tation through unconscious hypostatization of disposition, including aggressivity, pathogenicity and virulence, and of similar concepts common to physiology, pathology and genetics.

Details of the experiments will be published elsewhere.

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SPECIAL ARTICLES

THE ROLE OF THE CARBAMINO COMPOUNDS IN THE TRANSPORT OF CO_2 BY THE BLOOD[1]

SINCE Siegfried[2] first prepared salts of carbamic acid by the reaction of CO_2 and amino-acids, and demonstrated analogous compounds of CO_2 and proteins in quite alkaline solutions, the rôle of these carbamino compounds of protein, particularly hemoglobin, as carriers of CO_2 in the blood under physiological conditions has received support by Henriques,[3] Margaria and Green[4] and others. In a recent paper, particularly, Meldrum and Roughton[5] report experiments on the reaction between CO_2 and amino-acids as well as hemoglobin. In brief, they observed that CO_2 was taken up by amino-acids or hemoglobin (to which cyanide had been added to inhibit the rapid enzyme catalysis of the hydration of CO_2 to carbonic acid) in two phases: (1) a very rapid one which they assert, correctly we believe, to be due to the formation of CO_2 in the carbamino form and (2) a slow uptake which is due to the formation of carbonic acid. From their values of carbamino-CO_2 in hemoglobin solution calculated from the *rapid* uptake, they constructed "non-bicarbonate" or carbamino-CO_2 absorption curves for hemoglobin which they "assumed to be practically the same for normal blood and for cyanide blood." They came to the conclusion, which at first sight seems strongly supported by their observations, that carbamino-hemoglobin plays a very important rôle as a CO_2-carrier in the blood. We believe, however, that the above assumption is erroneous because the equilibrium system which they studied was entirely different from the equilibrium system (*i.e.,* normal blood without cyanide) to which they applied their experimental data. Therefore, we believe that their conclusions about the physiological rôle of carbamino-CO_2 derives no support from these experiments.

This paradoxical situation arises as follows. An aqueous solution of an amino-acid, *e.g.,* glycine, to which has been added one or less equivalents of base, and which hence contains a concentration of amphanion, COO^-CNH_2, equal to the concentration of base, when suddenly allowed by equilibration to react with a CO_2 gas phase, forms carbamate, *viz.*:

(1) $COO^-CNH_2 + CO_2 = COO^-CNHCOO^- + H^+$

In addition, CO_2 reacts to form carbonic acid, *viz.*:

(2) $CO_2 + H_2O = H_2CO_3 = H^+ + HCO_3^-$

Both reactions decrease pH, since both carbonic and carbamic acid are about a thousandfold stronger than glycine. In consequence the amount of COO^-CNH_2 diminishes in favor of $COO^-CHN_3^+$ and a greater pressure of CO_2 is needed to obtain a given concentration of $COO^-CNHCOO^-$.

Now reaction 1 is very rapid even at 0° C., whereas reaction 2 is very slow. It follows then that if the equilibration is allowed to go on for a short time only (*i.e.,* about one minute at 0° C.) the carbamate reaction will be practically complete, while the carbonic acid reaction will be scarcely begun. In effect, there is an equilibrium established which is one involving CO_2, carbamate and amino-acid *but in which no carbonic acid whatever is present* (Case 1). This equilibrium affords a convenient and illuminating laboratory dissection of the reaction but has no counterpart in nature.

On the other hand, if the equilibration is allowed to go on sufficiently long, reaction 2 will be completed and the equilibrium will also include carbonic acid (as well as its ions HCO_3^- and $CO_3^=$) and will be entirely different (Case 2). This *complete* reaction is the one which occurs in the blood and therefore the only one of physiological significance.

Now Meldrum and Roughton's experiments, both on amino-acids and hemoglobin, were especially designed to bring about the first equilibrium only, but the experimental facts so elicited were applied without modification to the second equilibrium state and conclusions drawn therefrom apparently without realization that the two systems were different.

The complete dissimilarity between these two cases can be shown by our own experiments (Case 1). In Fig. 1 is shown the equilibrium curve of carbamino concentration as a function of P_{CO_2} and pH in a 0.1 M glycine solution with 0.05 M of base. The curve calculated on the supposition that *no* carbonic acid or its ions are formed agrees with our (unpublished) experiments on amino-acids and hemoglobin and Meldrum and Roughton's work on hemoglobin, under circumstances eliminating the formation of H_2CO_3. From this curve it is possible to calculate the mass

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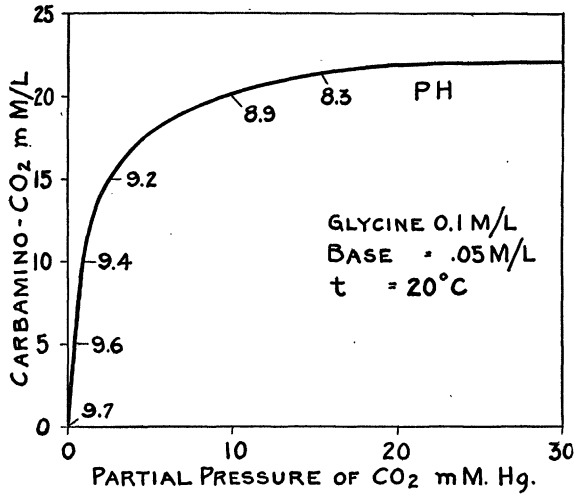

FIG. 1. Case 1. Equilibrium curve of 0.1 M glycine (with 0.05 M of base) and CO_2 showing the concentration of carbamino-CO_2 as a function of the partial pressure of CO_2. Equilibrium is assumed to exclude the formation of H_2CO_3 or its ions. t = 20° C.

action constant of the amino-acid-carbamino-CO_2 equilibrium.

Case 2: In Fig. 2 the curve of carbamino concentration for the same solution has been calculated from

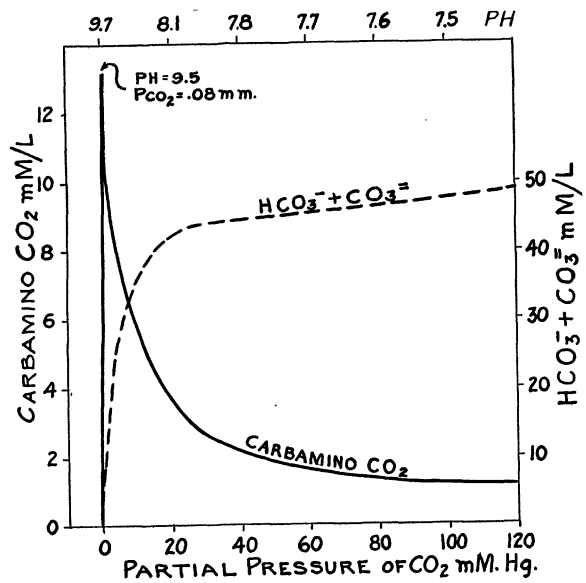

FIG. 2. Case 2. Complete equilibrium of 0.1 M glycine (0.05 M of base) and CO_2 showing (carbamino-CO_2) concentration as a function of P_{CO_2} calculated from the amino-acid carbamino-CO_2 mass action constant. H_2CO_3 and its ions are included.

this constant at 20° C., *but in this case the equilibrium includes H_2CO_3 and its ions.* The two cases, both in complete agreement with our experiments, are easily seen to be totally unlike. Fig. 1 shows that the total carbamino concentration approaches half of the base concentration as a limit at high P_{CO_2} and that at intermediate values of P_{CO_2} the carbamino concentration is high and *increases* appreciably per mm (Hg) change of P_{CO_2}. Whence by analogy one would conclude that carbamino hemoglobin, if it behaved in a similar way, would be an important carrier of CO_2 in the blood.

Fig. 2, however, shows that when total rather than partial equilibrium is considered the maximum of carbamino concentration is reached at P_{CO_2} 0.1 mm Hg and at a very alkaline pH. Moreover, it is only 13 per cent. of the base concentration. At higher P_{CO_2} the curve falls off sharply and at P_{CO_2} 50 mm Hg the carbamino concentration is low and *decreases,* but only by a trifling amount, as the P_{CO_2} is increased. Moreover, it can be easily shown that at pH < 8 the carbamino-CO_2 is only a small part (< 3 per cent.) of the total CO_2.

It is this total equilibrium state which corresponds to that of the blood under physiological conditions. If hemoglobin behaves similarly to amino-acids, the rôle of carbamino-hemoglobin as a carrier of CO_2 appears to be relatively insignificant.

In addition it must be remembered that carbonic anhydrase, a specific enzyme, enormously accelerates reaction 2 as has been shown by Meldrum and Roughton[6] and by Stadie and O'Brien.[7] Thus the discrepancy between the velocities of the two reactions is wiped out and the possibility of the occurrence of an equilibrium of the first type vanishes. This again emphasizes the necessity of considering only equilibrium 2 as being significant in the problem of the CO_2 transport by the blood.

WILLIAM C. STADIE

REFRACTORINESS TO OVARIAN STIMULATION IN THE RHESUS MONKEY

IN a series of publications Cole and Hart[1,2,3] and their collaborators have described the presence, quantity and biological activity of a gonadotropic substance in the blood serum of pregnant mares. Evans, Gustus and Simpson[4] have published a method for the purification and concentration of this gonadotropic substance and have also described its effects on the gonads of male and female rats.

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