The effect of putrescine, spermine and spermidine on auricle regeneration of the flatworm, *Dugesia tigrina*

Treatment	Number of animals with regenerated auricles/Total Number of animals for each day after decapitation						Mean time for auricle regeneration ± SD (days)
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	
Experiment 1							
Putrescine (1×10^{-4} M)	0/8	0/8	0/8	2/8	8/8	8/8	4.75 ± 0.46**
Control	0/8	0/8	0/8	0/8	4/8	8/8	5.50 ± 0.53
Experiment 2							
Spermine (1×10^{-4} M)	0/8	0/8	3/8	8/8	8/8	8/8	3.62 ± 0.51*
Control	0/9	0/9	1/9	5/9	9/9	9/9	4.33 ± 0.70
Experiment 3							
Spermidine (1×10^{-4} M)	0/9	0/9	4/9	9/9	9/9	9/9	3.55 ± 0.52***
Control	0/9	0/9	0/9	3/9	6/9	9/9	5.00 ± 0.86

*$p < 0.05$ versus control; **$p < 0.01$ versus control; ***$p < 0.001$ versus control.

when applied under specific conditions stimulates the cell division of cultured human embryo fibroblasts. Furthermore, Rupniak and Paul[5] inhibited the growth of rat embryo fibroblasts by use of methylglyoxal-bis (guanylhydrazone) which blocks the intracellular accumulation of spermine and spermidine and subsequently reversed the inhibition by appropriate addition of either spermine or spermidine. Precisely how putrescine and the polyamines are involved in growth processes is not well-defined. Although there are indications that they are vital to the regulation of nucleic acids, e.g. the regulation of DNA-synthesis[6].

In our study, we were interested in the effects that exogenously applied putrescine and the polyamines might have on a growth system more complex than that of cultured cells. We utilized a relatively simple, easily observable example of growth, that of flatworm auricle regeneration.

Methods and materials. 3 similar experiments were conducted (see table). Specimens of *Dugesia tigrina* were fasted 5 days in order to eliminate intestinal contents that might cause microbial infection. Then for each experiment, several animals were randomly assigned to either an amine treatment group or a control group. Each animal was then anesthetized for decapitation by being placed on a filter paper presoaked with saline solution (Betchaku's solution[7] without Neomycin sulfate was used for this study) mounted on a petri dish filled with ice, and then the animal's head was cut off immediately behind the auricles. After decapitation, control animals were each placed in 25 ml of saline solution, while amine-treated animals were each placed in 25 ml of saline solution containing putrescine, spermine or spermidine at a concentration of 1×10^{-4} M. The animals were then observed daily for the reappearance of auricles.

Results. The table summarizes the results. In each experiment, the amine treatment significantly decreased the mean time for auricle reappearance (t-test used).

Discussion. The results indicate that putrescine and the polyamines, at the concentration used, enhance the regeneration process of *Dugesia tigrina* and support the idea that the amines tested are needed for cell proliferation. The results are too preliminary to allow for speculation about how the amines act in flatworm regeneration, but are significant enough to suggest that putrescine and the polyamines be exogenously applied to other growth systems, such as embryonic development, mammalian wound healing and the regeneration processes of the rat liver and the salamander limb.

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Linalool, neral and geranial in the mandibular glands of *Colletes* bees – an aggregation pheromone[1]

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Summary. Mandibular glands of 3 species of *Colletes* were analyzed by combined gas chromatograph-mass spectrometry. Linalool, neral and geranial in 3:1:1 ratio were present and highly attractive to both sexes in field tests. Linalool has not previously been reported in Hymenoptera.

The mandibular glands of bees (Apoidea) produce substances that serve as alarm pheromones[2,3], markers of male territory[4–7], odor trail markers[8], and for host disorientation[8–10]. Netted female *Colletes louisae*[11], *Nomia melanderi*[12] and *Anthophora edwardsii*[13] attract conspecific females, but the source of identity of the attractant was undetermined. Freshly excised mandibular glands of female *Colletes thoracicus* have a highly attractive lemonlike odor[14].

Field experiments in Maryland showed intra- and interspecific attraction among both sexes of 3 closely related sympatric species, *C. thoracicus*, *C. inequalis* and *C. validus*. *C. inequalis* and *C. validus* shared the nest aggregation site

and food source in early spring; *C. thoracicus* began activity after males of the other 2 species had disappeared, although females were present. Attracted insect that flew upwind toward the pheromone were netted for identification. For pheromone analysis, bees collected at nesting sites were cooled over ice in the field. Whole heads or excised mandibular glands of at least 10 bees per test were extracted with methylene chloride. The extracts were analyzed on an LKB 9000 gas chromatograph-mass spectrometer utilizing an SE 30 capillary column. The first component, eluting at 140 °C, had a characteristic mass spectrum (MS) corresponding to a monoterpene alcohol with a molecular ion at m/z 154 and a base peak at m/z 71, and was identified as linalool (3,7-dimethyl-1,6-octadiene-3-ol). This component was identical to authentic linalool in its MS and GC retention time. 2 other components, eluting at 155 °C were identified as both isomers of citral, neral (3,7-dimethyl-2 trans-octadiene-1-al) and geranial (3,7-dimethyl-2-cis-octadiene-1-al) based on their MS and comparison with authentic compounds. These components were identified in both males and females of the 3 species in an approximate ratio of 3:1:1 (linalool:neral:geranial).

Field tests utilizing synthetic compounds were conducted at an aggregation of 100,000 nests of *C. thoracicus*. Assays with individual components and blends revealed these bees were attracted *only* to the mixture of the 3 components at the ratio found in their mandibular glands; citral or linalool alone were not attractive.

Neral and geranial have been previously reported in secretions of various bees. In *Trigona* it serves as trail and alarm pheromone[3]. In honey bees, it is produced by the Nassanoff gland and attracts foragers[15]. In *Andrena* spp., it is produced by the mandibular glands and may assist aggregation[7]. It is also reported in the colletid bee, *Hylaeus* (=Prosopis) but biological activity was not demonstrated[16,17]. Linalool, however, has not yet been reported from any Hymenoptera and constitutes a novel component of mandibular gland secretion. In the bark beetle, *Ips pini*, accompanied by cis-verbenol and ipsdienol, it serves as an aggregation pheromone[18]. The *Colletes* mandibular gland pheromone is released when the mandibles are used, as in pollen collection or nest excavation. Thus it may serve to assist feeding and nest aggregations, which are often seen in nature.

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Isolation of pathogenic treponemes from hare

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Summary. A presumably new species of pathogenic treponemes was isolated from a lesion on the male organs of hare (*Lepus* sp.) and the scrotum of a rabbit (*Oryctolagus* sp.) was infected with this treponeme. Lesion developed on the scrotum after a 110-day incubation.

Monographs on the infectious diseases of wild animals deny the occurrence of natural treponematosis in the hare (*Lepus* sp.)[1,2]. Mention was made of hare syphilis without any epidemiological proof[3]. Jaksits reported spiral organism (*Spirocheta pallida* subgenus *treponema*) in china ink preparations from lesions of the hare[4]. We have demonstrated serological reactivity in the hare[5] with the antigenes used for human serological tests for syphilis[6]. Having found the antibodies[7], we looked for the antigen and we succeded to demonstrate treponemes in the skin lesions of the scrotum of hares with dark field microscope.

In the present paper, we describe the skin symptoms of hare treponematosis and the successful experimental infection of rabbits with its pathogen.

Material and methods. About 1400 trapped hares were examined and in 15 cases a superficial ulceration or haemorrhagic crust without induration on the surface of genitals was found. Only 5 of them were positive on dark field microscopic examination. These were isolated. Their genital lesions were cleaned mechanically and washed with sterile saline. Then the cleaned surface was scarified and the juice was collected in a syringe and injected into the scrotum of seronegative New Zealand white rabbits. The challenged rabbits were isolated and the scrotum was controlled every 3rd day. The proof of treponeme infection was given by dark field microscopic examination.

Results and discussion. In 1 case, superficial lesions of about 5 cm diameter developed after 110 days on the surface of scrotum of the rabbit around the prick of the injection needle (figure). Removing the crust a great number of treponemes was demonstrated in dark field microscope. From the juice obtained by punction of the testes, treponemes could not be demonstrated.

The morphology of the treponemes isolated from hares and from the challenged rabbits was compared to that of *Treponema pallidum*. The treponemes from the lesion are