membrane proteins, their subsequent chemical characterization and the study of their interactions with lipids, as well as of their structural and morphological properties will provide the basis on which to build useful models of various membrane types.

Zusammenfassung. Neuere Resultate physikochemischer und chemischer Untersuchungen an Erythrozytenmembranen und Stromaproteinen, welche zum Teil im Widerspruch zur «unit-membrane»-Theorie stehen, werden diskutiert. Die Folgerungen aus diesen Ergebnissen werden anhand von vier kürzliche vorgeschlagenen Strukturmodellen dargelegt, wobei folgende gemeinsame Gesichtspunkte wesentlich erscheinen: 1. mosaikartige Untereinheitenstruktur biologischer Membranen; 2. Membranproteine als wesentliche Träger und Organisatoren der Membranstruktur; 3. völliges Durchdringen der Proteine durch die Membran; 4. hoher α-Helix-Gehalt der Membranproteine; 5. hohe Anteile hydrophober Wechselwirkungen zwischen Proteinen und Lipoiden.

Oxygen Exchange in the Erythrocyte

By G. THEWS

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Ever since 1927, when HARTRIDGE and ROUGHTON[1] experimentally determined the oxygen uptake rate by red cells for the first time, the erythrocyte O_2 exchange has been one of the most frequently examined biological transport processes. There are two main reasons for the great interest in this very specialized subject: (1) The O_2 uptake or delivery by the erythrocyte is a decisive factor in the transport chain conducting oxygen from the surrounding air into the cell. Under certain circumstances, this factor can limit the oxygen supply of the organs and tissues, and hence determine their functional capacity. (2) The erythrocyte is a particularly suitable object to study in connection with transport kinetics, because it enables very distinct experimental conditions on isolated cells to be produced.

The O_2 exchange on the erythrocyte is a diffusion process combined with a chemical reaction. Today, there is no longer any doubt that the O_2 molecules are exchanged passively following the O_2 partial pressure gradient, that is, according to laws of diffusion. This process, however, is complicated by the subsequent O_2 association with the hemoglobin or the O_2 dissociation from this chemical binding. Although the time required by reaction is short, it can, nevertheless, still influence the diffusing times.

In order to achieve an understanding of the whole process, we have 2 ways of approach: (1) mathematical analysis, and (2) experimental examination. Both yielded a number of separate results, which, although not giving a complete picture of the O_2 exchange processes, still present a picture that is basically clear.

(1) *Mathematical analysis of the O_2 exchange*

(a) *Differential equation of diffusion.* The simple process of diffusion is phenomenologically represented by the known partial differential equation

$$\frac{\partial[O_2]}{\partial t} = D \cdot \nabla^2[O_2] \qquad (1)$$

in which $[O_2]$ stands for the O_2 concentration, ∇^2 the Laplace operator, and D the diffusion coefficient. The O_2 diffusion coefficient for the inside of the erythrocyte has been determined serveral times (KLUG, KREUZER and ROUGHTON[2]; GROTE and THEWS[3]), and can be stated as approximately $D = 8 \times 10^{-6}$ cm²/sec. In some cases, particularly when representing the steady state, it is better to use the so-called Krogh's coefficient of diffusion, K, instead of D. K is connected with D by Bunsen's solubility coefficient α ($K = \alpha \cdot D$). Because of its physico-chemical basis, it has also been termed diffusion conductivity (compare THEWS[4]). The O_2 conductivity, K, for the erythrocyte is about 1×10^{-5} ml/cm per min/atm.

The partial differential equation of diffusion admittedly gives the general mathematical relationships for the process in question. However, data on the partial pressure distribution in the diffusing space, and its time change, can only be obtained by integrating it, taking into consideration the boundary and initial conditions. However, the introduction of boundary and initial conditions gives rise to the greatest of difficulties, especially in the case of biological problems (compare

[1] H. HARTRIDGE and F. J. W. ROUGHTON, J. Physiol. (Lond.) *62*, 232 (1927).

[2] A. KLUG, F. KREUZER and F. J. W. ROUGHTON, Helv. physiol. pharmacol. Acta *14*, 121 (1956).

[3] J. GROTE and G. THEWS, Pflügers Arch. ges. Physiol. *276*, 142 (1962).

[4] G. THEWS, Ergebn. Physiol. *53*, 42 (1963).

HILL[5]; JACOBS[6]; ROUGHTON[7]; THEWS[8,4]; CRANK[9]). The characteristic form of the human erythrocyte, for example, impedes a solution of the differential equation, by one of the classical methods, in closed form. Therefore, for purposes of an economical mathematical solution, the erythrocyte was replaced, in most cases, by a plane diffusion layer, the diffusion from the narrow sides being neglected. In this way, the problem is reduced to the simplest case of unidimensional diffusion. A closer assimilation to the actual conditions can be attained if the erythrocyte is replaced by a cuboid of the same surface and volume as the erythrocyte (see Figure 1). By taking a cuboid like this with constant boundary conditions, as a basis, it is possible to integrate the differential equation in closed form (THEWS and NIESEL[10]).

An even closer adaption, particularly if the chemical reaction is taken into account, is only possible if computer methods are used. Both electronic analog and digital computing methods are suitable for solving the O_2 diffusion problems on erythrocytes (compare NIESEL and THEWS[11]; MOLL[12]).

(b) *Molecular interpretation of the O_2 dissociation curve.* The hemoglobin in the erythrocyte considerably retards the simple diffusion process hitherto examined, first, because of the greater quantity of oxygen to be exchanged, and, furthermore, because of the time needed by the reaction. Under certain circumstances, though, an accelerating factor must also be considered. Providing there is a gradient for it in the inside of the erythrocyte, oxyhemoglobin could itself diffuse, and, in this way, transport considerable quantities of O_2.

To be able to take the kinetics of the hemoglobin-oxygen reaction in the transport equation into account, it is first necessary to get an idea of how the reaction takes place. This question is closely connected with the interpretation of the O_2 dissociation curve. The characteristic *S*-shape (see Figure 2), can by no means be explained by a simple second order reaction, between hemoglobin and O_2. Apart from a few set-ups which are only of historical importance today, it is mainly ADAIR'S[13] intermediate compound hypothesis that has been referred to for an explanation of the O_2 dissociation curve. The tetramer structure suggests that the oxygen combines with the hemoglobin in 4 stages:

$$\begin{aligned}
&Hb_4 + O_2 \underset{k_1}{\overset{k'_1}{\rightleftharpoons}} Hb_4O_2 && \frac{k'_1}{k_1} = K_1\\
&Hb_4O_2 + O_2 \underset{k_2}{\overset{k'_2}{\rightleftharpoons}} Hb_4O_4 && \frac{k'_2}{k_2} = K_2\\
&Hb_4O_4 + O_2 \underset{k_3}{\overset{k'_3}{\rightleftharpoons}} Hb_4O_6 && \frac{k'_3}{k_3} = K_3\\
&Hb_4O_6 + O_2 \underset{k_4}{\overset{k'_4}{\rightleftharpoons}} Hb_4O_8 && \frac{k'_4}{k_4} = K_4
\end{aligned} \qquad (2)$$

This is conditional upon the reaction at each stage being influenced by the foregoing O_2 associations, through a heme-heme interaction. The reaction constants, k'_n and k_n, then should have different values, like the equilibrium constants, K_n.

The intermediate compound hypothesis, which is widely accepted today, cannot explain though the dependence of the O_2 dissociation curve on the hemoglobin concentration. This shortcoming, together with a number of other factors, led to a completely new conception of the way in which the hemoglobin-oxygen reaction takes place (BARNIKOL and THEWS[14,15]). After

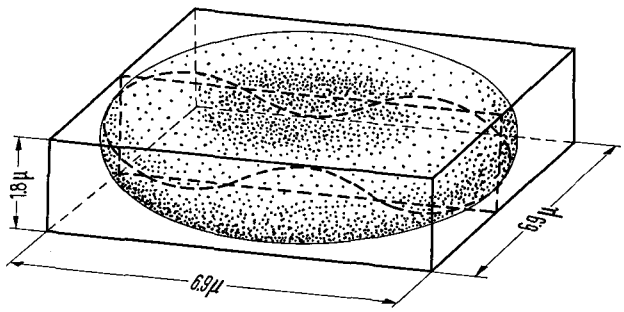

Fig. 1. Human erythrocyte with substitute cuboid drawn in, for which integration of the differential equation in closed form is possible (THEWS[4]).

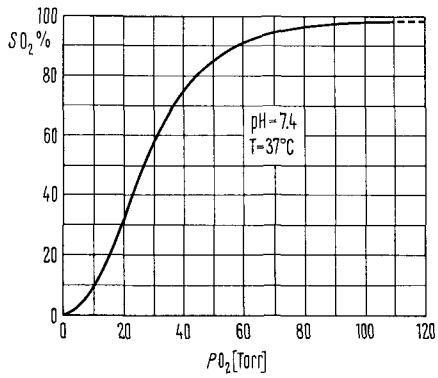

Fig. 2. O_2 dissociation curve of the human hemoglobin (pH 7.4, temperature 37 °C).

[5] A. V. HILL, Proc. r. Soc. B *104*, 39 (1928/29).
[6] M. H. JACOBS, Ergebn. Biol. *12*, 1 (1935).
[7] F. J. W. ROUGHTON, Proc. r. Soc. B *140*, 203 (1952).
[8] G. THEWS, Acta biotheor. *10*, 105 (1953).
[9] J. CRANK, *The Mathematics of Diffusion* (Clarendon Press, Oxford 1957).
[10] G. THEWS and W. NIESEL, Pflügers Arch. ges. Physiol. *268*, 318 (1959).
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[12] W. MOLL, Respir. Physiol. *6*, 1 (1968/69).
[13] G. S. ADAIR, J. biol. Chem. *63*, 529 (1925).
[14] W. K. R. BARNIKOL and G. THEWS, Pflügers Arch. ges. Physiol., im Druck.
[15] W. K. R. BARNIKOL and G. THEWS, Pflügers Arch. ges. Physiol., im Druck.

this hypothesis the tetramer hemoglobin dissociates symmetrically into its dimer and monomer subunits (see Figure 3). Each of these subunits has a specific O_2 affinity, which is the same for every stage of their oxygenation. An essential aspect of this hypothesis, moreover, is the effect of a low molecule intermediary substance, Z, which stabilizes the tetramer. The parameter values obtained from the numerical calculations allow the Z substance to be identified with Ca^{++} and/or Mg^{++}. By taking into consideration the complex-forming tendency of these bivalent ions, the other known properties of the O_2 dissociation curve can easily be qualitatively explained. Because of the great importance of this complex-forming property, the model is called a coordination hypothesis.

(c) *Diffusion combined with chemical reaction.* In order to describe the whole of the oxygen exchange process in the erythrocyte, it is necessary to combine the diffusion equation with the reaction equations, resulting from the intermediate compound hypothesis or the coordination hypothesis. The solution of a system of equations like this, necessitates unduly large mathematical calculations. Therefore, an attempt was made to reduce them by simplifying the differential equations. This was done mainly in 2 ways.

One way was to reduce the differential equation to the following form:

$$\frac{\partial[O_2]}{\partial t} = D \cdot \nabla^2[O_2] - k'[O_2][Hb] + k[HbO_2]. \quad (3)$$

The hemoglobin-oxygen association, then, is taken into account as a simple second order reaction. The apparent reaction constants k' and k, can then be regarded only to a limited extent as real constants. This was how ROUGHTON[16]; NICOLSON and ROUGHTON[17]; MOCHIZUKI and FUKUOKA[18] and FORSTER[19] solved the problem for various boundary and initial conditions. Recently, MOLL[12] also took the hemoglobin diffusion into consideration.

Another way of obtaining an approximate solution, was suggested by THEWS[4]. For the physiologically relevant case of O_2 uptake in the lung capillary, it may be shown that, during the diffusion process, a linear relationship exists between the O_2 pressure and the O_2 saturation of the hemoglobin. The reaction times retard the saturation process in such a way that the bent part of the O_2 dissociation curve (see Figure 2), becomes a straight line in effect. In this case, the problem can be attributed to the simple diffusion equation:

$$\frac{\partial[O_2]}{\partial t} = \frac{K}{\alpha'} \cdot \nabla^2[O_2]. \quad (4)$$

The solubility coefficient, α, in equation (1) ($D = K/\alpha$), must simply be replaced by the coefficient of 'apparent solubility', α', which consists of physical solubility and chemical binding quality. After this, the increase of the O_2 pressures in the erythrocyte, after a sudden rise in the outside O_2 partial pressure, can be calculated relatively easily. Moreover, by using an electronic analog computer method, the retarding effect of a plasma layer and the alveolar-capillary membrane in the lung, can be taken into account (see THEWS[4]).

(2) *Experimental examination of the O_2 exchange*

(a) *Methods.* Parallel to the mathematical methods, a series of experimental arrangements was developed, suitable for following the relatively rapid O_2 exchange processes in the erythrocyte. The rapid reaction and diffusion process field, as a whole, was opened up by HARTRIDGE and ROUGHTON[20], with the development of the rapid flow method. By means of this process, 2 reaction participants, e.g. erythrocyte suspensions and buffer solutions containing oxygen, are mixed in a mixing chamber, and then led at high velocity through an observation tube. Distinct times after mixing are assigned to each part of the observation tube. The time course of the O_2 saturation change in the hemoglobin, which is accompanied by an extinction change, can be followed photometrically at various points on the observation tube. FORSTER et al.[21] used a modified method to examine the O_2 uptake of the red cells.

The stopped flow method provides another opportunity for studying the exchange processes (CHANCE[22,23];

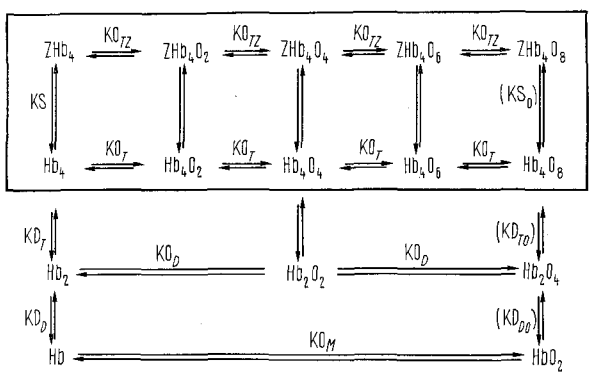

Fig. 3. Simplified molecular scheme of the hemoglobin in reaction with oxygen, after the coordination hypothesis (BARNIKOL and THEWS[15]).

[16] F. J. W. ROUGHTON, Proc. r. Soc. Edinb. B *111*, 1 (1932).
[17] P. NICOLSON and F. J. W. ROUGHTON, Proc. r. Soc. B *138*, 241 (1951).
[18] M. MOCHIZUKI and J. FUKUOKA, Jap. J. Physiol. *8*, 206 (1958).
[19] R. E. FORSTER, in *Handbook of Physiology (Respiration)* (Ed. W. O. FENN and H. RAHN; Amer. Physiol. Soc., Washington 1964), vol. I, Sect. 3, p. 827.
[20] H. HARTRIDGE and F. J. W. ROUGHTON, Proc. r. Soc. A *104*, 376 (1923).
[21] R. E. FORSTER, F. J. W. ROUGHTON, F. KREUZER and W. A. BRISCOE, J. appl. Physiol. *11*, 260 (1957).
[22] B. CHANCE, Rev. scient. Instrum. *22*, 619 (1951).
[23] B. CHANCE, Science *120*, 767 (1954).

GIBSON[24]). The solutions are mixed at high velocity here, as well, but photometric observation does not begin till the turbulence has stopped. The method of NIESEL, THEWS and LÜBBERS[25], may be regarded as a further development of the stopped flow method. An equilibrated erythrocyte suspension is injected into a solution, equilibrated with another O_2 partial pressure. The resultant diffusion-dependent and reaction-dependent O_2 saturation change in the hemoglobin, is followed by a rapid-measuring spectroanalyser, which records 100 complete absorption spectra per second.

Finally, we must mention the lamella method, THEWS[26] (see Figure 4). By utilizing the surface tension of the blood, blood lamellas, so thin that the erythrocytes lie side by side in a single layer, can be stretched in a metal ring. The monoerythrocyte blood lamellas are placed in the beam of a photometer, and exposed to a sudden change in the O_2 and CO_2 partial pressures. The O_2 saturation change is registered on a scope screen. This method is especially suitable for examining the exchange processes under physiological conditions, because the erythrocytes are surrounded by a plasma layer, just as they are in the capillary.

(b) *Results.* In view of the profusion of experimental examinations that have been made on the O_2 exchange in the erythrocyte, using these methods, it is absolutely impossible, here, to even give a summary of the results. Therefore, it seems more appropriate to single out those results that: (1) indicate the time required by the exchange processes, and, (2) show the relatively good agreement between data obtained experimentally, and calculated data. In Figure 5, three measured values (crosses), for the time course of the O_2 saturation increase in the erythrocyte, after an external O_2 partial pressure change, are shown (GIBSON et al.[27]). In an erythrocyte suspension, which was initially equilibrated at a hemoglobin O_2 saturation of 35%, the O_2 partial pressure outside the erythrocyte was suddenly in-

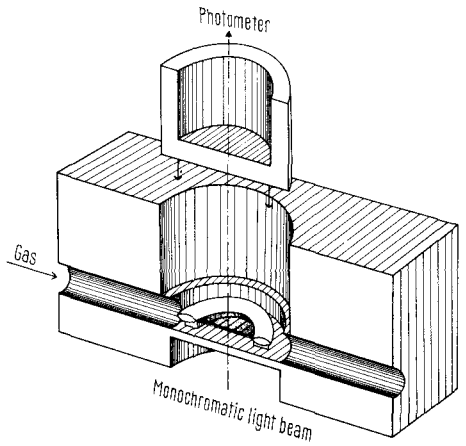

Fig. 4. Section of the measuring chamber used for examining the O_2 exchange on blood lamellas, after SCHULTEHINRICHS, VOGEL and THEWS[29].

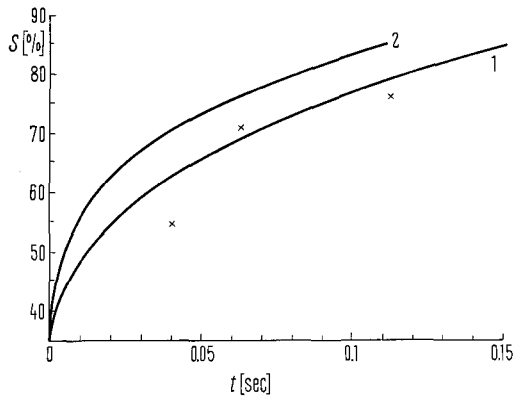

Fig. 5. Time course for the hemoglobin O_2 saturation increase in an erythrocyte suspension, after a sudden rise in the extracellular O_2 partial pressure to 85 mm Hg. Crosses, measured values; curves 1 and 2, calculated values.

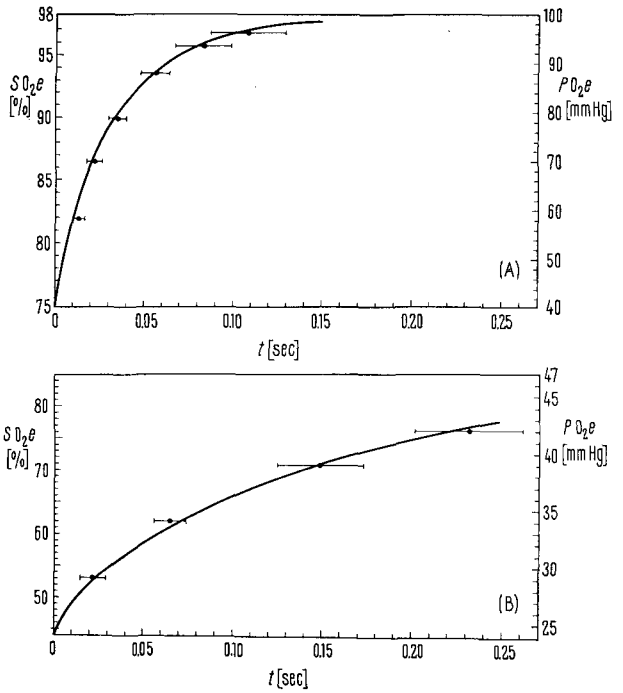

Fig. 6. Time course for the hemoglobin O_2 saturation increase in monoerythrocyte blood lamellas, when the O_2 and CO_2 partial pressures are suddenly changed. (A) P_{O_2} = 40 mm Hg, P_{CO_2} = 47 mm H → P_{O_2} = 100 mm Hg, P_{CO_2} = 40 mm Hg. (B) P_{O_2} = 24 mm Hg, P_{CO_2} = 40 mm Hg → P_{O_2} = 47 mm Hg, P_{CO_2} = 35 mm Hg. Dots, measured values; curves, calculated values.

[24] Q. H. GIBSON, Discuss. Faraday Soc. *17*, 137 (1954).
[25] W. NIESEL, G. THEWS and D. LÜBBERS, Pflügers Arch. ges. Physiol. *268*, 296 (1959).
[26] G. THEWS, Pflügers Arch. ges. Physiol. *268*, 308 (1959).
[27] Q. H. GIBSON, F. KREUZER, E. MEDA and F. J. W. ROUGHTON, J. Physiol. (Lond.) *129*, 65 (1955).
[28] W.-E. FRECH, D. SCHULTEHINRICHS, H. R. VOGEL and G. THEWS, Pflügers Arch. ges. Physiol. *301*, 292 (1968).
[29] D. SCHULTEHINRICHS, H. R. VOGEL and G. THEWS, Pflügers Arch. ges. Physiol. *301*, 302 (1968).

creased to 85 mm Hg (pH 7.1–7.4; temperature 37 °C). The measured values are compared with the saturation values calculated after THEWS[4], curve 1 standing for a plane, unlimited hemoglobin layer 1.8 μ thick, and curve 2, for a cuboid that replaces the erythrocyte (see Figure 1).

Another example is shown in Figure 6 (A and B). This concerns the hemoglobin O_2 saturation increase in monoerythrocyte blood lamellas, when the external O_2 and CO_2 partial pressures are suddenly changed. The dots, with their standard deviations, stand for the mean values obtained from 25 and 29 measurements, respectively (FRECH et al.[28]). Again the curves are calculated after THEWS[4]. In A, a change was made from a 'venous' to an 'arterial' gas mixture (P_{O_2} = 40 mm Hg, P_{CO_2} = 47 mm Hg → P_{O_2} = 100 mm Hg, P_{CO_2} = 40 mm Hg), and, in B, there is a corresponding change in the hypoxia range (P_{O_2} = 24 mm Hg, P_{CO_2} = 40 mm Hg → P_{O_2} = 47 mm Hg, P_{CO_2} = 35 mm Hg). Examinations of this kind indicate the physiological saturation processes taking place during the passage of the erythrocyte through the lung capillary. In this case, though, the retarding effect of the alveolar capillary membrane, which protracts the time by approximately the submultiple 1.3, must also be taken into account. According to the results shown in Figure 6, it is to be expected that the alveolar contact time of the erythrocyte, with the gas phase in the human lung, is about 0.2–0.3 sec.

Zusammenfassung. Es wird eine Übersicht über die mathematischen und experimentellen Aspekte des O_2-Austausches im Erythrozyten gegeben. Der Gesamtprozess kann als eine Sauerstoffdiffusion mit gekoppelter Hämoglobin-Sauerstoff-Reaktion beschrieben werden. Für die mathematische Analyse muss also die partielle Differentialgleichung der Diffusion durch ein Gleichungssystem erweitert werden, das dem chemischen Reaktionsablauf Rechnung trägt. Voraussetzung hierfür ist die Aufstellung eines Modells zur molekularen Interpretation der O_2-Bindungskurve. Die vereinfachten Differentialgleichungen für Diffusion und Reaktion lassen sich unter Beachtung der Rand- und Anfangsbedingungen nur näherungsweise integrieren.

Es werden ferner die experimentellen Methoden beschrieben, die für die Verfolgung der schnellen O_2-Austauschvorgänge im Erythrozyten geeignet sind. An einigen Beispielen werden die mit diesen Methoden gewonnenen Untersuchungsergebnisse erläutert. Es zeigt sich, dass die mathematische Analyse und die experimentellen Untersuchungen zu übereinstimmenden Ergebnissen führen.

Chemical Modifiers of Passive Ion Permeability of the Erythrocyte Membrane

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In current permeability research the use of chemical modifiers of cell membranes is part of the experimental routine, as is the application of enzyme inhibitors in the study of metabolic sequences and enzyme kinetics. Enzyme inhibitors are widely used in attempts to elucidate the nature of the enzymatic reaction sequence which accomplishes active transport. The inhibition of the K-Na-sensitive membrane ATPase by ouabain (SKOU[1]) is an indispensable tool for the identification of the transport enzyme in tissues and cells. The application of N-ethyl-maleimide facilitated the separation of the ATPase reaction into two distinct steps: A sodium activated P exchange between ATP and ADP and a potassium activated hydrolysis of some product of the reaction between ATP and membrane (FAHN et al.[2]). Finally, the effects of ethacrynic acid stimulated discussions about the possible occurrence of a specific sodium transport mechanism in addition to the K-Na-membrane ATPase (HOFFMAN and KREGENOW[3], WHITTEMBURY[4]).

Chemical modifiers of passive permeability have been employed even more extensively than inhibitors of active transport (cf. PASSOW[5]). It is the aim of the present paper to discuss a number of representative results obtained with a few selected types of modifiers of passive permeability and thus to demonstrate some of the special problems encountered in the interpretation of their actions. Obviously, such a discussion requires frequent reference to existing concepts of the molecular mechanisms which supposedly control passive permeability. Therefore, before focussing on the

[1] J. C. SKOU, Biochim. biophys. Acta *23*, 394 (1957).
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[3] J. F. HOFFMAN and F. M. KREGENOW, Ann. N.Y. Acad. Sci. *137*, 566 (1966).
[4] G. WHITTEMBURY, J. gen. Physiol. *51*, 303s (1968).
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