5. Compound E from alcohol eluant, needles, mp 102–104° (alcohol), Rf 0, insoluble in pet. ether, no colour with Liebermann-Burchard reagent. Found[8]: C, 78.92, 79.01; H, 13.69, 13.81. Calcd. for $C_{26}H_{54}O_2$: C, 78.32; H, 13.65; $C_{28}H_{58}O_2$: C, 78.80; H, 13.70. NMR-spectrum exhibited a strong methylene proton peak at 1.3 δ and a small peak at 1.55 δ. IR-spectrum showed the presence of 2 types of OH (3440 and 3200 cm^{-1}) and a $-(CH_2)_n$-group (722 cm^{-1} due to CH_2 rocking) besides other prominent peaks at 880, 1085, 1110, 1140, 1335 and a hump at 1550–1700 cm^{-1} in Nujol. Mass-spectrum showed a strong mass m/e 367 with other small mass fragments m/e 409, 396, 395, 390, 381, 368, 362, 353, 340, 339, 334, etc. and indicated that the compound was probably a mixture of long-chain fatty diols, $C_{26}H_{54}O_2$ and $C_{28}H_{58}O_2$.

Zusammenfassung. Isolierung und Charakterisierung verschiedener Stoffe aus der indischen Pflanze *Abroma augusta* Linn.

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Transport of Oxygen through Membranes Containing Haemoglobin Solutions

Transport of oxygen through membranes containing haemoglobin has been studied experimentally by SCHOLANDER et al.[1,2]. It has been shown that, in the presence of haemoglobin, oxygen moves through the membrane several times faster than it would otherwise do. The results of SCHOLANDER et al. have been theoretically discussed by WANG[3], and FATT and LA FORCE[4]. It is only recently that a non-equilibrium thermodynamic analysis[5] of SCHOLANDER's results has been attempted; but while doing this, the cross coefficients, relating flows to non-conjugated forces, have been neglected. This is obviously not in keeping with the linear formalism of thermodynamic theory of irreversible processes and influences the analysis approximately.

The present communication, therefore, aims at giving a thermodynamic analysis of facilitated transport of oxygen, taking into account the cross-coefficients relating flows to the non-conjugated forces.

The system used by SCHOLANDER for his investigation can be schematically represented as in the Figure. The membrane is composed of a filter soaked in a solution of haemoglobin. Oxygen gas at different pressures p^{I} and p^{II}, p^{I} being greater than p^{II}, is placed in the compartments on the 2 sides of the membrane. When oxygen passes through the membrane, some of it combines with the haemoglobin in the membrane by the following reaction:

$$n\,O_2 + Hb \rightleftharpoons HbO_{2n}. \tag{1}$$

It can be seen that the oxygen within the membrane may move in the form of HbO_{2n} as well as in the form of dissolved free oxygen. If the rate of the chemical reaction (1) is sufficiently much more rapid than that of diffusion, the chemical reaction (1) can be taken to be at equilibrium at every point in the membrane, i.e. affinity A of the reaction (1) can be taken to be zero. Therefore, we can write:

$$n\,\mu_1 + \mu_2 = \mu_3 \tag{2}$$

where μ stands for the chemical potential and the subscripts 1, 2 and 3 represent oxygen, haemoglobin and oxyhaemoglobin, respectively.

The dissipation function ϕ for the system like the one described above, can be written as[5]

$$\phi = J_1\,grad(-\mu_1) + J_2\,grad(-\mu_2) + J_3\,grad(-\mu_3) \tag{3}$$

where J's represent the fluxes of the species denoted by the respective subscripts. The linear phenomenological relations can now be written as

$$\left.\begin{aligned} J_1 &= -L_{11}\,grad\,\mu_1 - L_{12}\,grad\,\mu_2 - L_{13}\,grad\,\mu_3 \\ J_2 &= -L_{21}\,grad\,\mu_1 - L_{22}\,grad\,\mu_2 - L_{23}\,grad\,\mu_3 \\ J_3 &= -L_{31}\,grad\,\mu_1 - L_{32}\,grad\,\mu_2 - L_{33}\,grad\,\mu_3 \end{aligned}\right\} \tag{4}$$

where L's are the Onsager's coefficients. We know that under steady state conditions, the externally measured overall flow of oxygen, J_1^T, is constant throughout the system. J_1^T must, therefore, be equal to the total transport within the membrane. Hence, we can write for the steady state

$$J_1^T = J_1 + nJ_3. \tag{5}$$

Since the dependence of chemical potential on position is due to the local changes in the concentrations of oxygen, haemoglobin and oxyhaemoglobin, we can write:

$$\left.\begin{aligned} grad\,\mu_1 &= \mu_{11}\,grad\,c_1 + \mu_{12}\,grad\,c_2 + \mu_{13}\,grad\,c_3 \\ grad\,\mu_2 &= \mu_{21}\,grad\,c_1 + \mu_{22}\,grad\,c_2 + \mu_{23}\,grad\,c_3 \\ grad\,\mu_3 &= \mu_{31}\,grad\,c_1 + \mu_{32}\,grad\,c_2 + \mu_{33}\,grad\,c_3 \end{aligned}\right\} \tag{6}$$

where $\mu_{ij} = \partial\mu_i/\partial C_j$ and C_1, C_2 and C_3 represent the concentrations of oxygen, haemoglobin and oxyhaemoglobin respectively. From equations (4), (5) and (6) we can now write:

$$\left.\begin{aligned} J_1^T = &-\{(L_{11}+nL_{31})\,\mu_{11} + (L_{12}+nL_{32})\,\mu_{21} \\ &+ (L_{13}+nL_{33})\,\mu_{31}\}\,grad\,C_1 \\ &-\{(L_{11}+nL_{31})\,\mu_{12} + (L_{12}+nL_{32})\,\mu_{22} \\ &+ (L_{13}+nL_{33})\,\mu_{32}\}\,grad\,C_2 \\ &-\{(L_{11}+nL_{31})\,\mu_{13} + (L_{12}+nL_{32})\,\mu_{23} \\ &+ (L_{13}+nL_{33})\,\mu_{33}\}\,grad\,C_3 \end{aligned}\right\} \tag{7}$$

[1] P. F. SCHOLANDER, Science *131*, 585 (1960).
[2] E. HEMMIGSEN and P. F. SCHOLANDER, Science *132*, 1379 (1960).
[3] J. H. WANG, Science *133*, 1770 (1961).
[4] I. FATT and R. C. LA FORCE, Science *133*, 1919 (1961).
[5] A. KATCHALSKY and P. F. CURRAN, in *Biophysics* (Harvard University Press, Cambridge 1967), p. 203.

which in terms of diffusion coefficients can be re-written in the form[6]

$$J_1^T = -D_{11}\,grad\,C_1 - D_{12}\,grad\,C_2 - D_{13}\,grad\,C_3. \quad (8)$$

The inter-relationship between the diffusion coefficients Dij, the Onsager's coefficients Lij and the thermodynamic coupling coefficients μ_{ij} can be expressed as the product of the matrices.

$$\left.\begin{aligned}[D_{11}\,D_{12}\,D_{13}] = [L_{11}+nL_{31}\;L_{12}+nL_{32}\;L_{13}+nL_{33}] \\ \times \begin{bmatrix}\mu_{11}\,\mu_{12}\,\mu_{13}\\ \mu_{21}\,\mu_{22}\,\mu_{23}\\ \mu_{31}\,\mu_{32}\,\mu_{33}\end{bmatrix}.\end{aligned}\right\} \quad (9)$$

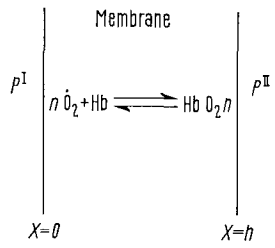
In view of the conservation equation

$$C_2 + C_3 = \text{Constant} \quad (10)$$

equation (8) can further be transformed into

$$J_1^T = -D_{11}\,grad\,C_1 - (D_{13} - D_{12})\,grad\,C_3. \quad (11)$$

Thus in equation (11) for the externally measured overall flow of oxygen, J_1^T, the cross phenomenological coefficients which were neglected in the analysis by earlier workers[6], have been taken into account. It is obvious from equation (11) that the overall oxygen flow, J_1^T, is composed of 2 components, the diffusional flow of free oxygen and the flow of haemoglobin-bound oxygen, represented respectively by the first and second terms on the right-hand side of equation (11).



Arrangement for the study of transport of oxygen through a membrane containing haemoglobin.

Integrating equation (11) between $x = 0$ and $x = h$, keeping in mind that J_1^T is constant in the steady state, we get:

$$J_1^T = \frac{D_{11}}{h}(C_1^0 - C_1^h) + \left(\frac{D_{13}-D_{12}}{h}\right)(C_3^0 - C_3^h). \quad (12)$$

In integrating equation (11) to get equation (12), use has also been made of the approximation that D_{11}, D_{12} and D_{13} can be treated as constants which do not vary with concentration. The superscripts 0 and h in equation (12) refer to the concentrations at $x = 0$ and $x = h$, respectively. The first term on the right-hand side of equation (12) which represents the diffusional flow of free oxygen is determined entirely by the external oxygen pressures.

It can be seen from equation (12) that when $p^{II} = 0$, $C_3^h = 0$ and hence J_1^T takes its maximum value. As p^{II} increases, the second term on the right-hand side decreases, thus lowering the value of J_1^T. Further, when p^{I} and p^{II} are both kept very large so that haemoglobin is saturated with oxygen at both the boundaries of the membrane, i.e. $C_3^0 \approx C_3^h$, the second term in equation (12) becomes negligible and all the oxygen transport is due to the diffusion of dissolved free oxygen only.

It must be mentioned here that the conclusions derived above from equation (12) are in conformity with the observations of SCHOLANDER et al.[1,2].

Zusammenfassung. Die Scholanderschen Befunde über den Sauerstofftransport durch Hämoglobinlösungen werden auf der Basis der irreversiblen Thermodynamik behandelt.

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[6] R. L. BALDWIN, P. J. DUNLOP and L. J. GOSTING, J. Am. chem. Soc. 77, 5235 (1955).

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On the Origin of Adenosine Triphosphate in Chromaffin Granules

The catecholamine (CA) granules have a high content of adenosine triphosphate (ATP), corresponding to about 15% of the dry weight of the granules[1]. The origin of this ATP is not known. There is no evidence that it is formed within the granules. However, adenylate kinase (AK), which is generally regarded as a mitochondrial enzyme, has been reported to occur in CA granule preparations[2]. This implies that the CA granules might be able to provide themselves with ATP by the reaction $2ADP \rightleftarrows ATP + AMP$. On the other hand, the results need confirmation, since mitochondrial contamination in these granule preparations was not ruled out. The aim of this study is to reinvestigate this question by using 2 different techniques for the purification of CA granules.

Methods. Bovine adrenals were obtained at the slaughterhouse within 20 min after the animals were killed, and immediately chilled with ice. The medulla was dissected out and homogenized in 5 vol. of 0.25 M sucrose by Potter-Elvehjem teflon glass equipment. Coarse particles were removed by low speed centrifugation (800 g for 10 min).

CA granules in the supernatant were isolated in 2 different ways: (a) The low speed supernatant was passed through a succession of membrane filters (Millipore Filter Corp., Bedford, Mass.) from 3 μ to 0.3 μ as described by OKA et al.[3]. The filtrates were centrifuged at 15,000 g for 15 min and the pellets were resuspended in 0.25 M sucrose solution. (b) The low speed supernatant was centrifuged at 15,000 g for 15 min. The pellet was resuspended in 0.25 M sucrose and layered onto continuous linear sucrose gradients (0.35–2.2 M) and centrifuged at

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[2] N.-Å. HILLARP, Acta physiol. scand. *42*, 144 (1958).
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