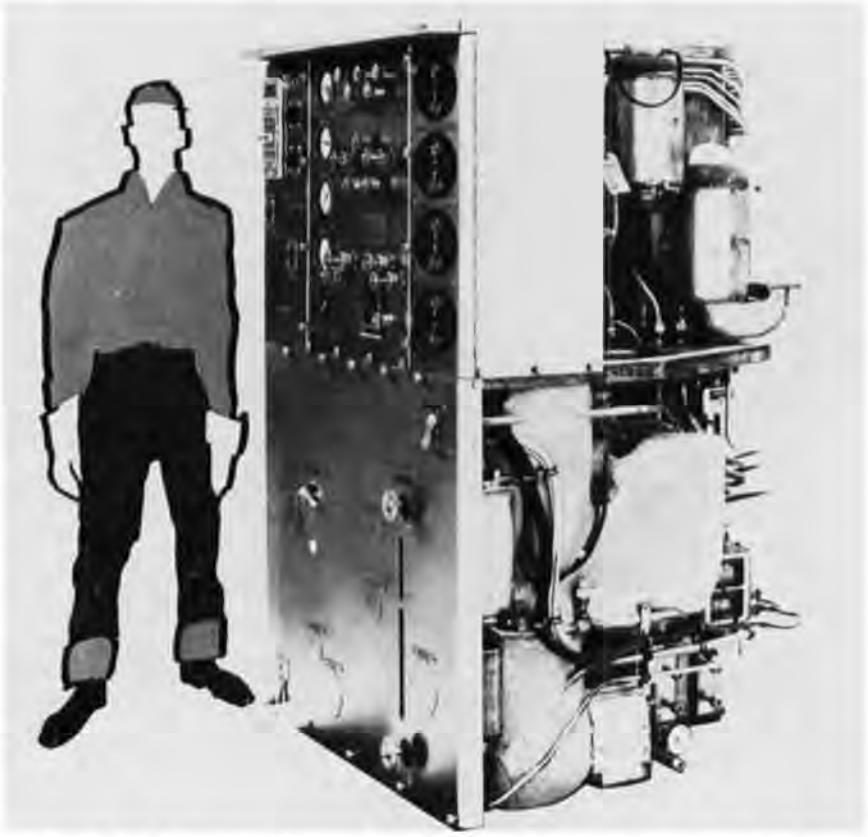

FIGURE 3. Submarine CO_2 removal plant, front and left-side view, at man-sized scale.[2]

tenance of constant CO_2 gas pressure up to the order of 2 to 3 per cent-atmosphere. The chief disadvantages of even the best of the amines tested were residual autoxidation that could not be entirely eliminated, even with 0.1 per cent thiourea; difficulty in satisfactorily attaining a physiological CO_2 partial pressure of about 5 per cent in the gas phase at about 1 atm. total pressure; and rates of absorption considerably lower than those shown by strong alkalis (such as KOH and NaOH) in small absorption containers.

The most interesting observation that can be made about the manometric use of ethanolamine CO_2 absorbents at present is that quite recently it has been

found that they are equaled or surpassed in virtually all aspects by concentrated potassium carbonate-bicarbonate buffers containing carbonic anhydrase (Warburg and Krippahl,[7] and Stambuk and Burk[8]). The carbonate-bicarbonate

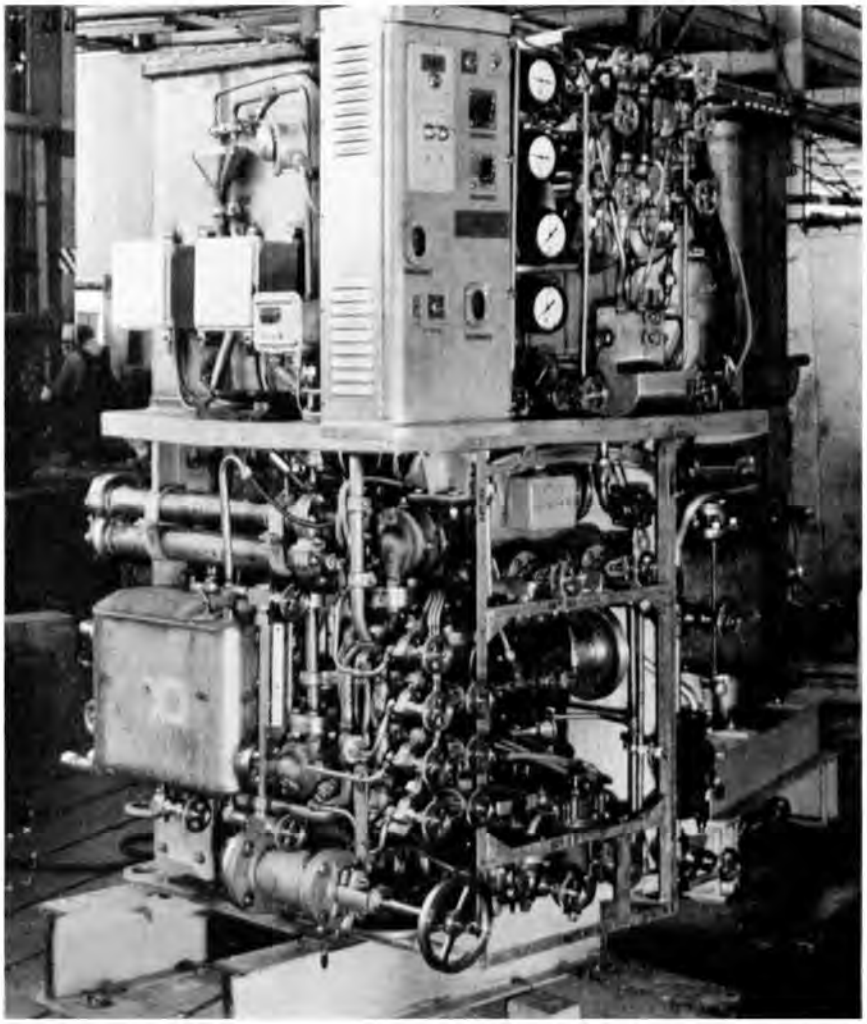

FIGURE 4. Submarine CO_2 scrubber, exposed view front and right side. Hardware rather than CO_2-absorbents occupies most of the space.[2]

buffers can be adjusted readily to provide fixed CO_2 pressures over a several thousandfold range from less than 0.01 per cent-atm. up to well above 10 per cent-atm., with high CO_2 retention, no autoxidation, and a satisfactory equilibration time of but a few minutes at most, providing that suitable manometric vessels are employed, notably and preferably the new vessels described by War-

burg and Krippahl.[9] In such vessels, illustrated in FIGURE 6, the small central well on the floor of the main compartment (of the vessel shown in FIGURE 1) is replaced by an elevated wide-area trough that is optionally further connected directly with a side arm; indeed there may be an additional independent side arm.[9] For high physiological CO_2 pressures of the order of 5 per cent-atm., the carbonate-bicarbonate buffers will be of the order of 1 to 3 *M* (and largely bi-

FIGURE 5. CO_2-canister with six LiOH units capacity.[2]

carbonate), and will contain 5 to 10 Meldrum and Roughton units[10] of carbonic anhydrase per cc. (that is, about 0.001 mg. highly purified enzyme/cc., or up to 1 mg. commercially prepared enzyme/cc., depending upon the purity of the latter). The vessels with a trough to contain buffer also improve the usage of the amine buffers by similarly decreasing the equilibration time, as a result of increased area and volume of buffer that may be employed conveniently in a given vessel.

The new manometric vessels, carbonate-bicarbonate buffers, and carbonic

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