nach der Drosselung zu beobachten und bleibt fast unverändert während der gesamten Versuchsdauer. Die festgestellten Differenzen zwischen Versuch und Kontrolle sind in beiden Gefässabschnitten nicht sehr gross. (3) Die Kontrollgruppe und die Versuchsgruppen weisen ein umgekehrtes Verhältnis zwischen den Mitochondrienwerten ober- und unterhalb der angenommenen bzw. bestehenden Stenose auf. Während bei den Kontrollen bei jedem Tier unterhalb grössere Zahlen als oberhalb vorhanden sind, ist bei 20 von 25 operierten Kaninchen oberhalb ein grösserer Wert als unterhalb zu beobachten. Das bedeutet nach dem sogenannten «Vorzeichentest» sowohl bei der Kontrolle als auch bei den Versuchen Signifikanz der Differenzen.

Versuchsdauer in Tagen	oberhalb der Stenose			unterhalb der Stenose		
	K	M	M/K	K	M	M/K
Kontrolle	538	10296	19,1	542	11491	21,2
1	635	13972	22,0	582	10670	18,3
3	630	13936	22,1	551	10237	18,6
7	623	13770	22,1	499	9328	18,7
14	586	12602	21,5	463	8759	18,9
21	655	14194	21,7	566	10223	18,1

K = erfasste «Kernbereiche» (= Zellen); M = Zahl der zu K gehörenden Mitochondrien; M/K = Mitochondrienbesatz pro Zelle.

Die erzielten Ergebnisse lassen wesentliche Hinweise eines physiologischen Anpassungsvorganges nicht erkennen, obgleich ein solcher nicht abzulehnen ist. Wir nehmen aber an, dass die geringen Verschiebungen der Mitochondrienwerte den veränderten Druckverhältnissen zuerkannt werden müssen. Es kommt durch die Erhöhung bzw. Erniedrigung des Druckes zu einer Änderung der Zellform und -grösse, die in einer Vergrösserung bzw. Verkleinerung der Kernflächen ihr Maximum erreicht[5]. Dadurch bedingt werden in einer optischen Ebene mehr bzw. weniger Mitochondrien abgebildet.

Summary. After inducing experimentally a stenosis of the aorta abdominalis in rabbits, the mitochondria of the aortic endothelium were fluorescence-optically demonstrated, and quantitatively investigated above as well as below the stenosis. The changes in the number of the mitochondria are proportional to the values of blood pressure, and are discussed in relation to changes in the shape of the endothelial cells.

R. Meyer und J. Götze

Pathologisches Institut der Humboldt-Universität, 105 Berlin (DDR), 30. Dezember 1965.

ATP-Dependent Ca^{++}-Extrusion from Human Red Cells

From the work of Dunham and Glynn[1] it is known that low Ca^{++}-concentrations (0.1 mM) inhibit the Na-K-activated membrane ATPase of human red cells which is held responsible for the active Na-K-transport across the membrane. For obvious reasons this Ca^{++}-sensitive site must be located on the internal surface of the membrane, and therefore the intracellular Ca^{++}-concentration in intact cells must be considerably lower than the Ca^{++}-concentration in the plasma or else the Na-K-pump would be incapacitated. The possibility was considered that this low intracellular Ca^{++}-concentration might be maintained by an active transport of Ca-ions out of the cell. Dunham and Glynn[1] had further pointed out that the membrane of red cells contains an ATPase which is strongly activated by Ca^{++}. This ATPase might be connected to active outward transport of Ca^{++} in the same way as the sarcotubular ATPase in muscle is related to the Ca-accumulation into these structures[2,3].

The question was approached making use of the reversal of hemolysis devised by Székely, Manay and Straub[4] in the version of Whittam[5]. Human red cells from freshly drawn defibrinated blood or from 1-2 days old citrated blood were washed 5 times at room temperature with a fourfold volume of glucose-free solution containing 130 μM/ml NaCl and 20 μM/ml *tris*-chloride at pH 7.2. Subsequently 8 ml of cells were hemolysed in 42 ml of water containing 5 μM/ml *tris*-buffer, 1 μM $CaCl_2$ and various amounts of Mg-ATPate (0.5–4 μM/ml). After 130 sec isotonicity was restored by adding 2.15 ml of a 3 M KCl-solution. After 5 min standing at room temperature, the ghosts were washed with about 100 ml of an ice-cold solution containing 130 μM/ml NaCl, 20 μM/ml *tris*-buffer, 5 μM/ml KCl and 1 μM/ml $CaCl_2$ and incubated in the same solution at 37 °C. The medium to ghost volume ratio was 1.85 on an average. Ca was determined in the medium directly with a complexometric titration in 0.2 ml samples[6] and in packed ghosts without previous washing after deproteinization with approximately the twofold volume of 10% trichloroacetic acid (TAA) and neutralization with 1-n NaOH. Magnesium, hemoglobin, inorganic phosphate and mersalyl in concentrations higher than those present in the samples did not interfere with the titration, nor did the TAA-precipitation remove any Ca from samples in recovery experiments.

The hemolysing procedure resulted in ghosts containing Mg-ATPate, Ca^{++} and, as major osmotic constituent, KCl. It caused the internal Ca-concentration (μM/ml ghosts) to be higher than 1 μM/ml. This effect is probably due both to the shrinking of the ghosts upon reversal of hemolysis and some binding of Ca. The initial Ca-content of ATP-treated and control ghosts was not different however. Figure 1 shows that at 37 °C Ca emerged from ATP-loaded ghosts at a high rate and that the release proceeded to a higher external concentration than with control ghosts treated in the same way except for the addition of Mg-ATPate. The Table and Figure 2 present evidence

[1] E. T. Dunham and I. M. Glynn, J. Physiol. *156*, 274 (1961).
[2] W. Hasselbach and M. Makinose, Biochem. Z. *333*, 518 (1961).
[3] S. Ebashi and F. Lipmann, J. Cell Biol. *14*, 389 (1962).
[4] M. Székely, S. Manay, and F. B. Straub, Acta physiol. hung. *3*, 571 (1952).
[5] R. Whittam, Biochem. J. *84*, 110 (1962).
[6] J. Raaflaub, personal communication.

that this ATP-dependent loss of Ca resulted in a reversal of the original gradient, the final internal Ca-concentration being considerably lower than the concentration for equal distribution of Ca between inside and outside calculated from initial concentration in ghosts and medium and the hematocrit value. The determination of the chloride distribution under identical conditions showed that the membrane potential in the ghosts was similar to that in intact cells, i.e. the interior was negative by 5–8 mV[7]. Therefore passive Ca^{++} distribution should lead to an even higher internal Ca^{++}-concentration than that calculated on the assumption of equal distribution.

From these facts it is clear that, in the presence of ATP inside the ghosts, Ca^{++}-movement against the electrochemical gradient took place during the incubation which was not observed in the absence of ATP. Several simple explanations for the effect could be ruled out:

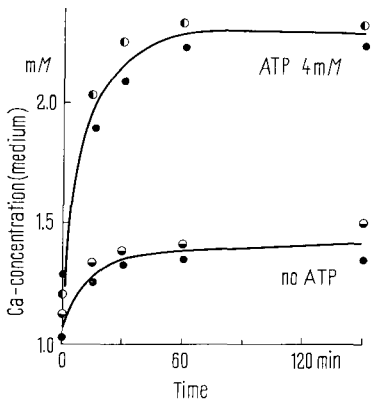

Fig. 1. Appearance of calcium in the external fluid at 37 °C from ghosts previously hemolysed in the presence of 1 m*M* $CaCl_2$, 5 m*M* *tris*-buffer pH 7.2 with and without 4 m*M* Mg-ATPate. Hemolysis was reversed with KCl. External medium: 130 m*M* Na, 20 m*M* *tris*, 5 m*M* K, 1 m*M* Ca, 157 m*M* Cl. Two single experiments: half symbols from blood sample I, dots from blood sample II. Lines drawn by eye.

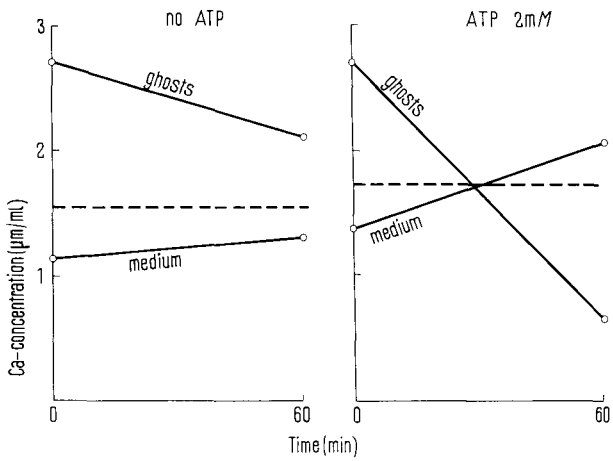

Fig. 2. Single experiment. Experimental conditions as in Figure 1. Ca concentration in ghosts given as μM/ml cells (not cell water). Notice difference in presence and absence of Mg-ATPate (2 m*M* = concentration in hemolysing fluid). Horizontal dotted line: Ca concentration calculated for equal distribution between ghosts and medium from initial concentrations and hematocrit. The straight lines joining the experimental points do not imply that the rates are constant.

(1) The P_i-gradient from ghosts to medium does not account for the movement, because the addition of 5 μM/ml phosphate to the medium did not alter the Ca-extrusion. (2) The Na-K-gradients are not involved since the Ca-extrusion also took place into a Na-free medium (Na being replaced by K). (3) Operation of the Na-K-pump is not required because 10^{-4} (w/v) ouabain in the medium did not abolish the Ca-movement. (4) It might be argued that the Ca found initially in the ghosts was adsorbed at the surface rather than dissolved in the interior water. If the warming up of the ghosts or a shift in pH during incubation had released such Ca, the result might be similar to what was observed. However, when ghosts were not precipitated directly with TAA but hemolysed in water – the membranes being removed by high-speed centrifugation and Ca determined in the supernatant – a high initial Ca-content and a marked drop during incubation was observed, much as in the standard procedure. pH measured in the medium surrounding ghosts loaded with 2 m*M* ATP did not change during 60 min incubation at 37 °C.

Mersalyl (salyrgan)[2] at a concentration of $5 \cdot 10^{-4} M$, applied both during hemolysis and in the external medium, reduced the Ca-loss from ATP-loaded ghosts to a value comparable to that observed in ghosts without ATP. At a concentration of $5 \cdot 10^{-5} M$, it had no clear-cut effect.

These observations suggest that human red cells are able to maintain low intracellular Ca^{++}-concentration by aid of an active transport mechanism for this cation which derives its energy from ATP-splitting and operates independently from the Na-K-pump mechanism.

Ca-concentration (μM/ml medium or ghosts ± 1 S.E.M.)

	0 min	60 min
	A	B
Medium	1.39 ± 0.024 (14)	2.36 ± 0.1 (11)
	C	D
Ghosts	2.39 ± 0.12 (11)	0.46 ± 0.046 (5)
Value calculated for equal distribution	E 1.73 ± 0.048 (11)	

Number of experiments in brackets. 2 or 4 m*M* ATP. Temperature 37 °C. Average hematocrit 0.351. *P*-values: B versus D < 0.0002; B versus E < 0.0002; D versus E < 0.0002.

Zusammenfassung. In einer Lösung mit 1,39 μM/ml Ca^{++} waren rekonstituierte menschliche Erythrozyten im Stande, ihren ursprünglichen Ca-Gehalt von 2,39 μM/ml Zellen in 60 min bei 37 °C auf 0,46 μM/ml Zellen zu senken, sofern sie Mg-ATPat enthielten. Dieser aktive Transport war durch g-Strophanthin nicht hemmbar, konnte aber mit $5 \cdot 10^{-4} M$ Salyrgan unterdrückt werden.

H. J. Schatzmann

Veterinär-pharmakologisches Institut der Universität Bern (Switzerland), March 15, 1966.

[7] According to whether the water content of ghosts was assumed to be 90 or 80%.