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.

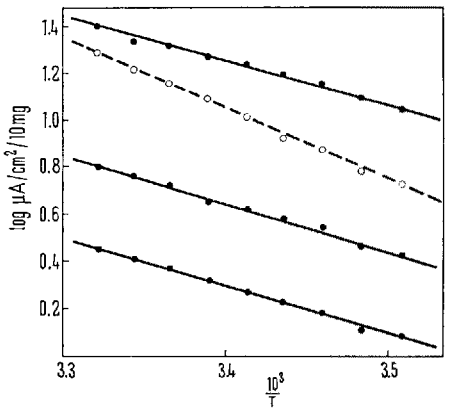

Fig. 2. A typical activation energy plot for the SCC measured across frog skin in aerated conditions (-O-O-) and following oxytocin (10 mU/ml) treatment (top plot). The results obtained following increasing conditions of anoxia in the presence of oxytocin are shown in the lower plots.

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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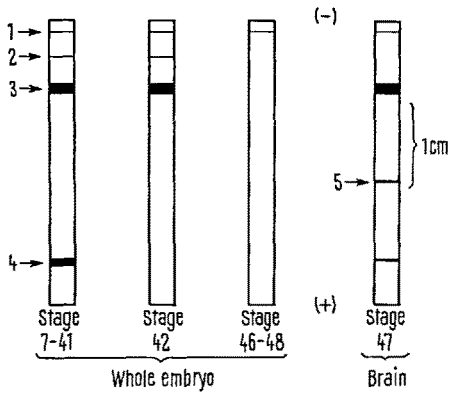
(an MAO inhibitor) was added to the anticonvection medium to a concentration of 0.25 mg/ml[13]. 100 µl of these samples were used for each run. A running buffer of *Tris* glycine at pH 8.3 was employed and the constant current supply was set to deliver 0.5 mA at 400 volts. A run required about 50 min for the bromphenol blue front to migrate through 3 cm of the running gel[11]. Gels were transferred to an incubation medium containing 25 mg tryptamine hydrochloride, 4 mg sodium sulfate, 5 mg nitro-blue tetrazolium, 5 ml 0.1 *M* phosphate buffer at pH 7.6, and 15 ml water. The gels were incubated at 37 °C for 40 min, washed in tap water, and fixed in 10% buffered formalin[13].

Results. It is possible to demonstrate 4 separate kinds of MAO in whole *Xenopus* embryos (Figure). All 4 are demonstrable for stages between blastula (Stage 7) and pre-swimming (Stage 41). One of these disappears by swimming (Stage 42), and only one remains by Stage 46. For the brain preparations at Stage 47 there are again four separate kinds of MAO. Three of these are similar to whole embryo MAO, but one is clearly different. Control runs with MAO inhibitor indicated no MAO in either whole embryo or brain preparations.

Discussion. Various investigators have been able to demonstrate multiple forms of MAO in rat liver by cellulose acetate electrophoresis[7] and polyacrylamide electrophoresis[4,8–10]. MAO assay on whole *Xenopus* embryos has shown a constant increase in enzyme activity during development. This proceeds in phases, showing a series of accelerations after hatching (Stage 38) and swimming (Stage 42), and a slowing down after Stage 46[14]. Measurements of 5-hydroxytryptamine, a major MAO substrate, show significant shifts in level at these same stages[15]. MAO bands 3 and 4 are identical to bands from similar preparations stained with the general protein stain amido black[16]. There is reason to believe that these 2 fractions are in part yolk products being consumed during differentiation[16]. This is in part supported by yolk utilization studies indicating that the terminal phase of yolk breakdown occurs around Stage 46[17]. In this same context, there is evidence for yolk formation in association with mitochondria[18], and MAO is a mitochondrial enzyme[19]. The possibility of a yolk association for MAO during development deserves serious consideration, and has been suggested previously[15]. The pattern of MAO for Stage 47 brain indicates that there are specific organ variations from the whole embryo pattern. At least one MAO band in brain is not demonstrable in whole embryo. Specific brain patterns have been demonstrated in both rat and human tissues[4,7–9,20].



Multiple forms of monoamine oxidase in developing *Xenopus laevis*.

Zusammenfassung. Das Isoenzymmuster der Monoaminoxidase verändert sich während der frühen Entwicklung der Larven von *Xenopus laevis*. Die Anzahl elektrophoretischer Banden verkleinert sich während der Entwicklung von vier auf eine.

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Combined Surgical and Radiation Injury V. The Effect of Bone Marrow Transplantation

Previous studies in this laboratory have demonstrated a delay in wound contraction in irradiated rodents which is most pronounced when the surgical wound follows irradiation by 4 days[1]. This retardation of wound contraction is corrected, in part, by the prior administration of radioprotective compounds[2] or partial bone marrow shielding during radiation[3], but not by the administration of antimicrobials[4]. The purpose of the present study was to evaluate and compare the wound contraction process in non-irradiated rodents, irradiated rodents and rodents subjected to whole body X-irradiation and subsequently transplanted with synginaic bone marrow.

Inbred female Lewis rats, 6–9 weeks of age, housed in a controlled environment and allowed water and food ad libitum, were used in all experiments. In preliminary studies, the LD 50/30 was determined to be 675 R X-ray. The rats were divided into 4 groups of 32 rats each. One group was not irradiated and marrow transplanted, a 2nd group was irradiated and given saline, a 3rd group was irradiated and marrow transplanted and the 4th group was not irradiated and given saline.

Harvesting of bone marrow was accomplished following sacrifice of donor rats by injecting Nembutal i.p. Femurs, tibias and humeri were removed and placed in Hanks' solution. The end of each bone was removed and aspirated in Hanks' solution at 37 °C using a 20 G needle. In order to obtain a homogenous suspension, a series of decreasing sized needles down to 25 G were used. The bone marrow suspension was then filtered through sterile gauze and washed twice in Hanks' solution at 37 °C. The average of 4 hemocytometer counts of nucleated cells were used to measure the cell concentration.