65% yield. The other compound absorbing at 435 nm was absent. It is known that TMTM initiates free radical polymerization of vinyl monomers under heat or photolysis by homolytic cleavage of carbon-sulfur bond[12]. It appears reasonable that the reduction of TMTM by N-benzyl-1,4-dihydronicotinamide should occur at the same site and it also strongly suggests that the product with absorption at 435 nm in TMTD experiment may well be the corresponding pyridinium N,N-dimethylperthiocarbamate, resulting from the reductive cleavage of carbon-sulfur bond in TMTD. The rather labile nature of this compound is certainly anticipated.

The fact that these 2 pyridinium salts absorb at much longer wave length than other pyridinium salts (410 and 435 nm versus 265 nm) suggests that these pyridinium salts are not typical ones but resemble N-methylpyridinium iodide, a charge transfer complex species[13] which possesses an UV-absorption maximum at a much longer wave length than 265 nm. The longer absorption maximum for the perthiocarbamate salt than that of the dithiocarbamate may be attributed to the fact that the dithiocarbamate ion can be stabilized by resonance and has a higher ionization potential[14].

*Résumé.* Le N-Benzyl-1,4-dihydronicotinamide réduit, par un mécanisme redox de transfert d'un électron, la liaison disulfide de plusieurs disulfides organiques tels que le diphényl disulfide, l'α-lipoamide et le tétraméthylthiuram disulfide. Les réactions chimiques sont comparées à l'oxydation enzymique de la nicotinamide-adénine nucléotide.

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## Activation of Sodium Transport Across Biological Membranes

Thermal activation of sodium transport across toad bladder and frog skin has been studied using the short circuit current (SCC) technique[1,2], and a reproducible activation energy of 14 kcal/mole determined for both tissues. Treatment of these tissues with octapeptide hormones, aldosterone or amphotericin-B leads to a stimulation of sodium transport[3-5], and the thermal activation energy measured during the period of maximum stimulation[1,2] has a value of 9 kcal/mole in all cases. We suggested that the observed lowering of the activation energy indicated an increased permeability of the mucosal surface of the epithelial transport cells to sodium. In the present work we have investigated this problem further in order to determine whether the 14 kcal/mole observed in the untreated tissue corresponds to the activation energy of the enzyme pump[6] or to a passive permeability barrier to sodium movement across the mucosal surface of the epithelial transport cells.

We have subjected frog skin to increasing conditions of anoxia by bubbling nitrogen through the bathing media during our thermal activation runs, and in this manner have obtained the results shown in Figure 1. The plot of log SCC (μA/10 mg/cm²) against reciprocal temperature is initially a straight line over the temperature range studied, having a slope corresponding to 14 kcal/mole. As the sodium transport is decreased by the steady fall in oxygen tension, this plot shows 2 slopes. At higher temperatures the activation energy corresponds to 14 kcal/mole and at lower temperatures the activation energy corresponds to 9 kcal/mole. A final plot obtained when the ion transport had been reduced to a low value gives a straight line corresponding to an activation energy of 9 kcal/mole.

Oxytocin (10 mU/ml) added to the serosal surface of frog skin, in aerated conditions, caused an increase in the SCC and as seen in Figure 2 the activation energy plot during this period of increased sodium transport gives rise to a straight line corresponding to an activation energy of 9 kcal/mole. Nitrogen gas was then bubbled through the bathing solutions and the thermal activation studied as the ion transport was reduced by the steady fall in oxygen tension. In each case, with oxytocin present, a straight line plot was obtained with an activation energy of 9 kcal/mole.

These results are interpreted as indicating that the 14 kcal/mole corresponds to the activation energy of the enzyme pump and that any treatment that predominantly affects the pump activity will produce activation energy plots parallel to but higher than those obtained for the

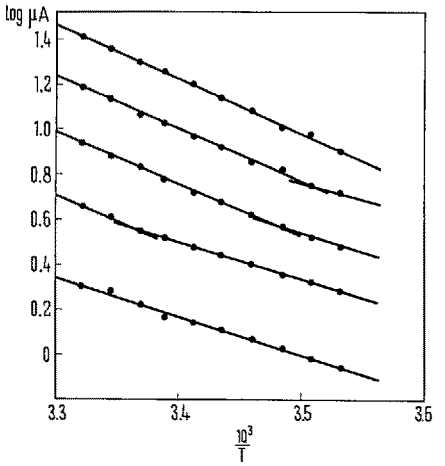


Fig. 1. A typical activation energy plot for the SCC measured across frog skin in aerated conditions (top plot) and during increasing conditions of anoxia (lower plots).

control tissue. It is believed that the 9 kcal/mole corresponds to a mobility activation energy for passive ion transport across the mucosal membrane. It is supposed that the effect of octapeptide hormones, aldosterone and amphotericin-B is to increase this passive movement of ions across the mucosal membrane to a rate that tends to exceed the activity of the enzyme pump. In the steady state the pump would limit the mucosal permeability by producing an increased level of sodium in the transport pool, whereas the reverse situation would exist if the mucosal permeability were limiting. During our anoxia treatment it is possible to achieve a state in which the pump is limited to such an extent that at lower temperatures the passive permeability tends to exceed pump activity whereas the reverse is true at higher temperatures once the pump has been sufficiently activated thermally.

Cyclic AMP ($10^{-3}M$) or theophylline ($10^{-2}M$) added to toad bladder in aerated conditions, led to an increased sodium transport. The thermal activation energy measured, following such treatment, gave a reproducible activation energy of 14 kcal/mole. The effect of cyclic AMP is therefore believed to involve an effect on the ion pump rather than on the permeability of the mucosal membrane, probably by acting as a source for increased supply of ATP to the ATPase pump.

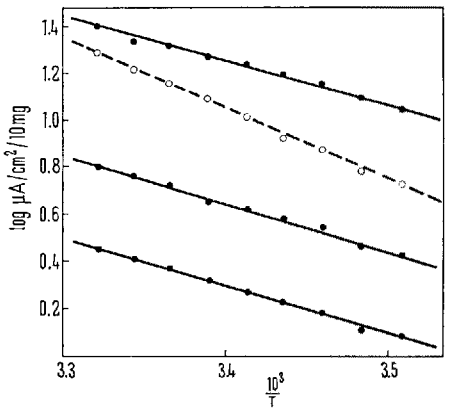


Fig. 2. A typical activation energy plot for the SCC measured across frog skin in aerated conditions (-O-O-) and following oxytocin (10 mU/ml) treatment (top plot). The results obtained following increasing conditions of anoxia in the presence of oxytocin are shown in the lower plots.

*Zusammenfassung.* Es wird die Temperaturabhängigkeit zur Entscheidung über den «aktiven» oder «passiven» Na-Transport in der Amphibienhaut benützt: Oxytocin verändert die Na-Permeabilität, während zyklisches AMP (+Thophyllin) einen aktiven Prozess zu beeinflussen scheint.

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## Multiple Forms of Monoamine Oxidase in Developing Xenopus

There has recently been a growing body of evidence to indicate that the enzyme monoamine oxidase (MAO) is not a single enzyme. Methods that have been employed to demonstrate its multiplicity include, drug and inhibitor effects[1-4], anion inhibition[5], thermostability[1,4], gel filtration and detergent action[6], cellulose acetate electrophoresis[7], polyacrylamide electrophoresis[4,8-10] and pH optima[4]. None of these studies has used embryonic tissues. A developmental study of MAO forms should be of interest to those concerned with problems of enzyme and monoamine differentiation. This project was undertaken using microelectrophoresis and embryos of the South African Clawed Toad, *Xenopus laevis*.

*Methods.* A Bionix polyacrylamide disc electrophoresis apparatus was used. It has been described in some detail by its designers, Pun and Lombroso[11]. A running gel of 18.75% at pH 8.7, and a stacking gel of 2.5% at pH 6.7 were used. *Xenopus* embryos were obtained by artificial ovulation and breeding, and staged using the Normal Table of Nieuwkoop and Faber[12]. Stages 7, 10, 18, 25, 38, 40, 41, 42, 46, 47, and 48 were selected to test. Whole embryos in groups of 4 were homogenized in 250 µl of a 15% detergent solution ('Lubrol' from ICI America Incorporated, Stamford, Conn.). This has been shown to be an effective agent in mitochondrial disruption for the release of MAO[7]. Each homogenate was left at 3 °C for 72 h, and then frozen at −30 °C until used. Brain tissue was prepared in the same way using 100 brains for each sample. Thawed homogenates were centrifuged in the cold at $20{,}000 \times g$ for 1 h. The supernatant was removed and mixed with an equal volume of anticonvection medium of $0.5M$ sucrose in $0.02M$ *Tris* at pH 6.7, containing bromphenol blue indicator[11]. Control preparations were made in the same way except that ipronizid

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