K^+ content concomitant to the decrease in Na^+ concentration should be expected since the Na^+ active transport appears to be coupled with transport of K^+ in the eel gills[3–13]. It thus seems that pressure acts in this experimental situation by decreasing the membrane permeability to Na^+ and Cl^-. On the other hand, the existence of a Na^+/K^+ coupled active transport process is in agreement with our findings that pressure steps of large amplitudes ($\geqslant 500$ kg/cm^2) affect in the opposite way the tissue concentrations of Na^+ and K^+. It can thus be tentatively concluded that the increase in Na^+ content and the decrease in K^+ concentration observed at high pressures are due to an inhibition of the active Na^+ transport system.
It is also interesting to consider that pressure acts differently on the Na^+ and Cl^- contents. Higher pressures indeed ($p > 350$ kg/cm^2 for Cl^- instead of 250 kg/cm^2 for Na^+) are needed to increase the Cl^- content, and the magnitude of the Cl^- increase measured at $p > 350$ kg/cm^2 is much larger than for Na^+ ions. This indicates that Na^+ and Cl^- movements are governed by independant mechanisms, as already suggested by Maetz[13]. If such is the case, the large increase in tissue Cl^- content observed at high pressures must be ascribed to an inhibition of the active Cl^- transport process, since application of hydrostatic pressures apparently decreases the passive permeability to both Na^+ and Cl^-.
The modifications observed in transport activities may be directly or indirectly induced by high hydrostatic pressures. In the first hypothesis, pressure would act directly on the configuration of structures implicated in ion transport. That possibility has been discussed at length in another paper[9]. In the second one, pressure may, for instance, modify the disposability of oxygen to the tissue; this would then induce modification in the transport of ions. However, an effect of pressure on the oxygen tension in the incubating saline can be neglected, since there is no gas phase in the experimental set-up we have been using. Therefore, a possible indirect effect mediated through changes in cellular oxygen disposability must implicate changes in the transport of oxygen to the intracellular medium. Up to now, there is no reason to believe that hydrostatic pressure would act more specifically on oxygen transport than on ion transport. On the other hand, direct effects of pressure on ionic permeability have already been described[8,9]. Moreover, the inhibition of the active Na^+ transport suggested by our results can be related to a direct effect of pressure on the activity of the ($Na^+ + K^+$) ATPase extracted from the gill epithelium. Indeed, we have been unable to demonstrate any effect of oxygen on the enzyme activity and, as shown in the table, pressure exerts an inhibitory effect which increases with the magnitude of the applied pressure step.
High hydrostatic pressures would thus directly induce, besides a decrease in permeability to Na^+ and Cl^-, an inhibition of the active processes involved in the movements of these ions. Studies on ion fluxes under high hydrostatic pressures are under investigation in this laboratory in order to assess this hypothesis.

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Differential sensitivity of newt limb regenerates to noradrenaline, as revealed by their production of cyclic AMP

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Summary. Regenerates of the newt forearm were incubated with noradrenaline. This increased cyclic AMP production more in the earlier than in the later stages of regeneration.

Amphibian limb regeneration depends upon the nervous system, and recent evidence supports the view that the neurotrophic agent is chemical[3,4]. Histofluorescent observations have shown the presence of catecholamines in the nerve invading the wound and in the regenerate, and inhibition of tyrosine hydroxylase retards limb regeneration[5]. Variations in the ratio of cyclic AMP to DNA during the development of the newt regenerate were reported[6]; and other papers have pointed out a possible importance of cyclic AMP during limb, lens and liver regeneration[7–11]. The purpose of the present study was to measure cyclic AMP concentrations in isolated newt limb regenerates of 2 stages, after incubation in the presence of noradrenaline. Cyclic AMP concentrations were compared with those of control regenerates at the same stages of development but which were not exposed to the drug.
Materials and methods. Newts, Triturus cristatus, collected in Italy were amputated through the forearm. Later, the regenerates were carefully dissected and isolated free from all stump tissue. 6–7 regenerates of early stage (bump) or 1–2 regenerates of late stage (palette) were pooled in 0.25 ml of Ringer and preincubated at 35 °C for 20 min. After addition of theophylline (final concentration 1.5 mM), an incubation was carried out at 35 °C for 10 min, with or without noradrenaline (concentration 10^{-4} M, total volume 0.33 ml). Homogenizer tubes containing the regenerates, were then plunged into boiling water bath for 10 min. The tissues were then homogenized (at 4 °C) and centrifuged. The pellets were used for protein determination[12]. Duplicate samples (50 µl) of the crude supernatant were taken for the satura-

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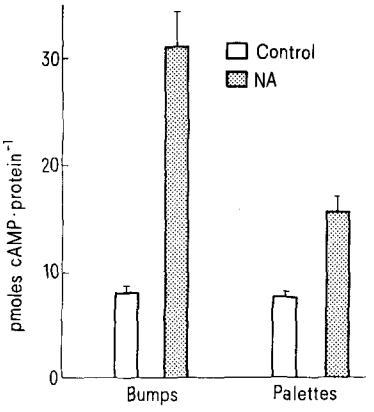
tion assay[13] and the same volume of Ringer containing theophylline was used for preparing the standard curve in the presence of 0, 1, 2, 4 and 8 pmoles of cyclic AMP. *Results and discussion.* Results are shown in the figure, and these confirm the presence of cyclic AMP in the Newt limb regenerate. Furthermore, it can be seen that cyclic AMP generating system is very sensitive to noradrenaline, both at early and late stages of regeneration. However, the increase in cyclic AMP concentration in response to noradrenaline is 2 fold larger in the early stage regenerate than in the late regenerate; the control values being similar in both cases (8.1 ± 0.5; 7.6 ± 0.2 pmoles · mg protein^{-1}). These results suggest that the main role of noradrenaline (and cyclic AMP) is in the phase of proliferation (bumps), rather than in the phase of proliferation/differentiation (palette). The role of cyclic AMP during the process of growth and differentiation has been reviewed recently[14], and conflicting results have been reported. Further investigations are clearly required, for tissue culture systems and investigations in vivo are not strictly comparable[6]. On the other hand, the variation in the catecholamine sensitivity of the nerve-dependent regenerate, reported in this paper, may provide a useful system in which to separate the different developmental stages on a biochemical basis.



Effects of noradrenaline (NA, 10^{-4} M) on cyclic AMP concentration in isolated regenerates of newt forelimb at early (bump) and late (palette) stages. Regenerates were pooled and four samples of each stage were treated with noradrenaline or served as controls. The final incubation was performed for 10 min at 35 °C in the presence of 1.5 mM theophylline. The data give the mean values ± S. E. M.

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Apparent arylsulfatase A activity in excretory fluids

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Summary. Arylsulfatase activity has been demonstrated in rat and human parotid and submandibular saliva indicating that oral bacteria are not the only source of salivary sulfatase activity. Activity was also observed in human sweat, tears and in snake venom.

The characteristics of arylsulfatase A (EC 3.1.6.1) from various tissues have been extensively studied[2–6]. While the in vitro activity is usually measured by the cleavage of sulfate from various organic sulfates[2], the substrate(s) upon which arylsulfatase A acts in vivo are unknown although recent investigations[6,7] strongly suggest that cerebroside sulfate may be the physiological substrate.

In the course of studies conducted in this laboratory on salivary secretion[8–10] a high level of arylsulfatase A activity was observed in salivary gland homogenates. Further investigation showed that the activity was also present in both human and rat parotid and submandibular saliva as well as secretions such as tears, sweat and some snake venoms.

Methods. Saliva collection. Rat saliva from parotid, submandibular and sublingual glands were obtained by cannulation of the gland ducts after pilocarpine stimulation as described by Abe and Dawes[11]. The saliva was stored at −10 °C until required. Parotid saliva was collected from 6 adult human males by means of a modified Lashley cannulae and submandibular saliva by means of individually fitted molded plastic cover[12]. Saliva was collected at a rate of 1 ml/min for 5 min by means of lemon drop stimulation. Enzymic activity was determined according to Yamato et al.[13]. Protein determination was performed by the method of Lowry et al.[14].

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