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

[1] Supported by a grant (C-2571) from the National Cancer Institute, U.S. Public Health Service.
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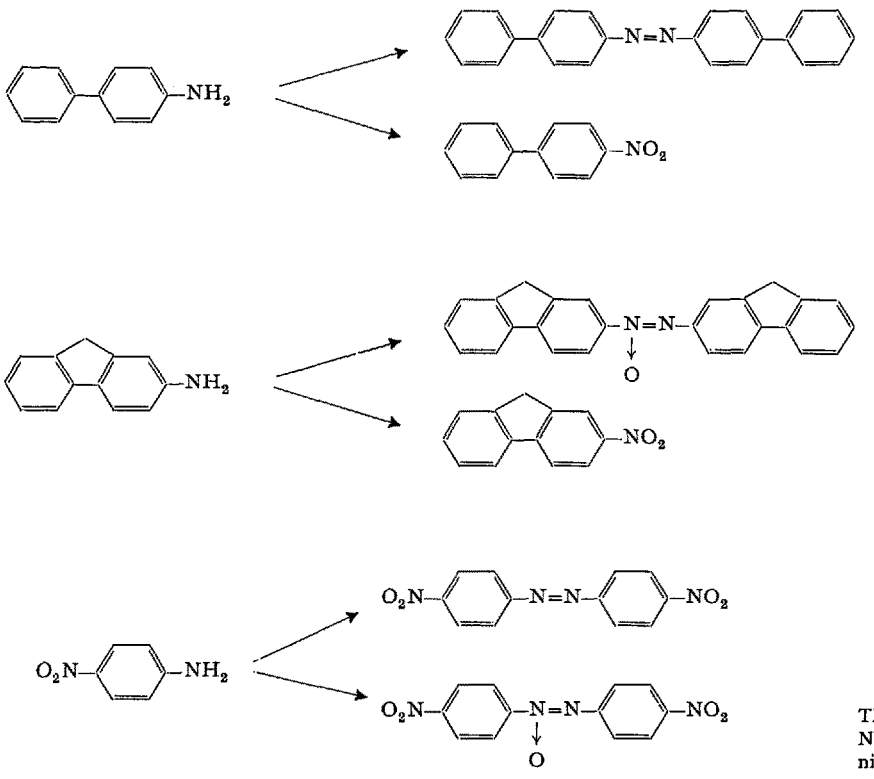
the synthesis of nitroso derivatives and that certain structural features are required to stop the oxidation at this point. Since the amines which had been oxidized by HOLMES and BAYER[4] were anilines, di- or trisubstituted with chlorine or bromine, the possibility that ring inactivation by electron-withdrawing substituents stabilizes the nitroso group was tested by oxidizing *p*-nitroaniline with peracetic[4] and persulfuric acid. The resulting mixtures were resolved by chromatography on Silica gel[7] into *p,p'*-dinitroazobenzene (m.p. 234–236°[8]. Anal. Calc. for $C_{12}H_8N_4O_4$: C, 52.9; H, 2.96; N, 20.6. Found: C, 53.2; H, 2.89; N, 20.8. $\lambda_{max}^{Acetone}$ 465 mμ; ε, 625μ. $\lambda_{max}^{Ethanol}$ 330 mμ ε, 30200) and *p,p'*-dinitroazoxybenzene (m.p. 195–197°; lit.[9] 192°. Anal. Calc. for $C_{12}H_8N_4O_5$: C, 50.0; H, 2.93; N, 19.4. Found: C, 50.5; H, 2.93; N, 19.2. $\lambda_{max}^{Ethanol}$ 343, 268 mμ; ε, 12050, 20300). In addition, unchanged *p*-nitroaniline was recovered from the mother liquor of the peracetic acid oxidation. These experiments suggest that ring activation is not sufficient to terminate the reaction at the nitroso stage and that the peracetic acid oxidation of primary aromatic amines affords nitroso derivatives only in special cases, in which the amino group is flanked by two bulky substituents[4]. When the method[4] is applied to N-2-fluorenamine or 4-aminobiphenyl the principal products of the reaction are 2,2'-azoxybisfluorene and 2-nitrofluorene or 4,4'-azobisbiphenyl and 4-nitrobiphenyl, respectively. The results of the present experiments are summarized in the Figure.

Zusammenfassung. Im Gegensatz zu neueren Befunden von anderen Autoren finden wir, dass die Oxydation von 2-Fluorenamin oder 4-Aminodiphenyl mit Peressigsäure keine Nitrosoderivate bildet, sondern die Azoverbindungen 2,2'-Azoxybisfluoren und 4,4'-Azobisdiphenyl. *p*-Nitroanilin wird zu einem Gemisch oxydiert, aus dem man *p,p*-Dinitroazoxybenzol und *p,p'*-Dinitroazobenzol durch Chromatographie an Kieselgel isolieren kann.



The products obtained in the oxidation of N-2-fluorenamine, 4-aminobiphenyl and *p*-nitroaniline with peracetic acid.

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[7] Silica Gel Powder, 80–200 mesh; J. T. Baker Chemical Company, Philippsburg, N. J. Chloroform was used as eluent.

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