($-14,3 \pm 6,8$ mm Hg; $p < 0.05$) et l'activité efférente du nerf rénal est réduite par rapport à l'activité de base ($-43,0 \pm 22,1$; $p < 0.05$). Cette valeur de l'activité sympathique sous aténolol est significativement différente de celle obtenue à des niveaux de pression comparables par hémorragie ($p < 0.001$) ou par perfusion de nitroprussiate de sodium ($p < 0.001$).

Les variations de la pression artérielle moyenne et de l'activité du nerf rénal étudiées au cours du temps de perfusion d'aténolol (entre 0,2 et 1 mg/kg) font apparaître une corrélation positive significative entre les deux variables ($r = 0,71$; $p < 0.01$; $n = 20$ mesures au cours des 6 expériences).

Un exemple de réponses obtenues sur préparations multifibres est représenté dans la figure. Au cours de cette expérience, 2 tests successifs ont été réalisés à 20 min d'intervalle. Le premier test montre, après 140 µg/kg d'aténolol (4 min de perfusion), une inhibition de la décharge de 2 fibres sympathiques spontanément actives (potentiels de petite amplitude et potentiels de grande amplitude). 8 min après l'arrêt de la perfusion, on note une remontée de la pression artérielle à son niveau initial. Il subsiste néanmoins une bradycardie, et la distribution spatio-temporelle de la décharge n'est pas la même que dans les conditions de base: une troisième fibre est recrutée (potentiels de moyenne amplitude) et la décharge des 2 précédentes est modifiée. Au cours du deuxième test, la pression artérielle est moins réduite et la dose nécessaire pour entraîner une inhibition complète de la décharge est plus importante.

*Conclusions.* Une réduction de l'activité sympathique postganglionnaire, déjà mise en évidence sous propranolol[4], s'observe donc également sous l'action de l'aténolol. Une action centrale du propranolol a été invoquée[3] pour expliquer la réduction du tonus sympathique. Nous avons cependant observé que l'activité baroréceptrice (nerf aortique) était augmentée malgré la baisse de la pression artérielle induite par différents béta-bloqueurs[4]. La réduction de l'activité efférente sympathique pourrait donc être le résultat d'un effet réflexe d'origine baroréceptrice.

Pour expliquer, au niveau des préparations multifibres, la non récupération qualitative de la décharge initiale après l'arrêt de la perfusion d'aténolol, on pourrait concevoir que la bradycardie persistante, résultant de l'action béta-bloquante de la drogue au niveau du cœur, pourrait conduire à une modification de la distribution spatiotemporelle des influx barorécepteurs et de leur intégration par les centres supérieurs.

Cette étude montre par ailleurs qu'il existe une corrélation entre la baisse de la pression artérielle moyenne et la réduction de l'activité sympathique sous aténolol. Il semble donc que l'action béta-bloquante de cette drogue ne soit pas essentiellement cardiosélective mais puisse se manifester au niveau vasculaire, par l'intermédiaire du système nerveux.

Les résultats obtenus au cours de traitements à long terme par l'aténolol chez des patients hypertendus[6] font apparaître que les effets cardiaques de la drogue ne peuvent à eux seuls expliquer les effets antihypertenseurs. En accord avec ces données, nous pouvons suggérer que la réaction vasculaire aux effets de l'aténolol doit contribuer, pour une large part, à l'action antihypertensive observée, non seulement au cours de traitements à long terme, mais aussi en expérimentation aiguë.

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## Effect of propylene glycol and ethanol on $^3$H-noradrenaline efflux from an isolated blood vessel

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*Summary.* Propylene glycol (14 mM), but not ethanol (17 mM), enhanced the late phase of passive $^3$H-efflux from rabbit isolated aortic rings preloaded with $^3$H-(–)-noradrenaline. We conclude that, with regard to $^3$H-noradrenaline release studies, propylene glycol is unsuitable for aiding the dissolution of e.g. corticosterone in physiological salt solution, while ethanol can be used for this purpose.

Corticosterone is often used as an inhibitor of extraneuronal uptake (uptake-2)[2] when the release of $^3$H-noradrenaline ($^3$H-NA) from adrenergic neurones is studied[3]. Since corticosterone is insoluble in water[4], its dissolution is often aided by the addition of small amounts of either propylene glycol[3,5,6] or ethanol[7] to the physiological salt solution (PSS). The aim of the present study was to examine if these 2 solvents, using concentrations in which they are used for this purpose, had any effect on the passive $^3$H-efflux from rabbit isolated aorta preloaded with $^3$H-NA.

*Methods.* The general method described previously was used[8]. Rabbit aortic rings were placed in isolated tissue baths filled with 2.00 ml PSS maintained at 37 °C. The rings were incubated with $^3$H-NA[9] ($10^{-6}$ M; 8.9 Ci/mmole; > 90% pure as determined by column chromatography) for 45 min. Thereafter the wash out of tritium took place by automatically emptying and refilling the bath with 1.75 ml PSS every 2 min for 210 min. In other experiments, the PSS used for the wash-out contained either ethanol (17 mM) or propylene glycol (14 mM). The bath fluid (1.75 ml/2 min) containing the $^3$H-efflux was collected directly in counting vials every 2 min (up to the 20th min) and every 10 min (2 min sample) thereafter. The $^3$H-content in the collected samples and in each ring at the end of the experiment was determined by liquid scintillation spectrometry[10]. The initial content of $^3$H-NA in the tissues was obtained from the sum of residual tissue radioactivity and effluent radioactivity and was expressed as 100%. The percentage of radioactivity remaining in the tissue at any time was calculated by subtracting the cumulative radioactivity recovered in the effluent from the initial tissue content. The experimental efflux data were plotted semilogarithmically as tissue desaturation against time. Each of the resultant curves were multiphasic exponential and may be considered to originate from more than a single exponential process[11]. Visual inspection of the curves indicated that the ex-

perimental data falls on a straight line from about 100 min after onset of wash-out, indicating a single exponential process. During this linear decline, the efflux of radioactivity presumably originated predominantly from a single compartment. The regression line for this compartmental efflux in each experiment was determined by least square linear regression analysis based on calculated $^3$H-content at 100 min and every 10 min calculated value thereafter. From the slope (k) of each regression line, the half-time of efflux ($t_{1/2}$ = ln 2/k) was calculated. Difference between means was determined by Student's t-test.

*Results.* Propylene glycol (14 mM) did not alter the initial (up to about 60 min) passive $^3$H-efflux from rabbit isolated aortic rings preloaded with $^3$H-NA. However later on, it enhanced the $^3$H-efflux (figure). Ethanol (17 mM) had no effect on the $^3$H-efflux during the initial or late wash-out period. Propylene glycol (14 mM) reduced the $t_{1/2}$-value for the late $^3$H-efflux by 52%, while ethanol (17 mM) did not alter this value (table).

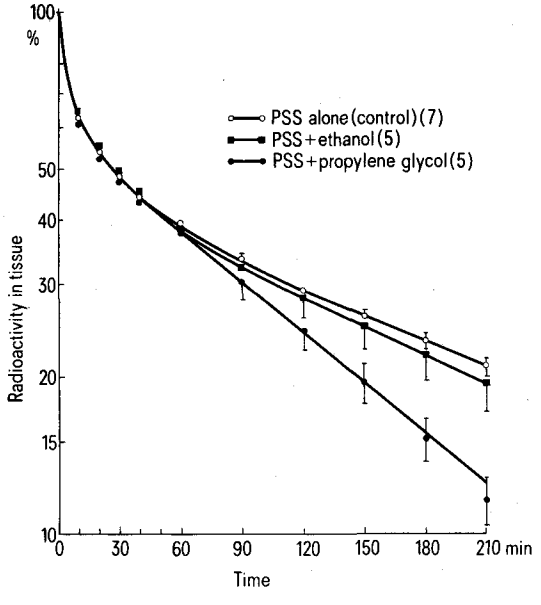
Effect of propylene glycol and ethanol on the $^3$H-efflux from rabbit isolated aortic rings preloaded with $^3$H-NA*

| Treatment | N | $t_{1/2}$** (min) |
|---|---|---|
| Untreated (control) | 7 | 185 ± 15 |
| Propylene glycol (14 mM) | 5 | 89 ± 3*** |
| Ethanol (17 mM) | 5 | 167 ± 17 |

*Aortic rings were incubated with $^3$H-NA ($10^{-6}$ M) in normal PSS for 45 min in vitro. Then, the $^3$H-efflux was measured during a wash-out period of 210 min.

**Half-time values (min; mean values ± SEM) of efflux curves individually analyzed from 100 to 210 min (see 'methods').

***Significantly different from untreated preparations; F-ratio: 32.96 ($p < 0.01$) and t-value: 6.30 ($p < 0.001$).




Effect of propylene glycol and ethanol on $^3$H-efflux from rabbit aortic rings preloaded with $^3$H-noradrenaline. Ordinate: Mean percentage tritium remaining in the tissue; logarithmic scale. Abscissa: Time in min after onset of wash-out. Aortic rings were preloaded with $^3$H-NA ($10^{-6}$ M) in normal PSS for 45 min in vitro. Then they were washed with either normal PSS (○); PSS containing ethanol (■; 17 mM) or PSS containing propylene glycol (●; 14 mM). Figures in parenthesis represent number of determinations on different aortic rings. The vertical bars represent ± SEM. For the sake of clarity, only the percent values obtained every half-hour are plotted from 60 min and onwards.

*Discussion.* The present data demonstrate that propylene glycol enhanced the passive efflux of tritium from rabbit aorta preloaded with $^3$H-NA, while ethanol had no effect. Propylene glycol in concentrations from 7 mM[3] to 56 mM[6] and ethanol[7] (17 mM) is used to aid the dissolution of corticosterone in PSS. The concentrations of propylene glycol and ethanol used in the present study were 14 and 17 mM, respectively. The use of the alcohols in these concentrations resulted in rapid and complete dissolution of corticosterone ($4 \times 10^{-5}$ M) in PSS.

The linear decline in the late phase (100–210 min) of the wash-out curve for untreated aortic strips indicates that the tritium originated predominantly from a single compartment, presumably the adrenergic neurones. This view is supported by the long half-time of efflux (table) from this compartment. The halftime is of the same order as that found by others[11,12] for the same tissue pretreated with inhibitors of monoamineoxidase and catechol-O-methyltransferase.

The tritium efflux represents unchanged $^3$H-NA and its $^3$H-metabolites[13]. However, it seems reasonable to assume that the tritium efflux during the late phase of wash-out directly represents the actual release of $^3$H-NA from adrenergic neurones less their neuronal re-uptake and possible extraneuronal binding.

The propylene glycol-induced enhancement of tritium efflux (figure and table) may be due to a facilitation of $^3$H-NA release from the neurones. Alternatively, the enhancement may be linked to an inhibition of neuronal re-uptake or extraneuronal accumulation. Further work will be required to decide if any of these explanations are correct.

It has been demonstrated previously that only very high concentrations (> 440 mM) of ethanol inhibits the electrically stimulated release of NA from rat brain cortical slices[14] and sympathetic nerve terminals of the isolated

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rabbit heart[15]. The lack of effect of a low concentration (17 mM) of ethanol in the present study is in agreement with that found by others[14,15]. The effect of propylene glycol on NA release has not been investigated to our knowledge.

It is concluded that ethanol is suited to aid the dissolution of e.g. corticosterone in PSS when the latter drug is used as an uptake-2 inhibitor in $^3$H-NA release studies. Propylene glycol, on the other hand, is not suitable for this purpose, since it enhances the passive $^3$H-efflux from vascular adrenergic neurones preloaded with $^3$H-NA.

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## Lens regeneration from cornea in tail ectopic eyes of Xenopus laevis tadpoles

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*Summary.* Evidence was found that cornea in tail ectopic eyes of Xenopus tadpoles has the capacity to regenerate a new lens.

It has long been known that the dorsal region of the iris of certain larval and adult urodeles has the capacity to regenerate a lens should the existing one be removed from the eye[2]. In Xenopus tadpoles, however, lens removal is followed by regeneration of a new lens from the inner layer of the outer cornea[3]. Lentoids have also been reported to form in cornea of Xenopus tadpoles cultured in vitro in the absence of other eye structures[4], but in contrast, cornea transplanted to the tail fin apparently does not form a lens[5]. In the latter studies[5], cornea transplanted to the anterior chamber of lentectomized eyes, or to the limb bud blastema of Xenopus tadpoles, sometimes did form a lens, implying that in the tail there is either inhibition or absence of stimulation. We were interested to know whether or not lens regeneration would take place from cornea arising from tail ectoderm in eyes formed in tadpole tails as a result of prior optic vesicle transplantation to the tail bud.

Optic vesicle transplants were carried out essentially after the method described in Billett and Wild[6]. Nieuwkoop and Faber stage 24/25 embryos were placed in sterile 10% Holtfreter's solution, pH 8.0, and the pigmented epithelium overlying and adjacent to the optic vesicle was completely removed using tungsten needles. The optic vesicle was then excised by means of a fine hair loop and vitally stained in 1:200,000 solution of Nile Blue Sulphate in 10% Holtfreter's solution; this was to make it visible in subsequent transplantation procedures. Stage 28/29 embryos (tail bud stages) were used as recipients of grafts. Following anaesthesia in a 1:3000 solution of MS222 in 10% Holtfreter's solution, a small incision was made in the tail bud with a tungsten needle and a tunnel excavated such that its roof consisted of tail bud ectoderm. Stained grafts were then inserted keeping the orientation the same as in the normal forming eye, i.e. presumptive retina apposed to ectoderm. After grafts had healed in, embryos were transferred to 250 ml finger bowls containing 10% Holtfreter's solution where they remained for 24 h. They were then transferred to aged tap water and reared to stage 49/50.

Direct examination of ectopic eyes under a dissecting microscope indicated that cornea, as well as a lens and eye cup, had developed in 22 out of 100 successful transplants. Examples of such eyes are shown in figures 1 and 2. Confirmation of the presence of cornea comprised of an inner and outer layer (as in the normal tadpole eye) was obtained by subsequent histological analysis in 2 selected cases (figure 3). Lentectomy was performed on the remaining 20 stage 49/50 anaesthetized tadpoles. A small semi-circular cut was made through the outer cornea along

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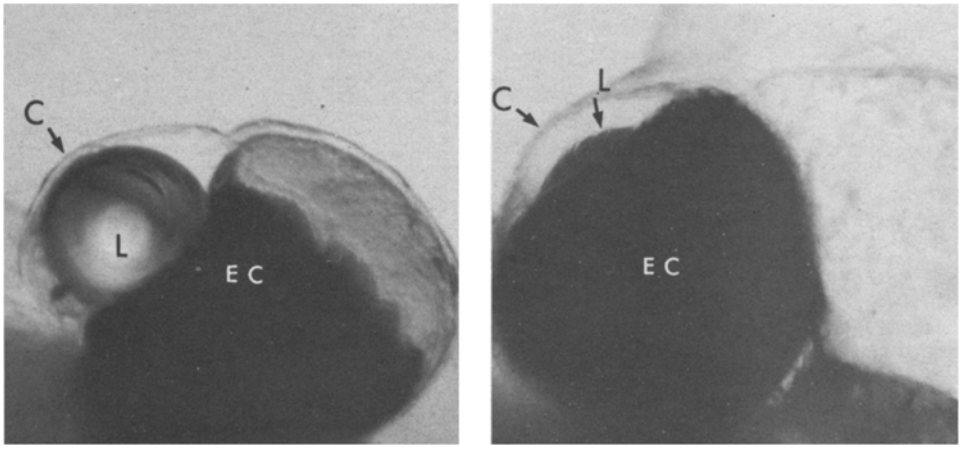


Fig. 1 and 2. Examples of ectopic eyes produced in tadpole tails showing cornea (C) and lens (L) differentiation. E. C., eye cup. ×60.