Treated/control SBP and HR ratios during aging in SHRs

Treatment		Age (weeks) 8	12	16	20
Atenolol	SBP	$0.91^{c} \pm 0.02$	$0.81^{c} \pm 0.01$	$0.81^{c} \pm 0.01$	$0.79^{c} \pm 0.03$
	HR	$0.86^{c} \pm 0.01$	$0.89^{c} \pm 0.01$	$0.83^{c} \pm 0.02$	$0.83^{c} \pm 0.03$
Propranolol	SBP	$0.93^{c} \pm 0.01$	$0.95^{c} \pm 0.02$	$0.88^{c} \pm 0.01$	$0.93^{c} \pm 0.02$
	HR	$0.83^{c} \pm 0.01$	$0.76^{c} \pm 0.01$	$0.76^{c} \pm 0.02$	$0.75^{c} \pm 0.01$
Nadolol	SBP	$0.92^{b} \pm 0.02$	$0.92^{b} \pm 0.02$	$0.94^{b} \pm 0.02$	$0.95^{a} \pm 0.02$
	HR	$0.88^{c} \pm 0.02$	$0.88^{c} \pm 0.01$	$0.87^{c} \pm 0.03$	$0.85^{c} \pm 0.02$
Pindolol	SBP	$0.87^{b} \pm 0.03$	$0.91^{a} \pm 0.04$	$0.94^{a} \pm 0.02$	0.95 ± 0.02
	HR	0.95 ± 0.03	$0.90^{b} \pm 0.02$	$0.90^{b} \pm 0.03$	$0.93^{a} \pm 0.03$
Penbutolol	SBP	$0.94^{a} \pm 0.02$	0.96 ± 0.02	1.02 ± 0.02	0.96 ± 0.03
	HR	$0.88^{c} \pm 0.02$	$0.81^{c} \pm 0.03$	$0.90^{b} \pm 0.02$	$0.94^{a} \pm 0.02$
Acebutolol	SBP	0.97 ± 0.03	0.98 ± 0.02	1.05 ± 0.05	0.94 ± 0.05
	HR	0.96 ± 0.02	$0.93^{b} \pm 0.02$	1.00 ± 0.02	0.96 ± 0.02
Ac-acebutolol	SBP	$1.08^{b} \pm 0.02$	1.02 ± 0.02	$1.07^{b} \pm 0.02$	$1.14^{c} \pm 0.03$
	HR	$0.91^{b} \pm 0.03$	$0.94^{a} \pm 0.02$	0.95 ± 0.03	$0.87^{b} \pm 0.03$

Values significantly different from unity. [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$.

467.9±11.0 and 468.8±8.5 beats/min. The table illustrates the changes in treated/control ratios for SBP and HR during aging as determined in the treated groups. In the doses used, only propranolol, atenolol, nadolol and pindolol were able to prevent GHD to a large extent while simultaneously inducing an important decrease in HR. Penbutolol reduced HR but did not oppose GHD, while Ac-acebutolol, an active metabolite of acebutolol in man that is formed only in small quantities by the rat[6], reinforced GHD despite reducing HR. Finally, acebutolol which in the dose used produced hardly detectable blood levels, had no effect either on HR or on GHD.

Thus, these results demonstrate that there is no correlation between reduction in HR and GHD prevention. If, with some drugs, e.g. atenolol[7], the induced bradycardia plays a major role in their antihypertensive effect, this factor is not the only one involved, since propranolol, which is more effective in reducing HR than atenolol, is less antihypertensive than the latter, and since Ac-acebutolol and penbutolol do not oppose GHD despite their lowering effect on HR.

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Isolation of a neurosecretory substance which stimulates RNA synthesis in regenerating planarians[1]

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Summary. An approach to the isolation of neurosecretory material from planarians is described. This material stimulated RNA synthesis, in a dose-dependent response, in regenerating *Dugesia tigrina*. The data support the concept that neurosecretion plays a key role in the process of regeneration in planarians.

The presence of neurosecretory cells has been established for a variety of species of planarians where they are believed to play a central role in regeneration[2-4]. Following transection of *Polycelis nigra*, the numbers of paraldehyde fuchsinophilic neurosecretory cells in the brain and the anterior nerve cords, as well as the amount of material within these cells, increases to a maximum within 3 days[5]. The number of cells then decreases precipitously and control levels are restored by the 4th day. Gabriel[6] found that following transection of *Dugesia lugubris* the quantity of RNA in the tissues of the animal increased during the first 3 days of regeneration. Similarly, in an investigation of the regenerative scissiparity cycle of *D. gonocephala*, Lender[7] found a close correlation between the numbers of stainable neurosecretory cells and RNA synthesis: when the numbers of neurosecretory cells rose so did the rate of uridine incorporation.

Classical ablation and replacement experiments in planaria are technically difficult. On the other hand, it is now well established that certain vertebrate neurosecretions are rich in cystine[8] and it is the presence of sulphur groups which forms the basis of their affinity for paraldehyde fuchsin. With this rationale in mind we expected a preferential incorporation of cystine into neurosecretory material of *Dugesia tigrina* during the initial stages of regeneration.

Asexual specimens of *D. tigrina* were transected just an-

terior to the pharynx, incubated in L-(3,3'-3H)-cystine (5 µCi/ml; sp. act. 1.9 Ci/mM). Amersham Corp. for 72 h at 18 °C, and subsequently homogenized in ice-cold distilled water (100 worms/ml.). The homogenate was centrifuged (10 min at 20,000×g) and ethanol added to the supernate to give a final concentration of 70%. Following concentration, the supernate was applied to DEAE A-25 (Sephadex) column, the components eluted with an increasing salt gradient and the fractions radioassayed.

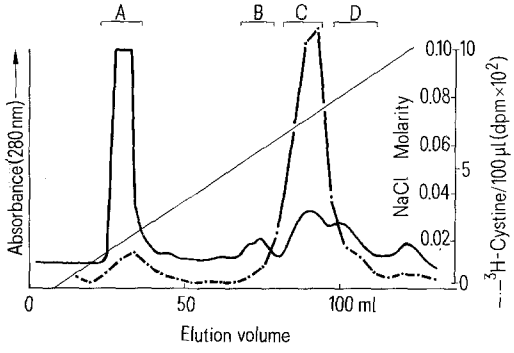

Fig. 1. Elution profile of distilled water/70% ethanol extract of regenerating *Dugesia tigrina* chromatographed on a DEAE Sephadex A-25 column (bed dimensions: 30×2.2 cm; flow rate: 20 ml/h). Elution was accomplished through a linear increase in NaCl concentration (200 ml; 0.0–0.18 M) buffered with Tris-HCl (pH 7.2; 0.025 M). Fractions indicated by brackets were combined, concentrated (PSAC Pellicon membrane, Millipore) and assayed for their ability to stimulate uridine incorporation. For the determination of ^{3}H-cystine labelled protein an aliquot of each fraction was spotten onto a disc of Whatman No. 42 filter paper and processed according to the method of Manns and Novelli[16].

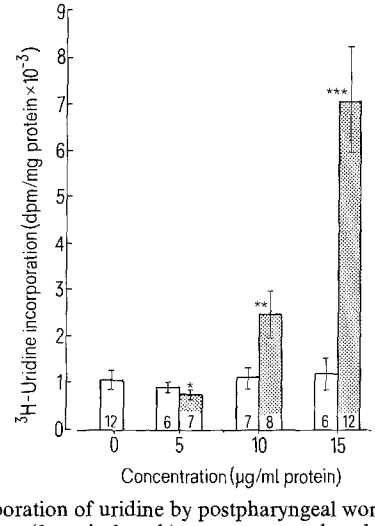

Fig. 2. Incorporation of uridine by postpharyngeal worm fragments. Asexual worms (1 cm in length) were transected at the level of the pharynx and the head piece discarded. The tail piece was cut transversely into approximately 3 equal pieces. Fragments from 3 worms each were incubated for 24 h in 400 µl of pond water containing 4 µCi ^{3}H-uridine (46 Ci/mM; Amersham Corp.), 100 U penicillin G and varying amounts of extract corresponding to peak A (open bars) and peak C (cross hatched bars) isolated from a DEAE Sephadex column. Worm pieces were washed in pond water homogenized in 100 µl 0.9% NaCl and 50-µl aliquots spotted on filter paper discs and processed for the uridine incorporated into nucleic acid[16]. Protein content of each aliquot was subsequently determined according to Bramhall et al.[17]. The number of replicates is written in each bar of the histogram. SE are illustrated by the vertical bars. Significance of difference was determined by the 't' test. *No significant difference; **$p < 0.05$; ***$p < 0.0001$.

The results (figure 1) show that although several components are eluted from the column, the majority of the label was associated with a single peak (C). In contrast, ^{3}H-cystine incorporation over the same period by normal (uncut) worms is much lower. Using antibodies prepared against this fraction (C) we have demonstrated immunohistochemically that this material is found only in neurosecretory cells associated with the brain and anterior nerve cords[9]. We therefore prepared unlabelled extracts from regenerating worms which were chromatographed as above and the various peaks assayed for their ability to stimulate uridine incorporation into nucleic acids of post-pharyngeal pieces of *D. tigrina*.

The results (figure 2) illustrate the ability of fraction C to stimulate uridine ([5,6-3H] uridine, sp. act. 58 Ci/mmole Amersham Corp.) incorporation into pieces of worm tissue. Exposure to higher concentrations of fraction C resulted in a significantly greater stimulation of uridine incorporation into nucleic acid. Thus fraction C appears to promote a dose-dependant response. In contrast, fractions other than C, i.e. fraction A, did not stimulate uridine incorporation. The maximum response observed in these experiments, at a concentration of 15 µg/ml represents a greater than 7-fold stimulation over control levels. This degree of stimulation is comparable to that observed in vivo, in the scissiparity cycle of *D. gonocephala* where at day 14 the level of uridine incorporation is approximately 10 times that at the 6th day[7].

The present observations are consistent with previous studies indicating that RNA synthesis is stimulated during the initial stages of regeneration in planaria[6,10,11]. More recently Sauzin-Monnot[12] demonstrated that an extract of blastemas (which are believed to be rich in neurosecretion) from regenerating *D. dorotocephala* will stimulate ^{3}H-uridine incorporation into neoblasts of posterior fragments from *Dendrocoelum lacteum*. However the posterior pieces of *D. lacteum* do not form heads following this treatment indicating that the initiation is of neoblast replication and or differentiation, but not brain formation. It is of interest to note here that the regeneration of lost segments of Nereid Polychaetes and Oligochaetes appears to be initiated by a cerebral hormone[13–15]. However, it has yet to be established whether these effects are the result of neurosecretions or other, as yet unknown, trophic factors.

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