from 30 to 100% of the control, probably in dependence on the metabolic state of the preparation. Even larger effect is regularly observed after wash-out and repeated application of epinephrine. It is to be noticed that the contractility reappears after epinephrine in Ca-free Tyrode. Time to peak tension is significantly shorter than the plateau phase. This dissociation is particularly marked in cats. It is assumed that the mechanical response originates from additional liberation of Ca from the reticular stores. Comparable effects were obtained after the administration of norepinephrine and isoproterenol in the same dose. The reaction to all 3 catecholamines was entirely prevented by β-adrenolytic agent propranolol (Inderal, concentration 10× exceeding that of epinephrine).

If the missing Ca is added to the solution at the period of fully developed effect of epinephrine, immediately a second contraction appears during the terminal phase of AP (not shown in the Figure), then the initial mechanical response rapidly increases and subsequently the AP shortens to the vicinity of the control values.

From these experiments it can be deduced that the reported absence of influence of epinephrine on AP duration might result from its 2 opposing effects: the first tends to prolong the time of repolarization; the second is indirect and appears to be related to increased intracellular concentration of Ca. At normal Ca concentration and medium rates of driving, both effects are in an approximate equilibrium and counterbalance each other.

Zusammenfassung. Im Gegensatz zur Kontrollbedingung (Tyrodelösung 1.8 m*M* Ca, 31°C, Reizfrequenz 30/min) ruft Adrenalin $6 \times 10^{-6}M$ im Ca-freien Milieu eine significante Verlängerung der Aktionspotenziale hervor. Die Zugabe von Ca^{2+} kehrt die Aktionspotenzialdauer mit voller Entwicklung der positiv inotropen Wirkung zu Ausgangswerten zurück.

P. Bravený, M. Šimurdová and J. Šumbera[6]

II. Department of Medicine, Medical Faculty, Research Institute of Medical Engineering and Department of Physiology, Medical Faculty, Brno (Czechoslovakia), 2 May 1973.

[6] Authors' addresses: P. B. II. Dept. of Medecine, Medical Faculty Brno; M. Š. Reserach Institute of Medical Engineering, Brno; J. Š. Dept. of Physiology, Medical Faculty Brno.

Respiratory Properties of the Blood of the Thornback Ray

Oxygen dissociation properties of whole blood of several elasmobranchs have been investigated, but few recent studies have been concerned with skates or rays. Arterial blood samples can be obtained via indwelling catheters without disturbing the resting fish. The average pH of arterial blood in vivo is 7.7 at 15°C. P_{O_2} is low (about 58 mm Hg) but higher values were obtained during hyperventilation, at low temperatures, and from anaemic fish.

Dissociation curves (Figure 1) were determined using a mixing method. Because of progressive changes in the P_{O_2} of such mixtures, each sample was used for no more than two points on the curves. The oxygen capacity (3.5 ml/100 ml blood, l.c. = 0.9) is relatively low. The sigmoid dissociation curve (Hill exponent n = 2.5, l.c. = 0.3) has a P_{50} of 30.2 mm Hg (l.c. = 2.8) at the physiological pH of 7.7 at 15°C. As in *Raia oscillata*[1] a definite Bohr shift (Δ log P_{50}/Δ pH = -0.25 ± 0.03) is found (Figure 1), but it is less than that of most fishes and of human blood. There is also evidence for a Haldane effect (Figure 2) which contrasts with results obtained on dogfish blood[2], but is consistent with the presence of a Bohr effect which theoretically requires a Haldane effect of similar magnitude[3].

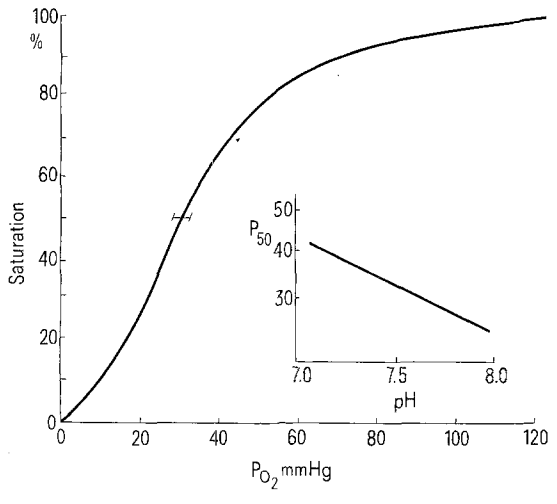

Fig. 1. *Raia clavata* O_2 dissociation curve of whole blood at pH 7·7, 15°C (average of 15 individuals). Horizontal bar indicates 95% confidence limits of P_{50}. Insert shows the Bohr effect.

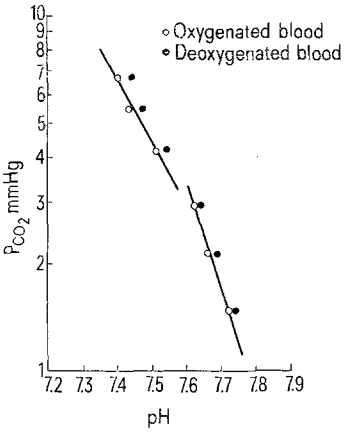

Fig. 2. *R. clavata* Log P_{CO_2}-pH diagram of whole blood. Each point represents a mean of 4–6 measurements. Fresh blood used for equilibration at each P_{CO_2}. Bicarbonate concentration calculated from $pK^- = 6.08$ at pH 6.75 and 15°C, $\alpha = 0.053$[5], was about 3.4 m*M*/l at pH 7.7. Buffer capacity (Δ HCO_3^-/l/Δ pH) was approximately 10 slykes between pH 7.4 and 7.7.

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P_{50}'s of about 30 specimens of different body weights (45 g–7.5 kg) were compared and there was some suggestion of a higher affinity among smaller specimens, but there was much variability. The presence of such a differential was tested because of indications that 'embryonic' haemoglobins have a higher oxygen affinity within the egg cases of skates[4] and other elasmobranchs.

It is concluded that the data reported here reflect conditions in the intact fish more closely than in previous studies because determinations were carried out within half an hour of blood having been withdrawn from catheters that had remained within the fish for several days. There are indications that changes in the respiratory properties of blood also occur in blood samples taken from other fish.

Résumé. Quelques propriétés respiratoires du sang de la raie (*Raia clavata*) ont été déterminées avec des échantillons de cathéter intravasculaire. Chaque échantillon était utilisé seulement pour deux points de la courbe de dissociation $-O_2-$, parce que les propriétés changent après une demie-heure. On a constaté un effet de Bohr (Δ log P_{50}/Δ pH $= -0.25 \pm 0.03$) ainsi que celui d'Haldane.

G. M. HUGHES and S. C. WOOD[6]

The Laboratory of the Marine Biological Association, Plymouth; and Research Unit for Comparative Animal Respiration, The University, Bristol BS8 1UG (England), 30 July 1973.

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A Computer Program for the Calculation of the Free Ligand Concentrations in Cases of Multiple Binding to Proteins

In many experimental situations in vitro or in vivo, a regulatory active compound, such as a drug or a hormone, is bound with considerable affinities by proteins which are not directly involved in the regulatory action exerted by the compound. Such unspecific binding diminishes the free active concentration of the compound and influences its metabolism as well as its regulatory functions. Therefore the half-maximal-effective dose of the compound may be overestimated, and also misinterpretations concerning the mode of action of the compound are possible when the dose response curve is not corrected for such secondary binding[1].

Many laboratories have directed their efforts towards the determination of the binding capacities and affinities of various plasma or tissue proteins for drugs and hormones[2,3,4]. The knowledge of these binding parameters permits the calculation of the active concentrations of these substances[1]. In the case of multiple classes of binding sites for one and the same compound, such calculations proved to be quite tedious. Therefore computer programs for a single[5] and two classes of binding sites[6], which were based on the explicit solution of a binding equation, have been published. The generalization for several binding sites was suggested to me by Dr. P. BALLY. In the present report a computer program which solves a binding equation with an implicit method for any number of classes of binding sites is described.

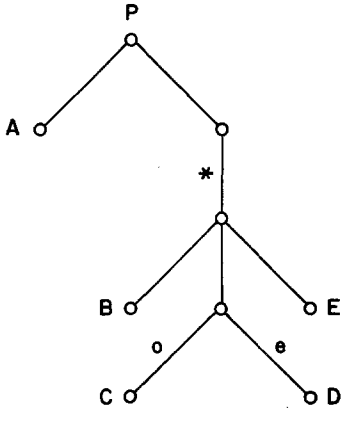

Fig. 1. Set graph of the procedure used for the implicit solution of the binding equation. – The nodes in the set graph designate sets of executable operations. For the meaning of the letters on the nodes and the symbols on the branches see text.

Algorithm and Computer program. The binding of a ligand H to multiple classes of binding sites can be described by the following binding equation:

$$H_t = H_f + \sum_{i=1}^{n} \frac{C_i H_f}{A_i + H_f}$$

where H_t designates the total concentration of the ligand, H_f the concentration of the free unbound ligand, C_i and A_i the binding capacities and affinities (expressed as dissociation constants), respectively of the different classes of binding sites and n the number of classes of binding sites.

The logic structure of the algorithm used for the implicit solution of this binding equation is depicted in the set graph in Figure 1. The algorithm starts with an opening set of operations (A) which initialize the procedure (P) by calculating a tolerated absolute error of the solution of the binding equation from a specified relative error and by assuming an initial value for H_f. This is done by setting H_f equal to the upper limit H_t. The following set of operations (B–E) is executed repeatedly as indicated by the starred symbol in the set graph until either the actual error of the solution is smaller or equal to the tolerated absolute error or the actual number of iterative cycles exceeds a specified upper limit. The binding equation is written in the form:

$$H_t - H_f - \sum_{i=1}^{n} \frac{C_i H_f}{A_i + H_f} = \text{error}$$

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