80% methanol The extracts were mixed and filtered. Part of the crude extracts was studied as such, another part was submitted to chromatography on alkaline alumina column which was then eluted with descending concentrations of ethanol.

Both crude extracts and eluates were submitted to paper chromatography, thin-layer chromatography and high voltage electrophoresis. Synthetic octopamine was available for comparison.

Results and discussion. Following chromatography on alumina column, the phenol derivative supposed to be octopamine emerged in the 70% ethanol eluate. Its identification as octopamine is based on the following criteria: a) both the unknown phenol derivative and synthetic octopamine showed the same colour shades with the Pauly reagent (yellow), Gibbs reagent (sky blue) and diazotized *p*-nitroaniline (lillac). b) on high voltage electrophoresis, the unknwon substance showed the same mobility towards the cathode as synthetic octopamine: $E_{1.2}$ = 0.9 *p*-tyramine, $R_{5.8}$ = 0.89 *p*-tyramine. c) on paper chromatography, Rf values were exactly the same for the unknown substance and for synthetic octopamine in 4 solvent systems: 0.42–0.49 in *n*-butanol:acetic acid:water (4:1:5); 0.21–0.23 in *n*-butanol: 35% methylamine (8:3), 0.74–0.78 in 1-penthanol:pyridine:water (4:4:1), and 0.77–0.80 in 20% KCl. d) superimposable Rf values were obtained also in thin-layer chromatography on silica gel: 0.12–0.14 in benzene:ethanol:methylamine (22:7:1); 0.45–0.47 in *n*-butanol-acetic acid:water (4:1:5).

The contents of octopamine in the different batches were 30, 60 and 45 µg per g fresh tissue, respectively.

In about 6–7 specimens of *Peristernia*, the hypobranchial body was separated from the remaining soft tissues (0.4 g versus 2.0 g) and analyzed. It contained 5 times as much octopamine as the other tissues (125 versus 25 µg/g). Thus, it appears probable that, like other active compounds (murexine, dihydromurexine, purple prepigments) also octopamine is concentrated in the hypobranchial gland.

In addition to octopamine, the soft tissues of *Peristernia* contained small amounts of imidazolepropionic acid, its methyl ester, and choline. It is possible that all these compounds are postmortal artifacts deriving from dihydromurexine by hydrolysis and transmethylation processes[9].

Small amounts of octopamine could be detected also in the soft tissues of other gastropod molluscs of the Philippines, such as *Bulla adamsi* (5–7 µg and 2 µg per g total soft tissues, respectively, in 2 different batches) and *Latirus polygonatus* (4 µg per g total soft tissues).

It appears that octopamine originates in tissues from *p*-tyramine by the action of dopamine-β-hydroxylase[10]. *p*-Tyramine has not been found in *Peristernia nassatula*, but it is present in considerable amounts (30 µg/g) in the soft tissues of *Bulla adamsi*.

The function of octopamine in the hypobranchial body of gastropod molluscs is as obscure as that in the posterior salivary glands of octopoda. It would be of interest to check whether this potential adrenergic transmitter could be used here for defense or for capturing prey.

After 21 days of incubation, 50 µCi $Na_2{}^{35}SO_4$ (NEN, specific activity 859 mCi/m*M*) were added to the medium

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Synthesis of Sulfated Glycosaminoglycans by the Three Cell Types of the Rabbit Cornea in Culture[1]

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Summary. Rabbit corneal cells were cultivated for 21 days and then exposed to $Na_2{}^{35}SO_4$, a precursor of sulfated glycosaminoglycans (GAG). All 3 cell types of the cornea, the fibroblasts, the epithelial as well as the endothelial cells, synthesize GAG. The fractionation-patterns of the epithelial and endothelial GAG are almost identical and differ clearly from the one of fibrolastic GAG.

Biosynthesis of collagen and glycosaminoglycans (GAG) is thought to be the domain of mesodermal cells, but evidence exists that also ectodermal cells, i.e. the epithelium of the chick and the rabbit cornea, produce these matrix components[2–6]. It is not yet established, however, whether all three cell types of the rabbit cornea do synthesize GAG and how far the fractionation-patterns of the GAG synthesized by these cells differ from each other.

Material and methods. Epithelial and fibroblastic cultures from the rabbit cornea were made as described elsewhere[3]. Endothelial cultures were prepared by treating the Descemet's membrane, stripped off from the corneal stroma, with 0.005 *M* Na_2EDTA and 1.25% trypsin for 30 min at 37 °C. These substances were dissolved in minimal Eagle's medium, Hanks BSS. The nutrient medium was TC 199 containing 20% fetal calf serum and antibiotics.

The cells originated from the corneas of 5 rabbits. All cultures were primary, 21 days old and at about saturation density at the time of labelling. Thus the experimental conditions were identical for all three cell types.

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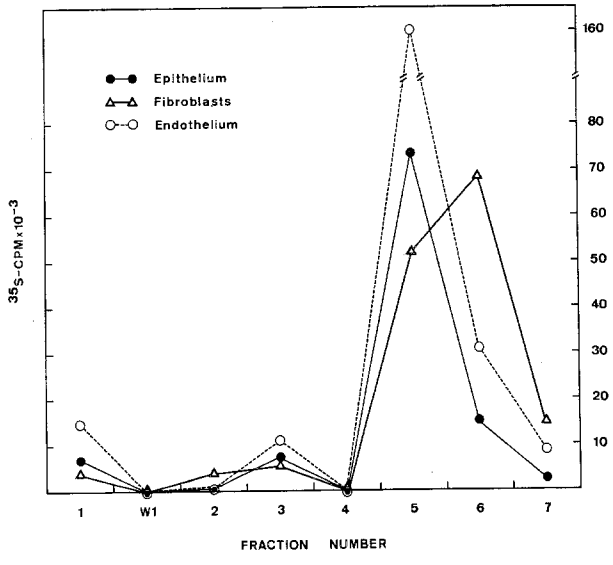
of each culture for 4 days. At the end of the exposure to the precursor, the medium was withdrawn and the cells homogenized. A small aliquot of the cell-homogenate was taken for protein determination. Cell-homogenate and medium were recombined, digested with pronase and thoroughly dialyzed. After treating with trichloracetic acid and ether, the infranatant containing the GAG was lyophilized. The powder was redissolved in a small volume 0.02 *M* NaCl/1% cetylpyridinium chloride (CPC) and added to a Whatman cellulose column. GAG-fractions were eluted with 7 specific solvents of increasing ionic strength according to ŠVEJCAR and ROBERTSON[7] and CONRAD[8,9] respectively. By this method, the GAG are separated into CPC-soluble GAG (fraction 1) and CPC-insoluble GAG (fractions 2–7). Between each fraction, the column was washed with 0.05% CPC. This CPC-concentration does not elute any GAG.

Radioactivity of each fraction expressed as percentage ± SD of the total eluted

Fraction number	Epithelium	Fibroblasts	Endothelium
1	7.6 ± 2.5	2.7 ± 0.1	5.7 ± 1.1
2	1.1 ± 0.3	1.9 ± 0.7	0.2 ± 0
3	6.6 ± 0.7	4.3 ± 0.7	4.9 ± 0.6
4	—	—	—
5	68.0 ± 3.2	33.2 ± 3.5	69.5 ± 4.1
6	13.9 ± 0.6	46.4 ± 3.1	15.3 ± 3.9
7	2.6 ± 0.3	11.4 ± 1.3	4.1 ± 0.7
Total protein of cultures (µg ± SD)	933 ± 25	361 ± 44	504 ± 43
CPM of total eluted (cpm × 10^{-3} ± SD)	98 ± 8	124 ± 31	204 ± 41

The data are calculated from 3 cultures of each cell type.



GAG-fractionation-pattern from one culture of each cell type. W_1 represents the missing radioactivity in the first wash, indicating that 0.05% CPC does not elute GAG.

Results and discussion. The results demonstrate that, in tissue culture, all three cell types of the rabbit cornea are able to synthesize GAG (Figure and Table). Interestingly the fractionation-patterns of the epithelial and the endothelial GAG are almost identical. The eluted GAG from both cell types have their highest peak of radioactivity in fraction 5. On the other hand, the radioactivity of the fibroblastic GAG is highest in fraction 6. According to CONRAD[9], the degree of sulfation increases from fraction 3 to fraction 7. Therefore the fractionation-patterns indicate that the epithelium and the endothelium synthesize predominantly GAG with a lower degree of sulfation, whereas the fibroblasts produce more highly sulfated GAG. Obviously the epithelium and the endothelium produce GAG which are not in common with the ones synthesized by fibroblasts. However, the endothelial cells and the fibroblasts possess some similarities: Both are of mesodermal origin and, referred to the total protein of cultures as a measure of the cells involved in synthesis, they incorporate about the same amount of sulfate into GAG, whereas the incorporation rate is considerably lower in the ectodermal epithelium.

The first fraction should contain keratan sulfate which is the only CPC-soluble GAG. The low radioactivity in this fraction makes it likely that only small amounts of keratan sulfate are synthesized by cultured corneal cells. This is in accordance with CONRAD and DORFMAN[10] who found that embryonic chick cornea stops synthesizing keratan sulfate after 48 h of culture.

In a recent communication[4], we have demonstrated by enzyme assay that fraction 3 and 5–7 of the epithelial and fibroblastic GAG comprise chiefly chondroitin sulfates.

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Dopamine-β-Hydroxylase Activity in Serum of Developing Rats

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Summary. After birth, the activity of dopamine-β-hydroxylase in rat serum increased up to 14 days maximal level and decreased gradually to reach a low level at 42 days which was about 14% of the activity of maximal activity at 14 days.

Dopamine-β-hydroxylase (DBH) is released into the blood stream from the sympathetic nerve terminals in sympathetically innervated organs and from the adrenals, and therefore serum DBH activity may reflect to some extent sympathetic nerve activity[2]. The activity of DBH in human serum was reported to increase markedly for the first 2 or 3 years of life reaching a high constant level during adulthood[3,4].

On the other hand, it has been found[5] that serum DBH activities both in spontaneously hypertensive (SH) rats[6]