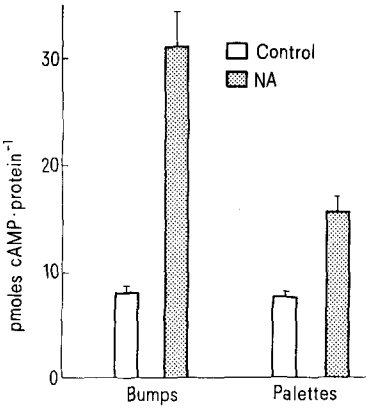
tion assay[13] and the same volume of Ringer containing theophylline was used for preparing the standard curve in the presence of 0, 1, 2, 4 and 8 pmoles of cyclic AMP. *Results and discussion.* Results are shown in the figure, and these confirm the presence of cyclic AMP in the Newt limb regenerate. 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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Arylsulfatase A activity in various excretions

Tissues and secretions	Substrate cleaved (nmole/ml)	Protein in sample (mg/ml)	Specific activity (nmole/mg)
Rat submandibular gland homogenate	1981	3.68	538
Parotid gland homogenate	1333	3.08	433
Sublingual gland homogenate	1876	1.32	1421
Rat submandibular saliva	62	2.92	21
Parotid saliva	1708	23	74
Sublingual saliva	1781	2.4	742
Human stimulated submandibular saliva	21.3	3.60	5.9
Stimulated parotid saliva	104	3.28	31.7
Tears	859	14.6	59
Sweat	806	1.7	474
Crotalus horridus horridus	315	43	7.3
C. viridis viridis	167	34	4.9
C. atrox	296	43	6.9
Agkistrodon piscivorus piscivorus	148	34	4.4

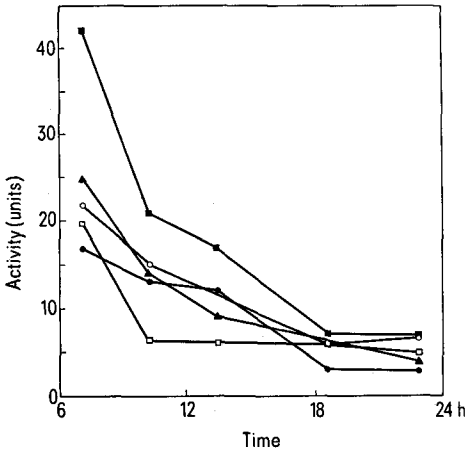
Salivary glands homogenates (10% w/v), sweat and tears were assayed for 1 h. Incubations of the assay mixture with saliva and snake venoms were terminated after 8 h. Onion stimulated tears were obtained from subjects by means of Pasteur pipet. Sweat was collected from exercizing individuals in a similar manner.

Results. Arylsulfatase A has been demonstrated to be a glycoprotein[15]. The content of the enzyme in secretions of the 3 major salivary glands (Table) is in keeping with the known general pattern of glycoproteins secreted by these glands, the sublingual gland secreting mainly glycoproteins. RINDERKNECHT et al.[16], who reported arylsulfatase A activity in whole saliva, implied that the enzyme may not be secreted from the glands since the enzymic level was raised in the case of pyorrhea. However, finding arylsulfatase A activity in cannulated saliva (i.e. pure saliva), satisfactorily demonstrated that an important contribution of oral arylsulfatase A is provided by glandular secretions. This evidence is substantiated by the fact that the enzymic activity exhibits diurnal rhythm (Figure).

Discussion. BENNICK and CONNELL[17] characterized human parotid saliva and identified 4 proteins rich in proline, glycine and glutamic acid. It is worth pointing out that the amino acid compositions of these proteins are similar to that of arylsulfatase A from ox liver[3]. Also MAYO and CARLSON[18] subjected human submandibular saliva to disc gel electrophoresis and showed 2 bands of unidentified glycoproteins. It is reasonable to believe that at least one of these bands could be arylsulfatase A.

Since the number of proteins in any biological excretion is limited, it is conceivable that each protein has evolved to fulfill essential and specific physiological function(s). Arylsulfatase A, found in all excretions examined, eg. saliva, sweat, tears and snake venoms may, therefore, be a common participant in exocrine processes. Sulfatides were reported present in epithelial glandular tissue[19] and it was postulated that they participate in the secretory mechanisms of glands, possibly as a part of the secretory sac membrane[20–22]. It is conceivable that the sulfatides are the sites acted upon by this enzyme in its capacity as cerebroside sulfatase. This is supported by the report of SEIGUER and CASTRO[20] who suggested an active role for arylsulfatase A when reactive Golgi vesicles fuse with membranes.

Arylsulfatase A may have evolved to appear in saliva, tears and sweat as a part of an antibacterial mechanism such as the role proposed for lysosyme, a major enzymic constituent in saliva and tears. GOREN et al.[23] demonstrated a significant correlation between bacterial viru-



Circadian rhythm of human salivary arylsulfatase A. Whole saliva was collected daily from a volunteer for 5 consecutive days at the time periods indicated above. Each line in the figure represents 1 day's pattern of arylsulfatase A activity. A unit of activity is defined as the cleavage of 1 nmole *p*-nitrocatechol sulfate per mg per hour.

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lence and the ability of certain bacterial strains to elaborate sulfatides. Thus while lysosyme may digest the carbohydrate part of the bacterial envelope, arylsulfatase A may act upon bacterial membrane sulfolipids.

Analysis of saliva and tears for hexosaminidase A was proposed for diagnostic purposes in early detection of Tay-Sachs syndrome[24]. Similarly, analysis of these excretions could be adapted into a convenient clinical tool for identifying metachromatic leukodystrophy, a syndrome associated with arylsulfatase A deficiency.

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On partial and differential bleaching experiments with the visual pigments in a fresh water euryhaline teleost (Etroplus suratensis)

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4 February 1976

Summary. Visual pigment was extracted from a fresh water toleost, *Etroplus suratensis* and the optical density measured over a range of 300–650 nm. The absorption spectrum indicates the λ_{max} at 550 nm and a small hump at 530 nm. Through partial bleaching at 630 nm, it was confirmed that the fish possesses a mixture of 2 visual pigments: the one with the λ_{max} at 550 nm predominating over the other.

Fishes of widely seperated habitats ranging from fresh water to sea water have received much attention in the analysis of visual pigments[1–6]. As a result, the influence of diverse factors, such as the habitat salinity of the natural environs and the artificial photoperiod in the laboratory, on the nature of their visual pigments has come to be understood only partially. Hence the discussion continues on the nature of the visual pigments in fishes[7,8]. The teleost, *Etroplus suratensis* is known to inhabit fresh water as well as brackish waters along the coasts of Ceylon and India[9], and thus offers a great potential for investigations into the nature of the visual pigments as related to the habitat[10]. As a first step in this direction, the present study was undertaken in *E. suratensis*, obtained from fresh water.

Material and methods. The fish procured from a local dealer were stocked in glass aquaria and fed on cooked rice everyday in the laboratory. Mostly they were used for the experiments within a week of their procurement from their natural habitat.

Extraction of the pigments. The method of CRESCITELLI[11,12] was followed for the extraction and analysis of the retinal pigment. The fish were dark-adapted overnight, before extracting the pigment. The eyes were dissected out and kept in 4% potassium alum solution for 1–2 h after corneal puncture. After repeated washing with distilled water and borate-KCl buffer at pH 8.3, retinal pigment was extracted into 2% digitonin in alkaline borate-KCl buffer. The homogenate was centrifuged at 4000 rpm for about 30 min. The optical density of the unbleached extract was measured at 300–650 nm in a Hilger-Watt UVISPEK spectrophotometer, using a microcuvette. In all, the optical density of 12 extracts

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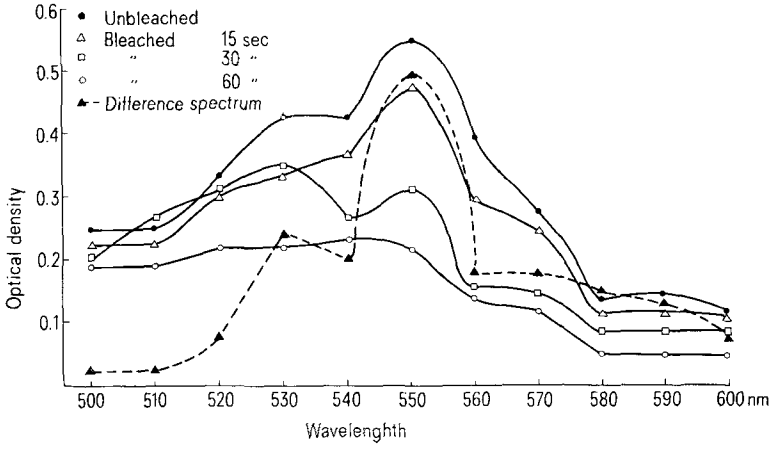

Fig. 1. Absorption spectrum of retinal extract of 24 eyes obtained from 12 animals. ●, Unbleached extract; △, partially bleached at 630 nm for 15 sec; □, – 30 sec; 0,60 sec; ◇, difference spectrum, obtained as a result of total bleaching with white light.