ically with a Clarke oxygen electrode (Rank Brothers, England) during magnetic stirring at 37 °C. The period chosen for the measurement of oxygen consumption was the first 4 min following a brief period (30–60 sec). Assay medium contained Krebs-Ringer phosphate buffer without Ca^{2+} (pH 7.4, KRPB), 2 mM KCN, 4×10^7 leukocytes and 10^9 heat-killed *Staphylococcus aureus* in a final volume of 2.12 ml. Lactate was assayed with the Biochemica test combination from Boehringer GmbH, Mannheim (Federal Republic of Germany). PMNs were incubated with bacteria (bacterium to cell ratio of 1:1) at 37 °C in 1 ml KRPB containing 10 µmoles glucose. After the addition of 1.5 ml of ice-cold KRPB in an hour, the incubation mixtures were centrifuged at 4 °C and the supernatant fluids were used for lactate assay.

Results and discussion. Since fluorescamine and dansyl chloride can be dissolved only in an organic solvent, the effect of acetone on the chemotaxis was studied. As seen in table 1, acetone did not affect chemotaxis at the concentration used for the chemical modification. Glycine, used to stop the reaction of modification reagents, produced no effect on chemotaxis even at 10 mM. Among modification reagents, TNBS, which is markedly hydrophilic due to the highly polar sulfonic acid moiety, and consequently may penetrate cells at a much slower rate, produced little effect on the chemotaxis even at 1 mM. On the other hand, fluorescamine and dansyl chloride, a hydrophobic reagent which may penetrate the membrane at a faster rate, strongly inhibited chemotaxis at 100 µM but no detectable changes were observed in viability between unmodified and modified PMNs. Neither glycine adducts nor hydrolysates of fluorescamine and dansyl chloride had any detectable effects on leukocyte motility. These results seem to indicate that the functional groups whose modification appears to be associated with the inhibition of chemotaxis are not located on the outside surface of the plasma membrane but within the membrane or cytoplasm. Next, we examined the inhibitory effect of 2 reagents on directed and random movements. As shown in figure 1, no remarkable alterations in directed and random movements were observed at a low concentration with either reagent. When the concentration was further increased the 2 reagents showed different inhibitory patterns. Namely, with fluorescamine 50% inhibition of chemotaxis was observed at 25 µM, and nearly complete inhibition occurred at 50 µM where modified PMNs were microscopically fairly rounded-up. Random movement was also inhibited almost in parallel with the decrease of the directed migration. On the other hand, dansyl chloride produced 50% inhibition of chemotaxis at 50 µM but did not exhibit any appreciable inhibitory effects on random movement until 75 µM. As can be deduced from the rapidity of the reaction of fluorescamine[6], the inhibition of random and directed movements by fluorescamine was completed within 30 sec (figure 2). As for dansyl chloride, the inhibition of chemotaxis was completed within 2 min, whereas the random movement (contrary to that seen with fluorescamine) was not appreciably inhibited until at least 5 min. The difference in the inhibitory effects shown by 2 reagents would depend on differences in the penetrating rate and specificity of the reagents, i.e. fluorescamine seems to penetrate the cell very rapidly and label nonspecifically functional groups concerned not only with chemotaxis but also with random movement, whereas dansyl chloride does not seem to modify functional groups associated with random movement, at least in a short modification time. We then studied the effect of dansylation on some functions other than motility. As can be seen in table 2, modified PMNs retained the same ingestive ability as unmodified PMNs. There were no remarkable differences found in the initial rate of the stimulated oxygen consumption during phagocytosis between modified and unmodified PMNs, although the rate was inhibited to a certain degree by dansylation. Lactate production, one of the glycolytic parameters, was inhibited to 90–75% of the control by a chemical modification, suggesting that the aerobic glycolytic process is not so much affected as the chemotaxis. Comparison of the degree of inhibition of the above parameters would indicate that dansyl chloride modified preferentially directed movement rather than the basic contractile, phagocytic and glycolytic processes.

As our attractant also had chemokinetic activity, preliminary studies were made of the effect of dansylation on the chemokinetic response. Chemokinesis of neutrophils was inhibited by dansylation in a parallel fashion to the inhibition of directed movement. At this stage, therefore, it seems possible that chemokinetic inhibition is at least one factor involved in the inhibition of directed movement by dansylation. Further detailed studies are in progress using serum albumin and formyl-methionyl peptide.

1 S. Boyden, J. exp. Med. *115*, 453 (1962).
2 P.A. Ward, Am. J. Path. *64*, 521 (1971).
3 T. Yamashita, N. Imaizumi and S. Yuasa, Cryobiology *16*, 112 (1979).
4 T. Yamashita, Y. Tanaka, T. Horigome and E. Fujikawa, Experientia *35*, 1054 (1979).
5 S.H. Zigmond and J.G. Hirsch, Exp. Cell Res. *73*, 383 (1972).
6 S. Udenfriend, S. Stein, P. Böhlen, W. Dairman, W. Leimgruber and M. Weigele, Science *178*, 871 (1972).

Acid-base equilibrium in the blood of sheep

L. Gattinoni[1] and M. Samaja[2]

Laboratory of Technical Development and Clinical Hematology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda (Maryland 20014, USA), 22 November 1978

Summary. The acid-base equilibrium in the blood of sheep is different from that of human blood mainly because of a lower concentration of 2,3-DPG. A nomogram relating pH, pCO_2, total CO_2 content and base excess has been developed.

Recently, we described the oxygen affinity pattern in the blood of sheep[3], which was found to be quite different from that of human blood. Some differences between the 2 species should be expected as regards the acid-base status also, mainly because of different concentrations of 2,3-diphosphoglyceric acid (2,3-DPG), which is known to be lacking in sheep[4]. The aim of this work is to investigate the in vitro relationship between pH, pCO_2, total CO_2 content and base excess (BE) and to provide a tool for computing the acid-base equilibrium in the blood of sheep.

Experimental titration curves for oxygenated sheep blood are shown in figure 1 (solid lines). The zero BE value, as defined by Siggaard-Andersen[5], was arbitrarily set at pH 7.4 and pCO_2 40 mm Hg (the temperature is always 37 °C) in order to facilitate the comparison with human blood (dashed lines), although the normal pH and pCO_2 values are given at 7.44 and 45 mm Hg, respectively[6], for a healthy adult sheep. As regards the acid pH range, the curves of human blood are significantly lower than those of sheep blood at corresponding pCO_2. It can be seen that sheep blood has a lower buffering power (i.e. the derivative d(BE)/d(pH) vs. pH (not shown) as defined by Van Slyke and Sendroy[7]) than does human blood in the pH range below 7.3. This can be attributed to the low level, in sheep, of 2,3-DPG, which has a pK of about 7.1[5] and therefore affects very much the buffering power in the low pH range in human blood. The state of oxygenation of hemoglobin does not seem to affect the buffering power, since no significant difference in the derivative was found between oxygenated and deoxygenated blood (not shown).

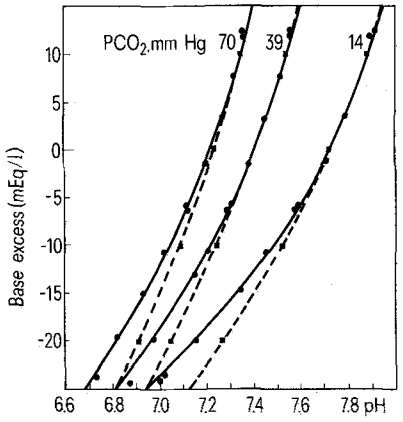

Fig. 1. Solid lines are the titration curves of heparinized blood from 4 healthy adult Dorset sheep. 1 ml of blood was equilibrated at 37 °C with selected gases at 5 different CO_2 tensions (namely, 70, 49, 39, 28, and 14 mm Hg; 3 only shown), balance O_2, or N_2 (not shown), in an IL 237 tonometer (Instrumentation Lab., Lexington, Mass.), and the pH measured in an IL 213 pHmeter (Instrumentation Lab.). A range of pH was achieved by adding to the blood various amounts of acid (0.2 N HCl) or base (0.2 N NaOH) in the constant volume of 0.1 ml (balance with normal saline solution) in order to keep a constant hematocrit. Dashed lines are the titration curves of oxygenated human blood calculated from the data of Siggaard-Andersen[5] at the sample pCO_2.

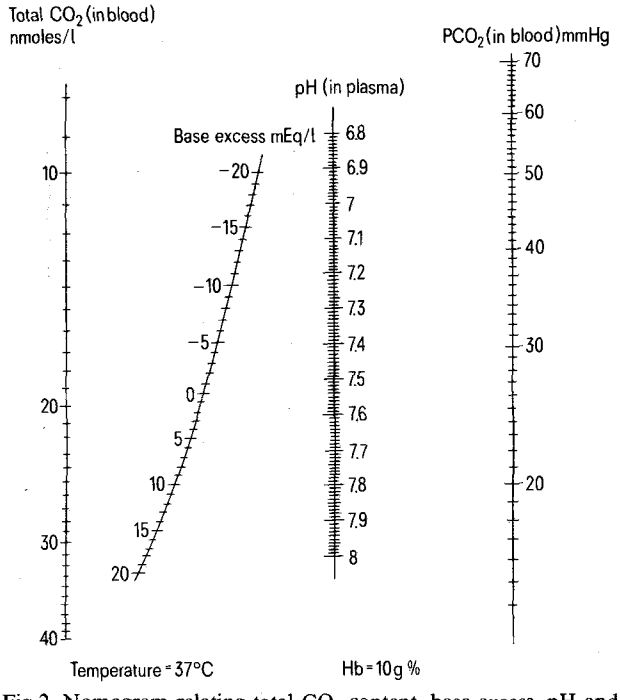

Fig. 2. Nomogram relating total CO_2 content, base excess, pH and pCO_2 in the whole blood of sheep. It is valid for any hemoglobin species and concentration within the physiological range. Total CO_2 content, on the other hand, is valid only when the hemoglobin concentrations is 10 g/dl.

At fixed pH, BE vs. pCO_2 forms a series of straight lines:

$$BE = A + B \times pCO_2. \quad (1)$$

A and B could be thought to be parabolic functions of pH, so that:

$$A = -13.09 \times (pH-7)^2 + 21.02 \times (pH-7) - 26.52 \quad (2)$$

$$B = 1.24 \times (pH-7)^2 + 0.36(pH-7) + 0.18. \quad (3)$$

BE can therefore be computed at any given pH in the range 6.8–7.6 and pCO_2 in the range 14–70 mm Hg by substituting equations 2 and 3 in equation 1. The values of the parameters were estimated by the least square rule and the root mean square error in calculating BE by such a procedure is 0.67 BE units, when expressed in meq/l. Part of figure 2 is a graphical representation of the previous functions, valid at 37 °C and any hemoglobin concentration.

In another set of experiments, total CO_2 concentrations were determined at 5 pCO_2 and varying pH, on both oxygenated and deoxygenated blood, by the same way described in the legend of figure 1, except that the volumes of blood and of the balancing solutions were 5 ml and 0.5 ml, respectively. A standard Van Slyke procedure[8] was used to calculate the total concentration of CO_2. At fixed pH, total CO_2 content in whole blood, at a hemoglobin concentration of 10 g/dl and 37 °C was found to be a linear function of pCO_2:

$$\text{total } CO_2 = B \times pCO_2. \quad (4)$$

This equation can be combined with the following one:

$$\log(\text{total } CO_2/pCO_2) = 0.91 \times pH - 6.99 \quad (5)$$

to give a relationship connecting three parameters:

$$\text{total } CO_2 = pCO_2 \times 10^{(0.91 \times pH - 6.99)}. \quad (6)$$

The root mean square error is 0.49 total CO_2 units, when expressed in mmoles/l. Equation 6 is also represented in the left part of figure 2, but it must be recalled that it refers to blood with a hemoglobin concentration of 10 g/dl.

1 Present address: Institute of Anesthesiology, University of Milan, via F. Sforza 35, I-20122 Milano (Italy).
2 Present address: Cattedra of Enzymology, University of Milan, via Celoria 2, I-20133 Milano (Italy).
3 M. Samaja and L. Gattinoni, Resp. Physiol. *34*, 385 (1978).
4 M.H. Blunt and J.H.J. Huisman, in: The Blood of Sheep, p. 155. Ed. M.H. Blunt. Springer, New York 1975.
5 O. Siggaard-Andersen, in: The Acid-Base Status of the Blood, 4th ed. Munksgaard, Copenhagen 1974.
6 S. Lahiri. Am. J. Physiol. *229*, 529 (1975).
7 D. Van Slyke and J. Sendroy. J. biol. Chem. *29*, 781 (1928).
8 D. Van Slyke and J. Plazin, in: Micromanometric Analyses. The Williams and Wilkins Co., Baltimore, Md. 1961.