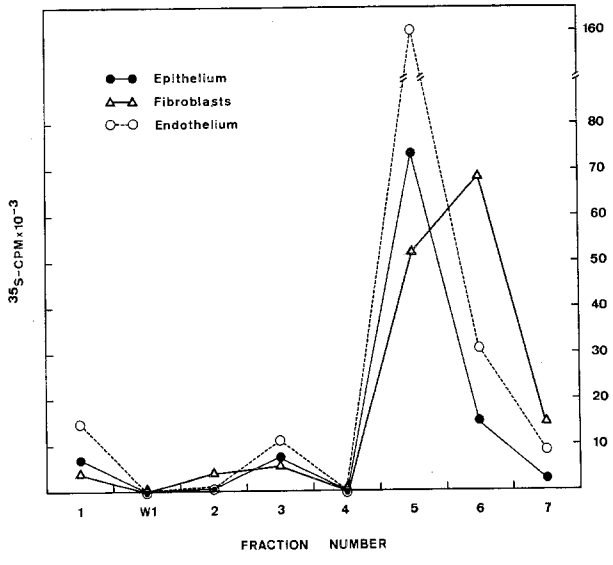
of each culture for 4 days. At the end of the exposure to the precursor, the medium was withdrawn and the cells homogenized. A small aliquot of the cell-homogenate was taken for protein determination. Cell-homogenate and medium were recombined, digested with pronase and thoroughly dialyzed. After treating 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]

Developmental changes in serum dopamine-β-hydroxylase activity serum protein and body weight of Wistar rats[a]

	Age (days)								
	1	4	7	10	14	21	28	42	
								(male)	(female)
Body weight (g)[b]	7.1 ± 0.3 (10)	10.6 ± 0.3 (10)	13.7 ± 0.5 (9)	18.1 ± 0.6 (9)	23.7 ± 0.9 (9)	40.0 ± 4.9 (4)	73.8 ± 4.3 (4)	139.0 ± 4.8 (5)	125.0 ± 2.9 (3)
Serum	Pooled serum from 5 rats[c]		Pooled serum from 3 rats[c]			Serum from a single rat			
Number of serum samples	2	2	3	3	3	4	4	5	3
Protein (mg/ml)	53.8 ± 1.2	59.8 ± 1.8	62.6 ± 4.0	65.2 ± 2.1	59.5 ± 5.0	57.6 ± 2.9	58.7 ± 1.8	63.3 ± 1.7	69.9 ± 3.9
Dopamine-β-hydroxylase activity									
(nmol/ml/min)	1.05 ± 0.13	0.44 ± 0.04	0.77 ± 0.36	0.87 ± 0.19	2.93 ± 0.54	1.90 ± 0.31	1.00 ± 0.16	0.41 ± 0.10	0.43 ± 0.04
pmol/mg protein/min	19 ± 2	7 ± 1	12 ± 5	13 ± 2	51 ± 13	34 ± 6	17 ± 4	7 ± 2	6 ± 1

[a] Values are mean ± SEM. [b] Number of rats in parenthesis. [c] Equal aliquots were obtained by the various animals pooled.

and normotensive Wistar-Kyoto rats, from which the SH rats originate were relatively high at 3 weeks of age decreasing markedly with age during development. The serum DBH activities in SH rats after 6 weeks of age were not significantly different from those in Wistar-Kyoto rats, but at 3 weeks of age about 2-fold higher serum DBH activity in SH rats was observed as compared with the activity in normotensive Wistar-Kyoto rats.

In order to see whether or not the decrease in serum DBH activity during development is a general phenomenon in the rat, we have examined in detail the developmental changes of serum DBH activity in another nonhypertensive Wistar strain.

Material and methods. Nonhypertensive adult male and female rats of Wistar strain were obtained from Shiibashi Co. in Tokyo. Male or female rats which were born from the adult rats in our laboratory were used for the experiments. Blood samples were obtained from rats by decapitation and exanguination into a test tube kept in ice. Serum was removed after centrifuging at $10,000 \times g$ for 10 min.

The enzyme activity was assayed by the method of NAGATSU and UDENFRIEND[7] as modified by KATO et al..[8] The assay is based on the spectrophotometric measurement of *p*-hydroxybenzaldehyde resulting from periodate oxidation of octopamine formed by DBH from tyramine. Octapamine (2.0 nmol) was added to a blank incubation as internal standard. The activity was expressed as octopamine formed, nmol/ml/min serum. To prove that the assay of DBH activity was not disturbed as a result of formation of endogenous inhibitors in serum during development, a sample of pure DBH from bovine adrenal glands was added to a serum sample as internal enzyme standard.

Protein was measured by the method of LOWRY et al.[9], using bovine serum albumin standard.

Results and discussion. Changes in body weight, serum protein and DBH activity during development of rats are shown in the Table. Body weight was increased slowly up to 14–21 days after birth and rapidly thereafter.

The concentration of serum protein changed only slightly after birth during the development, and therefore changes in serum DBH activity based on protein (nmol/mg protein/min) were also essentially similar to those based on the serum volume (nmol/ml serum/min).

Serum DBH activity was found to be fairly high at 1 day after birth, and decreased slightly up to 4 days. It then increased up to 14 days to reach a maximal level, which was about 3-fold higher than the level at 1 day. The activity decreased gradually after 14 days of age to reach a low level at 42 days which was about 40% of the activity at 1 day and about 14% of the maximal activity at 14 days.

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Rats were examined without selection of sex. However, at 42 days of age, there was not any significant difference in serum DBH activity between male and female rats.

The recovery of a pure DBH preparation from the bovine adrenal medulla that was added to incubation mixture was similar (95%) with each serum sample of various ages. This confirms that the observed developmental changes in rat serum DBH activity is not due to formation of endogenous inhibitors or activators in rat serum.

DBH activity in the brain was reported to increase progressively with maturation[10]. DBH activity in peripheral sympathetic nerves may also increase in a similar way as in the brain. In contrast, increase in body weight is slow during the first weeks and rapid after 2–3 weeks of age.

Developmental changes in serum DBH activity may be determined by the ratio between the rate of development of the sympathetic nerves and the rate of increase of the blood volume. The rapid increase in serum DBH activity up to 14 days may suggest that the development of sympathetic nerves after birth may be rapid up to 14 days as compared with the increase in the blood volume. Gradual decrease in DBH activity after 14 days of age may indicate that the rate of development of the peripheral sympathetic nerves in rat may become slow as compared with the increase of blood volume or serum protein after 14 days of age.

These results agree with our previous results in SH rats and Wistar-Kyoto rats in that serum DBH activities are relatively high only when they are young and decrease with age during development. Therefore, the elevation of serum DBH activity in young animals and its decrease during their development appears to be general phenomena in rats, and not to be specific for Wistar-Kyoto rats and SH rats.

Since serum DBH level can be determined as a steady state level between the rate of secretion from the sympathetic nerves and the rate of inactivation during circulation, the possibility of changes in the rate of inactivation of serum DBH during development must be also considered. This remains for further investigation.

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Die Cholesterin-7α-Hydroxylase-Aktivität der Rattenleber nach Hypophysektomie und nach Substitution mit Hypophysenhormonen

Cholesterol 7α-Hydroxylase Activity of the Rat Liver after Hypophysectomy and Administration of Hypophyseal Hormones

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Summary. In order to elucidate the role of the hypophysis in the regulation of cholesterol 7α-hydroxylase activity, male and female rats hypophysectomized on day 50 of life were treated with different hypophyseal hormones and tested on day 85 of life. Only a crude extract from human hypophysis and ovine prolactin was able to enhance the enzyme activity, thereby restoring the level of intact controls.

Nach Hypophysektomie erlischt der circadiane Rhythmus der Cholesterin-7α-Hydroxylase-Aktivität; sie bleibt auf ihrem in den Vormittagsstunden liegenden Tiefstwert stehen[2,3]. GIELEN et al.[2,4] führen die Folgen der Hypophysektomie auf einen Ausfall der Nebennierenrindenfunktion zurück, geben aber zu bedenken[4], dass «other factors might also control this enzymic activity». Auf der Suche nach solchen Faktoren wurden die Auswirkungen der Zufuhr verschiedener Hypophysenhormone auf die Cholesterin-7α-Hydroxylase-Aktivität hypophysenloser Tiere studiert.

Material und Methoden. Den hypophysektomierten Ratten (SPF-Stamm Chbb: THOM, Operation am 50. Lebenstag) wurde eins der folgenden Hormone bzw. Hormonpräparationen verabreicht: GH (human growth hormone, Präparation Dr. Schleyer, Ulm) 25 μg i.p. täglich, 9 Tage lang; PRL (ovine prolactin, Präparat NIH-P-S11) 125 μg s.c. 2× täglich, 11 Tage lang; TSH (bovine thyroid stimulating hormone, Thyreostimulin®) 40 mIU s.c. täglich, 16 Tage lang; ACTH (adrenocorticotropic hormone, ACTH-Depot-Schering®) 2 IU i.m. täglich, 10 Tage lang; PMS (pregnant mare's serum gonadotropins, Anteron®) 100 IU i.m. täglich, 6 Tage lang; CG (human chorionic gonadotropin, Primogonyl®) einmalige i.m.-Injektion von 100 IU 6 Tage vor Tötung der Tiere; HHE (human hypophyseal extract, Präparation Dr. Schleyer, Ulm) 0,1 ml (äquivalent 50 μg GH) i.p. täglich, 9 Tage lang. Tötung der Tiere am 85. Lebenstag, 24 h nach der letzten Injektion, 8 Uhr vormittags.

Zusammensetzung des Testansatzes in Anlehnung an MAYER et al.[5]: 0,1 ml Substrat-Puffer-Gemisch, pH = 7,4 (0,2 μCi [4-^{14}C]-Cholesterin, Phosphat-Puffer 100 m*M*, Cysteamin 35 m*M*), 0,05 ml Wasser mit den Komponenten eines NADPH-generierenden Systems (Glucose-6-phosphat 50 m*M*, NADP 5 m*M*, Glucose-6-phosphat-Dehydrogenase 0,035 IU) und 0,02 ml Mikrosomensuspension äquivalent 5 mg Leber in 0,25 *M* Saccharose. Präparation des Substrat-Puffer-Gemisches: Zusatz von Tween 20 (0,16 mg/μCi) zum in Benzol gelösten Substrat, Entfernung des Benzols im Vakuum unter N_2, Zugabe von Puffer-Cysteamin-Lösung (0,5 ml/μCi). Start der

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