occurs equally in common inflammatory processes, e.g. in pneumonia, where serum bactericidin level is high. Considering the fact that from the functional aspect leukins behave in the same way as does serum bactericidin, one cannot rule out the possibility that they may represent the same substance. Neither can it be ruled out that the bactericidin level may be raised by the haemoblastic process itself through a hitherto unknown mechanism.

Zusammenfassung. Die bakterizide Eigenschaft des Serums gegen grampositive Testbakterien *B. anthracoides* wurde an 136 Hämoblastosekranken untersucht. Bedeutend erhöhte Bakterizidinmittelwerte wurden bei akuten Leukämien, chronischen myeloiden Leukämien, M. Hodgkin, Retikulosarkom und Lymphosarkom, Myelom und Myelofibrose im Vergleich mit der gesunden Kontrollgruppe gefunden. Einzig bei chronischen lymphatischen Leukämien war der Bakterizidinmittelwert nicht erhöht.

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Effect of Ouabain on the Active Potassium Accumulation

Schatzmann[1] was the first to observe in 1953 that cardiac glycosides, even in the low concentration of 10^{-5}–$10^{-6}\,M$, inhibit the active cation transport of erythrocytes. A similar effect was found in other tissues, such as nerve[2], muscle[3], and ascites tumour cells[4] as well. At the same time, a number of authors stated that cardiac glycosides – while inhibiting the ion transport – do not in the least influence the carbohydrate metabolism of the cells[1,5]. This finding led to the conclusion that cardiac glycosides act directly on the carrier located in the cell membrane. This is supported by the fact that ouabain – the most effective of cardiac glycosides – is able to act in a very low concentration. $10^{-7}\,M$ ouabain exerts a 50% inhibition on the active ion transport, which seems to prove that the ouabain molecules directly block the active sites of the cell membrane involved in cation transport.

We tried to approach this transport inhibitory mechanism by experiments carried out on guinea-pig brain cortex slices and in human erythrocytes.

By our method described in previous papers[6,7], we incubated guinea-pig brain cortex slices, 100 mg wet weight per vessel at 37°C in air, by shaking in 2 ml of a standard medium containing $0.136\,M$ NaCl, $0.006\,M$ KCl and $0.03\,M$ tris HCl buffer pH 7.4. The incubation in the vessels was stopped at different intervals by treating the slices with trichloroacetic acid. The slices were then homogenized, centrifuged and washed, and the potassium content in the slices determined from the supernatant by flame-photometer. The analytical data were corrected for the swelling of the slices. Our results are shown in Figure 1.

If the brain cortex slices were incubated in a standard medium without substrate, they lost 60% of their K-content after the first 5 min of incubation, and on further incubation a continuous, slow K-outflow was evidenced. When after 5 min $0.02\,M$ glucose and $0.01\,M$ l-glutamate were added to the system, then an intense K-accumulation took the place of K-outflow. If, besides glucose and glutamate, $5 \cdot 10^{-6}\,M$ ouabain were added to the medium, the active K-accumulation was completely prevented; and instead a further strong K-outflow could be observed, which reduced the K-content in the slices to 10–15% of the original value.

The inhibition of K-accumulation in erythrocytes was investigated with ATP-rich erythrocyte ghosts prepared by our method[8]. Human red blood cells were partially haemolyzed in a 0.42 osmolar medium at 0°C, in this hypotonic state the cells were enriched with ATP, then after 2 min isotonicity was restored by 9% NaCl. These

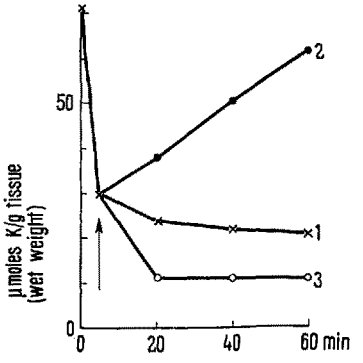

Fig. 1. Changes in the K content of brain cortex slices in the presence of ouabain at 37°C. 1 = Substrate-free control, $2 = 2.10^{-2}\,M$ glucose and $10^{-2}\,M$ l-glutamate, $3 = 2 \cdot 10^{-2}\,M$ glucose, $10^{-2}\,M$ l-glutamate and $5 \cdot 10^{-6}\,M$ ouabain. The substrates and ouabain were added at 5 min.

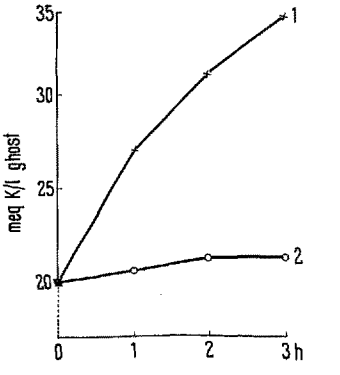

Fig. 2. The effect of ouabain on the K accumulation of ATP-rich erythrocyte ghosts at 37°C. 1 = Control, $2 = 10^{-5}\,M$ ouabain. The ATP content of the ghosts was 3400 µg/ml at 0 min.

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ghosts contained 3–4 mg/ml ATP, that is 4–5 times the normal ATP content of the erythrocytes. The cation permeability of the ghosts was the same as that of the normal erythrocytes and proved to be very suitable for model experiments of 3–4 h at 37°C. As isotonicity was regained by hypertonic NaCl, the cells contained mainly Na and, in the presence of ATP as energy source, were able to produce active K-accumulation to a great extent.

As shown in Figure 2, the ATP-rich control cells accumulated potassium at a very high rate, while the ghosts containing $10^{-5}M$ ouabain in addition to ATP were practically unable to transport K to the cells.

The next step was to study if ouabain could be removed by washing from the erythrocyte ghosts, and if the inhibitory effect on ion accumulation could be prevented. The experiments showed ouabain to be so strongly bound to the cell membrane that it could not be removed by washing; even when washed 4–5 times with a 10-fold volume of the ghosts, a 50% inhibition of ion accumulation still remained. The inhibition could not be entirely suspended, although in the case of a steady dilution such intensive washing should decrease the concentration of ouabain to $10^{-9}M$, at which concentration – according to our experiments – ouabain has no effect at all.

Our experiments suggest that ouabain, due to its lipophilic character, is strongly bound to the cell membrane and affects the active centres involved in K-accumulation. The question arises: of what nature is this active centre, located in the membrane?

The erythrocyte membrane includes several enzymes, such as ATPase[9,10], DPNase[11], nucleoside phosphorylase[12] and various proteinases[13]. Among these enzymes, membrane-ATPase can be activated by K and Na[14,15], thus it might be considered as part of the carrier mechanism. Our investigations showed cardiac glycosides to have a strong inhibitory action on membrane-ATPase. These findings were supported by a number of authors, e.g. POST et al.[14], DUNHAM and GLYNN[15], as well as WHITTAM[16]. Some other authors found an ATPase – that could be activated by monovalent cations and inhibited by cardiac glycosides – in heart muscle[17], in brain microsomes[18], in kidney[19], in liver[20], in nerve[21,22] and in a number of other tissues. Thus this 'transport-ATPase', which is, in all probability, a complex enzyme system[23–25], seems to be part of the carrier in the active cation transport in every tissue.

Zusammenfassung. 10^{-5}–$10^{-6}M$ Ouabain hemmt die aktive K-Akkumulation in Gehirnrindenschnitten von Meerschweinchen und im ATP-angereicherten Erythrocytenstroma. Die Versuche weisen darauf hin, dass Ouabain, infolge seiner lipophilen Struktur, fest an die Zellmembran gebunden ist und den in der Membran befindlichen Carrier der «Transport-ATPase» blockiert.

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The Oxidation of N-2-Fluorenamine and 4-Aminobiphenyl by Peracetic Acid[1]

The detection of 2-nitrosofluorene and 4-nitrosobiphenyl in the blood of animals dosed with 4-aminobiphenyl and in liver microsomal systems incubated with N-2-fluorenamine has recently been reported[2,3]. The nitrosos compounds were identified[2,3] by comparing the spectra of extracts of the biological systems with the spectra of reference compounds prepared by the oxidation of N-2-fluorenamine and 4-aminobiphenyl with peracetic acid according to HOLMES and BAYER[4]. Since the identity of the oxidation products, presumed to be nitroso compounds, was not established[2,3], the peracetic acid oxidation of 4-aminobiphenyl and of N-2-fluorenamine has been reinvestigated and the oxidation products have been fully characterized. Peracetic acid oxidation[4] of 4 aminobiphenyl gave 4,4'-azobisbiphenyl (m.p. 255–257°; lit.[5] 249–250°. Anal. Calc. for $C_{24}H_{18}N_2$: C, 86.2; H, 5.42; N, 8.38. Found: C, 86.2; H, 5.56; N, 8.27; $\lambda_{max}^{95\%\ Ethanol}$ 363, 242 mμ; ε, 33400, 18700). 4-Nitrobiphenyl, m.p. 113–115°, was isolated from the mother liquor and identified by mixed melting-point determinations and the infrared spectrum. Oxidation of N-2-fluorenamine with peracetic acid[4] yielded 2,2'-azoxybisfluorene (m.p. 279–280°; lit.[6] 279°. Anal. Calc. for $C_{26}H_{18}N_2O$: C, 83.4; H, 4.84; N, 7.48. Found: C, 83.7; H, 5.19; N, 7.56. $\lambda_{max}^{Ethanol}$ 382, 248 mμ; ε, 33600, 17200). 2-Nitrofluorene, m.p. 157–159°, was obtained from the mother liquor. A mixed melting point of the material with authentic 2-nitrofluorene was not depressed.

The failure to obtain the nitroso derivatives indicated that peracetic acid oxidation[4] is not a general method for

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