Table 2. Comparison of the N-terminal sequences of human serum albumin (HSA) and human urine albumin (HUA)

	1	5	10	15	20	25
HSA:	Asp-Ala-His-Lys-Ser-Glu-Val-Ala-His-Arg-Phe-Lys-Asp-Leu-Gly-Glu-Glu-Asn-Phe-Lys-Ala-Leu-Val-Leu-Ile-					
HUA:	*Asp-Ala-His-Lys-Ser-Glu-Val-Ala-His-Arg-Phe-Lys-Asp-Leu-Gly-Glu-Glu-Asn-Phe-Lys-Ala-Leu-Val-Leu-Ile-*					
	(Gly)*		(Glu)		(Glu)	

		30	33
HSA:	Ala-Phe-Ala-Gln-Tyr-Leu-Gln-Gln		
HUA:	*Ala-Phe-Ala-Gln-Tyr-Leu-Gln-Gln*		

* Data in brackets show very weak admixtures detected in the degradation steps.

were observed in the HUA preparation. The amino acid composition of HUA (table 1) was based on the presence of 61 leucine residues per mole of protein. Differences in the amino acid composition of HUA (table 1) from the values for HSA, in this case largest in the content of lysine, glycine and alanine, had been observed to a certain extent even with individual analyses of various HSA preparations. The amino acid composition of HUA is very close to that of HSA. HUA was subjected to stepwise degradation in a sequencer (table 2). Its N-terminal sequence of 33 residues does not show any differences from the N-terminal region of HSA[7]. By cyanogen bromide cleavage of the native proteins with intact disulfide bonds, in both cases 3 corresponding fragments were obtained. We marked them similarly as in the previous studies[16,17] as fragments N, M and C (N-terminal, middle and C-terminal fragment). The distribution of the fragments in the elution diagrams is identical for both proteins and reveals identity in positions of the methionine residues in both molecules. The peptide maps of both oxidized albumin preparations are practically identical (figures 1A and B) and also the tryptic peptide maps of isolated fragments are very similar (figures 2A and B, to 4A cyanogen bromide digests of native HUA and HSA, in each case 3 fragments (N, M and C), and compared their tryptic peptide maps. Peptide maps were prepared from tryptic digests (1:100, w/w; 16 h at 23 °C) of the oxidized preparations (3 mg) by paper electrophoresis[18] at pH 5.6 (30 V/cm) and chromatography in the system n-butanol:pyridine:acetic acid:water (15:10:3:12, v/v).

Results and discussion. The electrophoretic and immunological properties of HSA and HUA were found to be identical. As the only difference, minor additional bands and B). Also the amino acid composition of fragments N, M and C from both proteins does not show significant differences.

The results provide information that the primary structure of urine albumin is at least very close to that of serum albumin and practically exclude the possibility of any sequential differences in the N-terminal region. They permit us to draw the conclusion that albumin after passage through the glomerular barrier does not show any substantial differences in covalent structure from HSA. This conclusion is supported by the observation of Hoffsten et al.[19], that murine albumin from urine has the same mol.wt as albumin isolated from serum, and that by chromatography no fragments of albumin could be detected in the urine.

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Stimulation of *Dugesia tigrina* auricle regeneration by exogenous putrescine, spermine or spermidine

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Summary. Specimens of a flatworm, *Dugesia tigrina* were decapitated and then cultured in a solution of 1×10^{-4} M putrescine, spermine or spermidine. Subsequent observation for the reappearance of auricles indicates that the amine treatment stimulates the flatworm regeneration process.

An increased synthesis of the diamine putrescine and the polyamines, spermine and spermidine, has been shown to occur in a variety of systems undergoing growth, such as rat liver regeneration[1], embryonic development[2] and mammalian cells induced to proliferate in vitro[3]. Studies involving exogenous application of these amines indicate that they are necessary for cell proliferation. For example, Pohjanpelto and Raina[4] have presented evidence that putrescine

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 $\pm$ 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