conarii transection (cutting the post-ganglionic sympathetic fibres immediately prior to their entrance into the superficial pineal)[8], or to superficial pinealectomy[9]. 4 to 8 weeks later, hamsters were decapitated and their brains were quickly removed. The epithalamic region was frozen in isopentane cooled by liquid nitrogen. The tissues were prepared for fluorescent histochemistry according to the technique of FALCK-HILLARP[6]. 8 to 10μm thick paraffin sections were examined with a Zeiss dark-field microscope. High Speed Ektachrome and Tri-X films were generally exposed for 1 to 3 min.

Results. The terms, superficial and deep pineal, refer to the 2 pineal components as described by SHERIDAN and REITER[3]. In all 12 intact (sham operated) hamsters, the superficial pineal contained an extensive network of green to yellow-green fluorescent fibres which ranged in size from thin, individual fibres to thick fibrous bundles. Many of these fibres appeared to be associated with connective tissue trabeculae and were judged to be pervivascular nerves. In some favorable sections large green fluorescent fibre bundles were observed adjacent to the lateral aspect of the superficial pineal and were considered to be fibres of the nervi conarii. The deep pineal of the intact hamsters had an extensive network of intensely green to yellow-green fluorescent fibres. In 2 hamsters, the plexus was seen to be continuous with a large fibre bundle located in the stalk.

After bilateral superior cervical ganglionectomy, the number of green to yellow-green fluorescent fibres in both the superficial and deep pineal was greatly reduced in 75% (9 of 12) of the hamsters, while in 25% of the animals there was little discernible difference in fibre density with respect to sham operated controls. After bilateral transection of the nervi conarii, no intrapineal fluorescent fibres were observed in either pineal component in 44% (4 of 9) of the hamsters. In the remaining 56%, the superficial pineals contained very few fluorescent fibres while the deep pineal was completely devoid. After superficial pinealectomy, no green fluorescent fibres were observed in the deep pineal.

Discussion. Under appropriate conditions, the green to yellow-green fluorescent product, as seen in the present study, is due to the presence of norepinephrine (NE). NE has been shown to be the sympathetic neurotransmitter in the pineal[10,11]. In the present study, both the superficial and deep pineal components of intact hamsters were found to contain numerous green fluorescent fibres. In several animals many of these fibres were also seen in the stalk which connects the superficial and deep pineal.

The primary autonomic innervation of the mammalian pineal is believed to arise from the superior cervical ganglia[12]. In the rat, the green fluorescent fibres within the pineal gland disappear after superior cervical ganglionectomy[13,14]. After bilateral removal of the superior cervical ganglia or after bilateral transection of the nervi conarii in the hamsters, both the superficial and deep pineal lost most of their green to yellow-green fluorescence. Ganglionectomy also incapacitates the hamster pineal in terms of its antigonadotrophic capabilities[5,7], probably due to the fact that pineal complex cannot function without an intact sympathetic innervation.

The present study shows that the deep pineal mass remains intact after superficial pinealectomy. However, the green fluorescent product was almost completely lost after removal of the superficial pineal. Thus, as with the superficial pineal, the deep pineal also seems to be non-functional with respect to its inhibitory influence on reproduction if it is denervated, since in light-deprived hamsters with only superficial pinealectomy the deep pineal is incapable of suppressing gonadal function[5]. On the other hand, it is possible that the function of the deep pineal is entirely different from that of the superficial gland as suggested by WIKLUND[15]. The results suggest that the postganglionic sympathetic fibres which terminate in the deep pineal either pass in the vicinity or through the superficial pineal and are interrupted at the time of superficial pinealectomy.

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Effect of Chemical Sympathectomy, Adrenalectomy and Adrenergic α- and β-Blocking Agents on the Development of Hyperglycemia Induced by Streptozotocin in the Rat

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Summary. The sympathetic nervous system in the rat does not play any significant role in the streptozotocin-induced and tolbutamipe-induced hypoglycemia.

Recent investigations have shown that the effect of a number of pharmacological agents on insulin secretion may be mediated by α- and β-adrenergic pancreatic islet cell receptors; α-receptor stimulation by adrenaline inhibited glucose induced insulin release in the rat[1,2], monkey[3] and man[4-6]. However, β-receptor stimulation by isoprenaline stimulated insulin secretion in man[7], dog[8] and rat[9]. α-Adrenergic blocking agents, such as phentolamine, increased insulin secretion in man[10], baboon[11,12] and dog[13], whereas β-adrenergic blocking agents, such as propranolol and MJ-1999, inhibited insulin release in mice[14], dog[8] and man[15]. Both in vitro and in vivo studies showed that glucose-induced insulin release could be blocked in the rat sympathectomized by 6-hydroxydopamine[16].

The present study was designed to see the role of the sympathetic nervous system in the development of hyperglycemia following i.v. administration of streptozotocin. Chemical sympathectomy was produced by 6-hydroxydopamine (6-OHD). This substance has been

shown to have a selective effect in destroying the sympathetic nerve endings[17]. We also investigated the effect of adrenalectomy and α- and β-adrenergic blocking agents on the development of hyperglycemia induced by streptozotocin. In addition, it was thought of interest to see the effect of sympathectomy and α-adrenergic blocking agents on the hypoglycemic response induced by tolbutamide or phenformin.

Table I. Effect of adrenalectomy and 6-hydroxydopamine on the development and maintenance of hyperglycemia produced by i.v. administration of streptozotocin (50 mg/kg) in the rat

Treatment	Blood sugar mg/100 ml			
	24 h after		7 days after	
Controls	331 ± 29[a]	(17)	317 ± 29	(13)
6-Hydroxydopamine	267 ± 42	(14)	264 ± 15	(13)
6-Hydroxydopamine + adrenalectomy	336 ± 35	(16)	340 ± 35	(16)

[a] Mean ± SE. Nos. in parentheses indicate the number of rats. Adrenalectomy was done 5 days after i.v. administration of 6-hydroxydopamine (1 × 50 mg/kg). Streptozotocin (50 mg/kg i.v.) was injected 24 h later and blood samples collected 24 h and 7 days later.

Table II. Effect of phentolamine or Oxprenolol pretreatment on streptozotocin (50 mg/kg i.v.) induced hyperglycemia in the rat

Treatment	Blood sugar mg/100 ml			
	24 h after		7 days after	
Controls	448 ± 33	(14)[c]	407 ± 35	(13)
Phentolamine[a] (20 mg/kg i.p.)	396 ± 8	(16)	365 ± 18	(6)[b]
Oxprenolol[a] (20 mg/kg i.p.)	328 ± 16	(16)	332 ± 19	(12)

[a] Injected 30 minutes before Streptozotocin. [b] 9 animals out of 16 in this group died before 7 days. [c] Mean ± SE. Numbers in parentheses indicate the number of rats.

Table III. Hypoglycemic effect of tolbutamide and phenformin in normal and 6-hydroxydopamine treated rats[a]

Treatment and dose	Time between injection and blood collection (h)	Change in blood sugar Normal		6-OHD treated	
Tolbutamide (50 mg/kg p.o.)	2	− 29 ± 2	(14)	− 30 ± 1	(14)
Phenformin (100 mg/kg p.o.)	3	− 16 ± 6	(12)	− 19 ± 8	(11)

[a] 6-Hydroxydopamine was injected (100 μg/day) every alternate day for 14 days. In the case of tolbutamide, glucose primed rats fasted for 16 h were used. In the case of phenformin, streptozotocin induced diabetic rats were used (fasted for 3 h), since phenformin does not show activity in normal glucose primed rats. Animals were used 45 to 47 days after the last injection.

Methods. Female rats weighing from 140 to 160 g were rendered diabetic by i.v. administration of streptozotocin (50 mg/kg i.v.). All animals were fasted 16 to 18 h before giving streptozotocin. Blood samples were collected by orbital bleeding from these rats at 0, 4 and 24 h, and 1 week after i.v. administration of streptozotocin. 6-OHD was given in 2 dosage schedules. In one series it was given to rats of either sex beginning from the day of birth (100 μg/g s.c.) every alternate day until day 14 (totally, 7 injections) and the animals were used for the experiments after 45 to 47 days (Table II). 6-OHD was given at birth since it has been reported that chemical sympathectomy produced is complete and longer lasting[18]. In the second series, a single i.v. dose of 50 mg/kg was given and the animals were used 1 week later. Adrenalectomy was performed bilaterally and the animals were kept on 0.9% saline.

Results and discussion. Table I shows the effect of 6-OHD (50 mg/kg i.v.) on the development of hyperglycemia produced by i.v. administration of streptozotocin. As can be seen, 6-OHD pretreatment did not produce any significant change in the diabetogenic properties of streptozotocin. The blood sugar values of all the 6-OHD treated rats were comparable to controls (Table I). In the second series, the effect of streptozotocin on the adrenalectomized rats was observed. As can be seen from the Table, again there was no difference in the hyperglycemia produced in the normal and adrenalectomized rats following streptozotocin (Table I). 6-OHD at these doses has been shown to have a selective effect in destroying the sympathetic nerve terminals in the adult rat[19, 20].

Table II shows the effect of α-and β-adrenergic blocking agents on the hyperglycemia produced by streptozotocin. Pretreatment of rats with either phentolamine or

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Table IV. Effect of phentolamine or phenoxybenzamine pretreatment on tolbutamide-induced hypoglycemic response in the rat

Treatment	Dose (mg/kg)	Blood sugar (mg/100 ml/1 1/2 h)		p-values
Controls	—	93 ± 3.3	(14) [a]	—
Na-tolbutamide (s.c.)	100	63 ± 7.5	(16)	< 0.001
Phentolamine [b] (i.p.)	20	79 ± 2.3	(14)	< 0.01
Phenoxybenzamine [b] (i.p.)	5	86 ± 4.0	(8)	N.S.
Phentolamine [b] (i.p.)	20			
+		67 ± 4.3	(16)	N.S.
Na-tolbutamide (s.c.)	100			
Phenoxybenzamine [b] (i.p.)	5			
+		62 ± 1.3	(8)	N.S.
Na-tolbutamide (s.c.)	100			

[a] Mean ± SE. Number in parentheses indicate number of rats. [b] Injected 30 min before Na-tolbutamide. Blood was collected 1 1/2 h later.

Table V. Effect of Oxprenolol on tolbutamide-induced hypoglycemic response in the rat

Treatment	Dose (mg/kg)	Blood sugar (mg/100 ml/1 h)		p-values
Controls (saline)	—	86 ± 3.1 [a]	(8)	
Oxprenolol (i.p.) [b]	20	84 ± 3.5	(8)	N.S.
Na-tolbutamide (s.c.)	100	53 ± 2.1	(8)	< 0.001
Oxprenolol (i.p.) [b]	20			
+		50 ± 1.7	(8)	< 0.001
Na-tolbutamide (s.c.)	100			

[a] Mean ± SE. Number in parentheses indicates the number of rats. [b] Injected 30 min before Na-tolbutamide administration. Blood was collected 1 h after Na-tolbutamide injection.

Oxprenolol did not produce any significant change in the hyperglycemia produced by streptozotocin. Both these procedures did not have any effect on the acute (24 h) or maintenance of hyperglycemic response (7 days) following streptozotocin injections. Both these results seem to indicate very little involvement of sympathetic nervous system in the streptozotocin-induced hyperglycemia in the rat.

The hypoglycemic response of tolbutamide and phenformin in normal and 6-OHD treated rats is shown in Table III. There was no significant difference between the hypoglycemic response observed in the 2 groups. Hypoglycemic response to tolbutamide was also unaltered by pretreatment of rats with α-adrenergic blocking agents such as phentolamine and phenoxybenzamine (Table IV), or β-adrenergic blocking agents such as Oxprenolol (Table V). Our results with Oxprenolol are in accordance with the observations made by BORN and SPRATTO[21] using propranolol. These authors have shown that β-adrenergic blocking agents have significant effect on blood glucose response in the mouse but practically no effect in the rat. They have therefore emphasized that a species difference exists between the rat and the mouse with respect to the action of β-adrenergic blocking agents on blood glucose response. These observations seem to lend support to our inability to show any significant effect of α- and β-adrenergic blocking agents on the hypoglycemia produced by oral hypoglycemic agents, and any significant difference in the development and maintenance of hyperglycemia between normal and sympathectomized animals.

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Evolution des prostates rudimentaires d'*Ellobius lutescens* (Microtinae) en culture organotypique; action des androgènes[1]

Development of the Rudimentary Prostates of *Ellobius lutescens* (Microtinae) in Organ Culture; Effects of Androgens

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Summary. The structure of the rudimentary prostates of *Ellobius lutescens* is maintained intact after 6 days of organotypic culture in the absence of male hormones. Comparison with controls even shows a noticeable increase in the size of the epithelial cells. Adding male hormones to the culture medium does not modify the morphology of adult prostates, while it induces a sharp stimulation of immature prostates. In accordance with our previous results, these experiments show that the prostates of *Ellobius lutescens* lose their sensivity to androgens after puberty.

L'état rudimentaire des prostates que nous avons décrites chez *Ellobius lutescens* atteint d'oligospermie[3], nous a incités à entreprendre des essais de stimulation de celles-ci par des traîtements aux androgènes. In vivo, aucun traîtement par des doses fortes ou physiologiques de propionate de testostérone n'a réussi à modifier la structure des prostates d'*Ellobius* entiers, adultes ou impubères[4,5]. Ce n'est que chez des animaux castrés à l'état immature (à l'âge de 4 à 5 semaines) et traités pendant un mois par du propionate de testostérone que l'on observe une nette stimulation des cellules épithéliales prostatiques aboutissant à une significative augmentation du volume des prostates[5]. L'ablation précoce des testicules favorise donc la réponse des prostates aux androgènes. Ainsi, pour soustraire les prostates aux diverses influences de l'organisme, nous avons eu l'idée de nous placer dans les conditions de la culture organotypique. Les travaux de FEYEL-CABANES et al.[6], portant sur des organocultures de rats castrés traîtés par des androgènes, ayant montré qu'une restimulation de l'épithélium prostatique pouvait être obtenue in vitro, nous ont encouragés à entreprendre de telles expériences sur *Ellobius*. Nous rapportons ici nos observations histologiques concernant l'évolution des prostates d'*Ellobius* adulte et impubère, en culture organotypique, en présence ou en l'absence d'androgènes.

Matériel et méthodes. Animaux: environ 150 *Ellobius*, adultes et impubères (âgés de 5, 6, 7 ou 8 semaines) pro-