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.

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

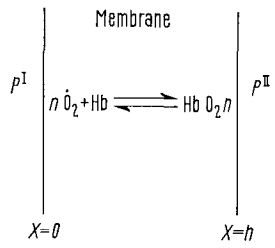
In view of the conservation equation

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

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.

R. C. SRIVASTAVA and RAJ PAL[7]

Department of Chemistry,
Harcourt Butler Technological Institute,
Kanpur-2 (U.P., India), 23 May 1969.

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

[7] Department of Physics, Punjab Agricultural University, Hissar (Haryana, India).

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

[1] N.-Å. HILLARP, Acta physiol. scand. *47*, 271 (1959).
[2] N.-Å. HILLARP, Acta physiol. scand. *42*, 144 (1958).
[3] M. OKA, T. OHUCHI, H. YOSHIDA and R. IMAIZUMI, Life Sci. *5*, 427 (1966).

286,000 *g* (max) for 75 min in a Beckman L2 65 B (SW 40 rotor). The gradients were fractioned with Pasteur pipettes.

AK was assayed spectrophotometrically[4], cytochrome oxidase polarographically[4], catecholamines fluorimetrically[5], monoaminooxidase (MAO) radiometrically[6] and protein with phenol-reagent[7].

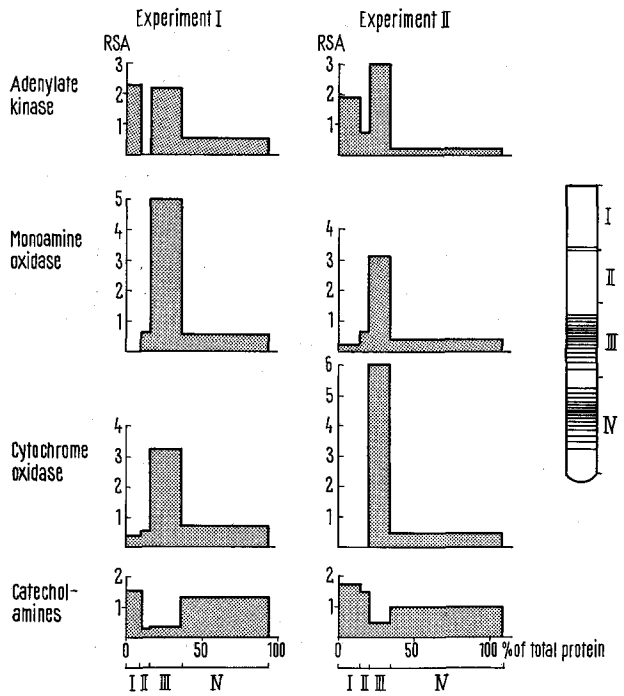
Results and discussion. No AK activity was found in the 0.3 μ fraction obtained by the membrane filter technique, even after the addition of detergent (Lubrol, ICI). This technique has been reported to be the best method to get pure CA granules[3]. However, it is necessary to be cautious in interpreting the present results, since we were not able to obtain the high recoveries of CA granules previously reported[3]. Furthermore, there is evidence of some partial destruction of CA granules by squeezing through the filters[8].

The density gradients formed are shown in the Figure. The data are presented diagrammatically according to DE DUVE et al.[9]. The relative specific activities in relation to protein in the different particulate fractions as well as in the low speed supernatant are drawn on the ordinate. The distribution of protein in the 4 different subfractions is plotted along the abscissa. The figure reveals 3 different distribution patterns: one for cytochrome oxidase and MAO, a second for AK and a third for CA. The distribution pattern of MAO was qualitatively identical with that of cytochrome oxidase. Since this enzyme is generally believed to be localized exclusively in the mitochondria and is widely used as a mitochondrial marker in tissue fractionation studies, this finding supports the concept that MAO in the adrenal medulla is likewise located in the mitochondria[10].

AK was enriched in 2 subfractions: in a soluble fraction on top of the gradient, corresponding in volume to the load of the gradient, and in a particulate fraction, in which the marker for mitochondria, i.e. cytochrome oxidase, was also accumulated. However, in the fraction containing most of the CA, the relative specific activity of AK was lower than that of cytochrome oxidase and MAO. This may be due to the fact that AK is very readily released from the mitochondria in contrast to other soluble enzymes, as for instance those associated with the citric acid cycle[11].

Our fractionation data and the fact that AK is readily realeased from the mitochondria favour the interpretation that AK in the intact cell is mainly or exclusively associated with the same organelles as are cytochrome oxidase and MAO: i.e. the mitochondria. However, some AK as well as CA was also found in fraction I, probably reflecting leakage from damaged particles during the preparative procedures.

On the basis of our results one cannot exclude the possibility that a minor part of the AK in the intact cell is loosely associated with the CA granules, although this interpretation does not seem very probable. Thus the present results do not give any direct support for production of ATP in the CA granules by means of a local system involving AK[12].



The fractionation of sucrose density gradient tubes is shown to the right. The protein content in the different fractions in per cent of the load is plotted on the abscissa. The relative specific activities (RSA) of the enzymes and the catecholamines are drawn in this figure. Total amounts, activities and recoveries. Experiment I: Protein 9.5 mg/ml (93.8%); AK, 0.63 μmol NADH/min mg protein (96.0%); MAO, 0.46 nmol tryptamine/min mg protein (137.7%); Cytochrome oxidase 0.9 μmol O_2/min mg protein (111%); CA, 0.18 mg/mg protein (94.8%). Experiment II: Protein 11.2 mg/ml (110.8%); AK, 0.38 μmol NADH/min mg protein (88%); MAO, 0.40 nmol tryptamine/min mg protein (81.4%); Cytochrome oxidase 0.66 μmol O_2/min mg protein (118%); Catecholamines 0.18 mg/mg protein (113.4%).

Résumé. Les granules chromaffines contiennent de fortes quantités de nucléotides (ATP). L'origine de cet ATP est inconnue, mais il a été admis implicitement que la réaction ADP $\rightleftarrows$ ATP + AMP est catalysée par l'enzyme adénylate kinase. Nous avons recherché la présence de cet enzyme dans des granules isolés en gradient de densité de sucrose.

H. LAGERCRANTZ, B. KUYLENSTIERNA and L. STJÄRNE

Department of Physiology I, Karolinska Institutet, and Department of Biochemistry, University of Stockholm, S-104 01 Stockholm (Sweden), 11 November 1969.

[4] G. L. SOTTOCASA, B. KUYLENSTIERNA, L. ERNSTER and A. BERGSTRAND, Meth. Enzymol. *10*, 448 (1967).
[5] U. S. v. EULER and F. LISHAJKO, Acta physiol. scand. *52*, 137 (1961).
[6] R. J. WURTMAN and J. AXELROD, Biochem. Pharmacol. *12*, 1439 (1963).
[7] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RAMGALL, J. biol. Chem. *193*, 265 (1951).
[8] S. MALAMED, A. M. POISNER, J. M. TRIFARÓ and W. W. DOUGLAS, Biochem. Pharmac. *17*, 241 (1968).
[9] C. DE DUVE, B. C. PRESSMAN, R. GIANETTO, R. WATTIAUX and F. APPELMANS, Biochem. J. *60*, 604 (1955).
[10] H. BLASHKO, J. M. HAGEN and P. HAGEN, J. Physiol., Lond. *139*, 316 (1957).
[11] M. KLINGENBERG and E. PFAFF, *Regulation of Metabolic Processes in Mitochondria* (Eds. J. M. TAGER, S. PAPA, E. QUAGLIARIELLA and E. C. SLATER; Elsevier, Amsterdam 1966), BBA Library, vol. 7, p. 180.
[12] Acknowledgements. We want to thank Prof. ERNSTER for valuable discussions. The investigation has been supported by the Swedish Cancer Society, by Stiftelsen Therese and Johan Anderssons Minne and by Svenska Sällskapet för Medicinsk Forskning.