into the cultures 1.5 h prior to harvesting the cells, and autoradiography is used to reveal the late S labeling pattern. Occasionally in these autoradiographs, polyploid cells are found which show 2 different types of labeling within the cell, 1 for each diploid set as demonstrated in Figure 1.

This unusual labeling pattern is seen rarely in *Peromyscus maniculatus* cultures. However, in our studies on a male cell line of the Indian Muntjac, *Muntiacus muntjak* ($2n = 7$) established by WURSTER and BENIRSCHKE[1], this labeling pattern was observed frequently in polyploid cells. The culture had been pulse labeled with H^3TdR (2 μc/ml of growth medium) for 30 min. After labeling, the cells were rinsed with warm Hanks' solution and grown in fresh medium containing non-radioactive TdR (2 μg/ml). Samples were collected at 2 h intervals after labeling, and squash preparations were made for autoradiography.

Figures 2, 3, and 4 show polyploid cells containing 14 chromosomes which were collected at 5 h after H^3TdR addition. In Figures 2 and 3 one diploid set of chromosomes is labeled, while the other is not. In Figure 4 both diploid sets are labeled, but one shows an earlier DNA replication pattern than the other. The chromosomes in some of the diploid sets can also be differentiated by the degree of condensation. In Figure 2 the chromosomes with incorporated H^3TdR are 1.5–2 times as long as the unlabeled set, suggesting that one set lags behind the other in prophase coiling as well as in DNA synthesis. Figure 3 is an extreme example in this regard, with one diploid set in prophase and the other in metaphase. The frequency of occurrence of these heterophasic polyploid cells is presented in the Table.

These heterophasic cells are interpreted as the products of fusion between 2 diploid cells that are at different stages of the cell cycle. The 2 diploid elements may synchronize and enter S phase following the first mitosis after fusion. However, it is probable that some of these mitoses will be 'terminal' mitoses due to the inability of the 2 individual genomes to synchronize prior to division.

SANDBERG et al.[2] have described tetraploid mitoses in which one diploid set of chromosomes is labeled and one is unlabeled in a cell line derived from the blood of a patient with acute myeloblastic leukemia. In contrast to the Indian muntjac cells, the chromosomes within a given metaphase in the human cells did not vary in degree of condensation. The authors suggested that the polyploid cells observed originated from multinucleated megakaryocytes, plasma cells, or normoblasts.

Recently, JOHNSON and RAO[3] used synchronized HeLa cultures to show that fusion can be accomplished between 2 cells in different stages of the cell cycle. According to these authors, when G_2 or metaphase cells are fused with G_1 or S cells, an induction of chromosome condensation may occur resulting in aberrant chromosome morphology in the lagging nucleus. They called this phenomenon, which is very similar to the fragmentation induced by myxoviruses[4] or prolonged Colcemid treatment[5], premature chromosome condensation. Our observations confirm their conclusions that cells may fuse while in different stages of the cell cycle, but we found no examples of premature chromosome condensation. Perhaps this is because the fused cells we observed were not widely separated in the cell cycle. However, it is also possible that the technology employed by JOHNSON and RAO[3] (Colcemid treatment, double thymidine block, etc.) in synchronizing the cell populations resulted in metabolic imbalances which enhanced the occurrence of premature chromosome condensation. Under more normal growth conditions the induction of chromosome condensation in fused heterophasic cells may be reduced or absent[6,7].

Zusammenfassung. In Kulturen von *Peromyscus maniculatus* und in *Muntiacus muntjak* wurden Tetraploidzellen gefunden, die als Produkte von Zellfusionen aufgefasst werden. Es wird angenommen, dass die ursprünglichen diploiden Elemente sich im Zeitpunkt der Fusion in verschiedenen Phasen des Zellzyklus befunden haben.

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Frequency of heterophasic polyploid cells expressed as percent of mitotic cells[a]

Hour after H^3TdR pulse	Diploid mitotic cells (%)	Homophasic polyploids (%)	Heterophasic polyploids (%)
1	87.0	9.0	4.0
3	91.3	5.0	3.7
5	85.8	8.7	3.3

[a] Mitotic cells counted at each collection point = 250.

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Short Adrenergic Neurons Innervating the Female Urethra of the Cat

Fluorescence histochemistry has demonstrated that the uro-genital tract of different mammals receives an adrenergic nerve supply originating from ganglion formations located within or in the vicinity of the effector organ. This special type of short adrenergic neurons innervates the internal male accessory genital organs[1–3], to a certain extent the female reproductive tract[4–6] and the trigonum of the urinary bladder[7,8]. Besides, the

hypogastric nerves participate in the innervation of the female reproductive tract[5,6] and supply the bladder trigonum together with the pelvic nerves[7].

In the course of denervation experiments undertaken to clarify the origin of the adrenergic nerves to the female reproductive tract of the cat, it was observed histochemically that neuronal noradrenaline in the urethra was not affected by removal of the inferior mesenteric ganglia together with the hypogastric nerves. This prompted a further examination of the organization of the sympathetic innervation to this part of the female urinary tract.

Material and methods. The material included a total of 39 female cats, weighing between 2.0 and 3.3 kg. In 12 animals maintained under nembutal anesthesia (30 mg/kg i.p.) the inferior mesenteric ganglia together with 5 cm of the hypogastric nerves were excized through a midline abdominal incision. In 3 animals the sympathetic chain was removed bilaterally from the ganglion levels L_1 to S_4. All animals were killed 7 days postoperatively. The outcome of the surgical procedures was compared with that of 3 sham-operated and 19 unoperated animals.

The urethra from 14 of the unoperated animals and 7 of the animals subjected to hypogastric denervation was dissected out for fluorometric determination of noradrenaline[9,10]. Two unoperated animals were taken for measurement of urethral 5-hydroxytryptamine[11].

Pieces from the urethra of the remaining animals were processed for fluorescence microscopic demonstration of certain monoamines according to the formaldehyde method[12].

Results and discussion. In the normal cat, fluorescence microscopy revealed in all parts of the urethra a fairly large number of varicose nerve terminals emitting an intense green catecholamine fluorescence (Figure 1a). This finding agreed well with the presence of 3.55 ± 0.38 (S.E.M.) μg/g noradrenaline in the organ; neither adrenaline nor dopamine occurred in measurable amounts.

The nerve fibres ran isolated or in small fascicles in particularly large number in the circular smooth muscle layer (Figure 1a), intimately following the course of the muscle cells. Some nerves also occurred in plexus formations around blood vessels. Also the other layers of the smooth muscle wall received adrenergic fibres, though in a smaller number. The over-all amount of nerves was larger in the proximal portion of the urethra; the thickness of the wall did not differ overtly in the various regions, indicating that the innervation was more dense proximally.

In the wall of the proximal urethra, large ganglion formations were found (Figure 2) consisting of nerve cell bodies exhibiting a moderate green fluorescence as well as non-fluorescent, probably cholinergic cells[8]. Smooth, preterminal axons with a low fluorescence intensity

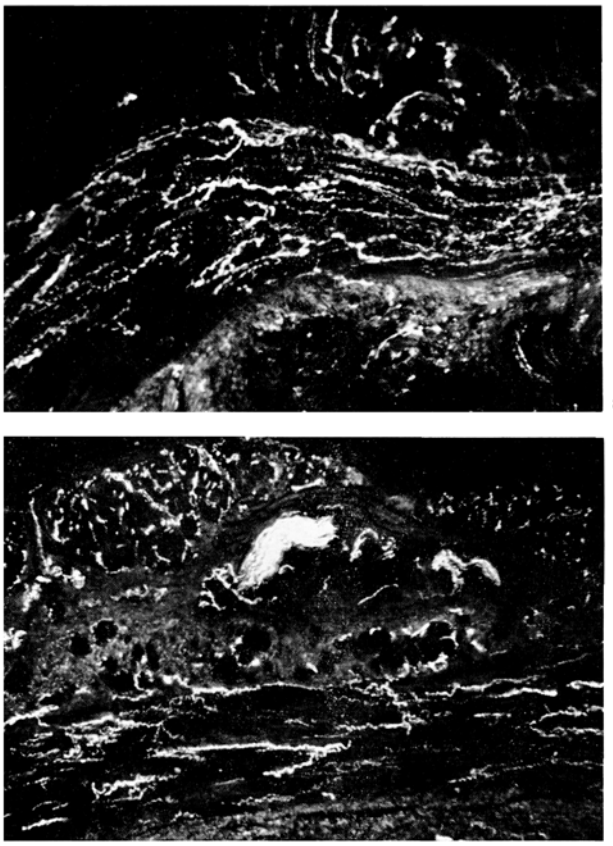

Fig. 1. Fluorescence photomicrographs of transversely sectioned cat urethra; lumen outside lower margin of the pictures. ×75. a) Untreated cat. Considerable number of varicose nerves, particularly in the circular muscle layer. b) After removal of the hypogastric nerves together with the inferior mesenteric ganglia, the number of nerve terminals is reduced, especially evident in the circular muscle layer. Note the thick bundle of fluorescent nerves between the outer longitudinal and the circular muscle layers.

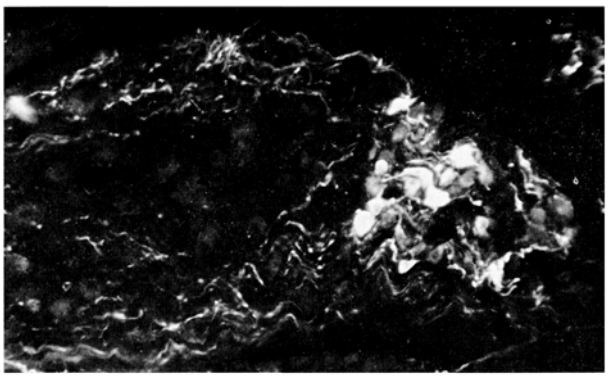

Fig. 2. Cat, hypogastric denervation. Large ganglion formation in the wall of the proximal urethra. Adrenergic nerve cells with green fluorescence of varying intensity to the right. The remainder of the ganglion consists of non-fluorescent probably cholinergic ganglion cells. Slightly fluorescent, smooth preterminal fibres leave the adrenergic cells and run through the ganglion (upper and lower parts of the picture). ×120.

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were seen to leave the cell clusters in bundles together with non-fluorescent fibres. Both types of perikarya were enclosed by fluorescent terminal nerve fibres in a manner suggesting a synaptic arrangement.

Flask-shaped cells resembling enterochromaffin cells were distributed in quite large number among the epithelial cells of the mucosa (Figure 3a). They emitted an intense yellow cytoplasmic fluorescence, probably derived from 5-hydroxytryptamine, which occurred in

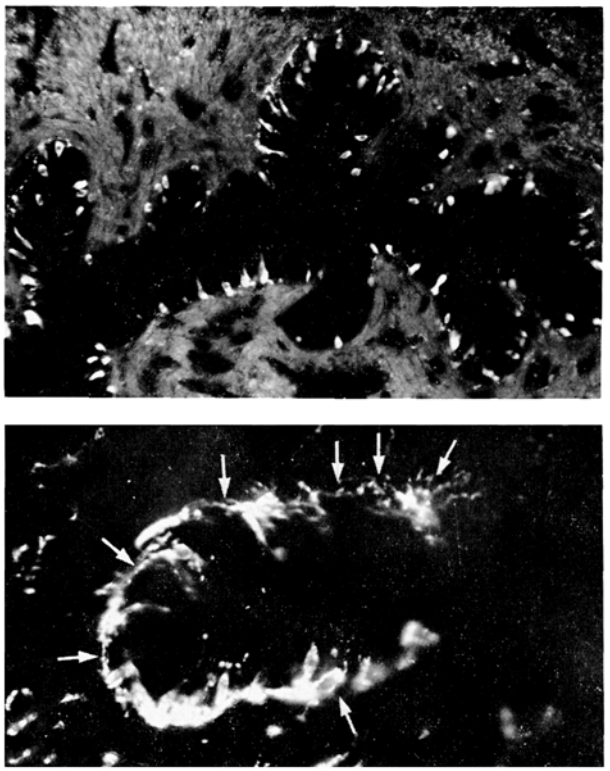

Fig. 3. Mucosa of urethra from cat subjected to hypogastric denervation. a) Large number of yellow-fluorescent, flask-shaped cells in the epithelium. ×100. b) At higher magnification, an abundancy of adrenergic nerve terminals (arrows) are seen to run in close contact with the base of the yellow enterochromaffin cells. ×240.

0.37 and 0.48 μg/g (2 determinations) in the urethra. A conspicuous accumulation of delicate adrenergic nerve terminals occurred in the immediate vicinity of, and even contiguous to, the base of the cells (Figure 3b). It is not unlikely that the arrangement represents a direct adrenergic innervation of the cells.

Hypogastric denervation produced a slight but clear decrease in the number of adrenergic nerves running in the smooth muscle wall (Figure 1b). This was consistent with the fluorometric determinations which revealed a significant reduction (Student's t-test: $0.02 > P > 0.01$) of urethral noradrenaline to 2.15 ± 0.44 μg/g.

No change was found in the number or fluorescence intensity of the urethral adrenergic nerves after removal of the lumbosacral portion of the sympathetic chain.

Conclusions. Fluorescence microscopy has shown the presence of adrenergic ganglion formations in the wall of the proximal urethra of the female cat. These ganglia contribute to the major portion of the urethral adrenergic innervation by way of short neurons. Fluorometric determinations of noradrenaline in combination with denervation experiments indicate that about $^1/_3$ of the postganglionic adrenergic innervation to the urethra derives from the inferior mesenteric ganglia via the hypogastric nerves. The sacral sympathetic ganglia (pelvic nerve) do not seem to contribute significantly to the sympathetic innervation[13].

Zusammenfassung. Die adrenergische Innervation der weiblichen Urethra bei der Katze wird mit Hilfe fluoreszenzmikroskopischer Technik dargestellt. Adrenergische Ganglien in der proximalen Urethra bestreiten den Hauptanteil der urethralen adrenergischen Innervation (kurze Neurone). Ein Drittel der adrenergischen Bahnen stammen aus dem Nervus hypogastricus.

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Proliferation of Spleen Cells from Mice Infected with Friend Virus in the Spleens of Unirradiated and Irradiated Mice

Infection of mice with Friend virus (FV) results in splenomegaly, which is due to proliferation of reticulum cells in the spleen[1]. Although injection of a spleen homogenate from FV-infected mice into normal hosts can cause proliferation of host spleen cells, it is not known whether FV-infected cells injected i.v. into normal mice can proliferate in the hosts. Chromosome marker, CBA/HT_6T_6, was used to identify the origin of cells[2,3].

CBA/HT_6T_6 mice were infected with a homogenate of FV-infected spleens. 7 days later, the spleens of the infected CBA/HT_6T_6 mice were teased, and samples of spleen-cell suspension, each containing 1×10^7 cells, were injected i.v. into normal CBA/H mice, unirradiated or irradiated with 400 R. T_6T_6 cells were scored in the spleens of the CBA/H mice 7 and 14 days after injection of the cell suspension, as described previously[4]. DNA synthesis was measured by injecting ^{125}I-UdR into the mice, and counting ^{125}I-UdR uptake in the spleen 18 h later[5].

The Figure shows ^{125}I-UdR incorporation and spleen weight in CBA/HT_6T_6 mice before and after infection with FV. For a normal CBA/HT_6T_6 mouse, IUdR uptake was 1.47 ± 0.24 ($\times 10^{-3}$) μCi per spleen, and the weight of the spleen was 56.5 ± 4.2 mg. For an FV-infected