der Mitochondrien sowie im sarkoplasmatischen Retikulum. Elektronendichtes TNBT-Formazan lässt sich weiterhin in optisch leeren Räumen der Muskulatur nachweisen, die durch den Frier- und Tauprozess während der Kryostatschnittanfertigung bedingt sind. Herdförmig stellen sich durch feinstgranuläre oder linearе Ablagerungen besonders die Membranen des sarkoplasmatischen Retikulums sowie die Aussenmembranen der Mitochondrien dar (Figur 2). Ob die TNBT-Formazanablagerungen vorzugsweise in, neben oder – wie bei der Negativkontrastierung – zwischen den Membranen erfolgt, ist zumeist nicht eindeutig zu entscheiden. In einigen Ultradünnschnitten konnten wir trotz gleichem präparativem Vorgehen keine Formazanablagerungen nachweisen. Die in den Gewebsschnitten gefundenen Granula entsprechen hinsichtlich der Elektronendichte den im Reagenzglas hergestellten TNBT-Formazan-Granula, deren Beständigkeit gegenüber Fixierung, Einbettung und Elektronenstrom nachwiesen werden konnte.

TNBT wird also bereits nach kurzen Inkubationszeiten der Kryostatschnitte an Gewebsstrukturen angelagert, so dass die Aussagekraft und Interpretation licht- und submikroskopischer Dehydrogenasenachweise durch eine unspezifische, nichtenzymatische Reduktion des TNBT («Fehllokalisation») beeinträchtigt werden kann. Die Bedingungen, die zur TNBT-Formazan-«Fehllokalisation» führen, sind komplexer Natur und gegenwärtig noch nicht völlig überschaubar. Als Faktoren, die für diese «Fehllokalisation» des TNBT-Formazans in Frage kommen, sind zu nennen: 1. eine unspezifische Reduktion des TNBT durch gewebseigene reduzierende Komponenten («nothing-dehydrogenase»-Reaktion). 2. eine Diffusion enzymatisch gebildeten Formazans, zum Beispiel in Gewebslücken, die während der Kryostatschnittanfertigung durch den Frier- und Tauprozess entstehen. Dementsprechend haben wir Formazanablagerungen in optisch leeren Räumen nachweisen können. 3. eine sclektive Bindung entweder des TNBT oder des TNBT-Formazans an bestimmte Zellstrukturen (Mitochondrien, sarkoplasmatisches Retikulum, Zellkernmembranen) ist sehr wahrscheinlich. Elektronenmikroskopisch haben wir TNBT-Formazanablagerungen in unmittelbarer Nähe von Lipoproteinmembranen nachweisen können. Damit liegen im Gewebsschnitt den Modellexperimenten von PEARSE und HESS[4] vergleichbare Bedingungen vor, bei denen TNBT eine Affinität zu Lipoidfraktionen zeigte. LOJDA[1], BROOKE und ENGEL[2] sowie FAHIMI und KARNOVSKY[3] haben ebenfalls aus lichtmikroskopischen Untersuchungen auf eine Bindung des TNBT an die Lipoproteinstrukturen geschlossen.

Bei Verwendung von TNBT als Reduktionsindikator zum licht- und submikroskopischen Nachweis oxydativer Enzyme muss daher ebenso wie mit Nitro-BT (PEARSE und HESS[4], BROOKE[5], BROOKE und ENGEL[2]) mit einer unspezifischen, nichtenzymatisch bedingten Lokalisation des TNBT-Formazans gerechnet werden, die die Ergebnisse experimenteller Untersuchungen beeinflussen kann.

Summary. Light- and electron-microscopic investigations reveal a non-enzymatic, non-specific deposition of TNBT-formazan in the sarcoplasma and along some membranes in the heart of the rat. These results are of interest with regard to the interpretation of histochemical identifications of oxidative ferments with TNBT as reduction-indicator.

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Adrenergic Innervation of the Male Reproductive Ducts in Some Mammals. II. Effects of Vasectomy and Castration

The normal distribution of adrenergic nerves to vasa deferentia of various species was observed by several authors[1–4] using a highly specific fluorescence histochemical method[5–7]. Cell bodies of adrenergic neurons in ganglia near the prostatic end of the vas deferens synapse with preganglionic fibres of the hypogastric nerves[2,4,8], and give rise to adrenergic axons of the vas deferens and adjacent organs[9–11]. The present authors[12] recently surveyed the testis and its associated ducts in several mammals with the aim of determining differences in distributions of adrenergic neurons in various regions. This study was undertaken to obtain experimental evidence concerning the course of the adrenergic innervation, and to determine if alterations might result from castration and reduced male sex hormone activity.

Histochemical procedures used were referred to previously[12], and followed the routine technique of this laboratory for noradrenaline (NA) localization by fluorescence microscopy[2,13,14]. 6 Sprague-Dawley albino rats (160–200 g) were uni- or bilaterally vasectomized by removing a one cm section of the duct. In unilateral cases, opposite unoperated ducts served as normal controls. Comparisons also were made with unoperated specimens. In bilateral operations, one vas deferens was completely

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sectioned, but blood vessels along the anterior margin were not disturbed on the opposite side. Tissues were obtained from the remaining ends of the operated ducts at 2, 6 and 14 days after operation, but 1 rat was retained for 6 months. One unilaterally vasectomized cat was sacrificed after 6 days.

On the epididymal side of the site of vasectomy, fluorescent adrenergic terminals, normally present, were not evident in the cauda epididymidis, indicating that the reactive NA disappears from the axons in 2–3 days (compare Figures 1 and 2). When blood vessels along the anterior border were not removed, adrenergic terminals were observed in the anterior muscle wall of the vas deferens, but were absent mainly from its posterior wall and the cauda epididymidis. Muscular atrophy was not apparent even after long-term vasectomy (6 months), but, in this case, the existence of widely separated, less frequent, and uniformly distributed terminals suggested reinnervation from the severed axons at the prostatic end.

On the prostatic sides of sectioned vasa deferentia no alterations of the adrenergic terminals or their varicosities were apparent. However, a striking increase of fluorescence intensity was observed in the non-terminal nerve trunks in this region, indicating an accumulation of reactive substances in the axons[14,15].

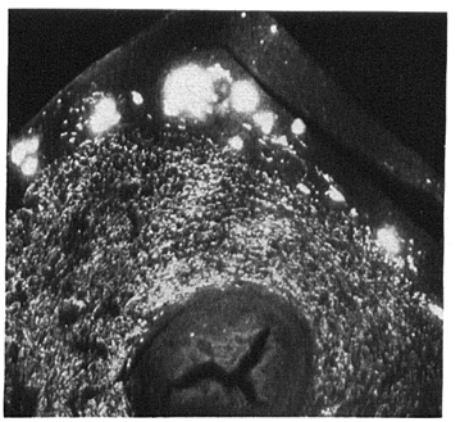

Fig. 1. Vas deferens, rat, from the prostatic side after vasectomy. Varicose terminals in musculature appear normal but accumulation of fluorescent material is extensive in the non-terminal nerve trunks. × 45.

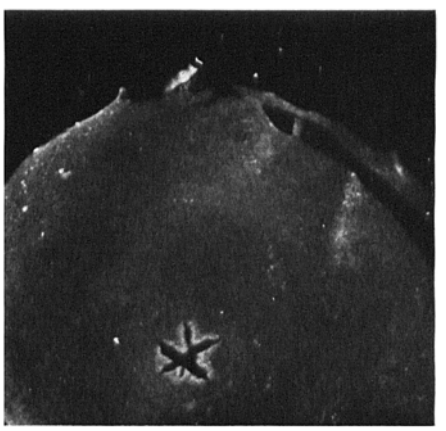

Fig. 2. Vas deferens, rat, from the epididymal side after vasectomy. Complete absence of fluorescent terminals and trunks. All white spots in photograph are artifacts. × 45.

Ten adult rats were castrated totally and sacrificed 30, 60 and 180 days later. The effects were associated with the general reduction in size of epididymides and vasa deferentia. Normally, adrenergic terminals are distributed in smooth muscle walls of the vas deferens and cauda epididymidis. Before castration, adrenergic terminals penetrate the muscle walls of epididymal ducts, but afterwards, they are located mainly along the outer margins of the muscle layer, as they normally appear in blood vessels. In the vas deferens, the reduced thickness of the muscle wall results in a much closer and more dense adrenergic ground plexus. Terminals are thrown into slight folds, and varicosities seemed less sharply defined, smaller, and more difficult to identify (Figures 3 and 4). Background fluorescence also was somewhat increased in castrates. While these differences in appearance might result from technical errors, the observed effects were consistent and appear to reflect altered tissue states after castration.

The occurrence of synapses between the hypogastric nerves and the adrenergic neurons innervating the vas deferens has been demonstrated by the failure of denervation after hypogastric nerve section[8,11]. The present results provide the further positive evidence that sectioning of the vas deferens results in the disappearance of the green fluorescent materials from the terminal axons on the post-ganglionic side of the lesion. In the cat, however, adrenergic nerves to the ductuli efferentes and caput epididymidis[12] remained present after vasectomy, but

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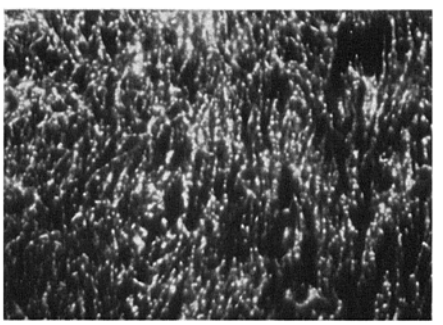

Fig. 3. Vas deferens, normal adult rat, showing typical varicose adrenergic terminal plexus in smooth muscle. Compare with Figure 4. × 210.

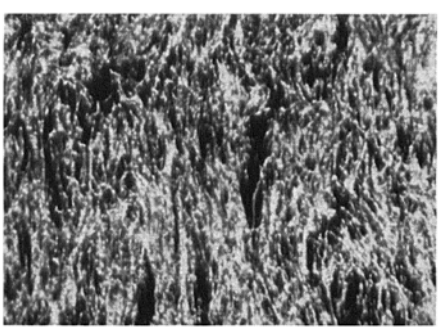

Fig. 4. Vas deferens, castrate rat. Abundant numbers of terminals occur, often superimposed upon each other because of muscle atrophy. Note smaller varicosities. Compare with Figure 3. × 210.

the plexus to the cauda epididymidis and epididymal vas deferens was not visible. Adrenergic nerves evidently reach the caput epididymidis by way of the superior spermatic nerve from the lumbar sympathetics in this species.

After castration, the adrenergic plexus was distributed to the smooth muscle in a manner that recalled the distribution of the cholinesterase reactive plexus of the rat epididymis[16,17]. In the cauda epididymidis, it appeared also to be confined mainly to the peripheral surface of the duct walls. In normal animals this is true for the more weakly innervated ducts of the upper cauda and lower corpus epididymidis. The effect was more readily apparent in the ChE-positive plexus than in the adrenergic one, where no clear reduction in fluorescence intensity could be recognized in individual axons. Absence of male sex hormones has only slight effects on the morphology of the adrenergic nerves to the male reproductive ducts, attributable mainly to the simultaneous atrophy of smooth muscle elements and associated tissue changes[18].

Résumé. Après vasectomie complète, pratiquée sur des rats, la fluorescence des terminaisons nerveuses, ainsi que la substance transmettrice, disparaît le long du canal déférent et de l'extrémité de l'épididyme en deçà de la lésion. Chez les chats, les terminaisons nerveuses dans les canalicules efférents et la tête de l'épididyme ne disparaissent pas après l'opération. Dans la direction de la glande et du ganglion prostatique, la matière fluorescente s'accumule dans les troncs nerveux, et les terminaisons nerveuses restent visibles. Après castration, quelques modifications compensatrices ont été observées.

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Influence of Hypercapnia on the Noradrenalin Content of Sympathetically Decentralized and Innervated Skeletal Muscle

Hypercapnia results in increased sympathetic discharge with augmented catecholamine release from the sympathetic nerves[1] and the adrenal medulla[2]. EULER and HELLNER-BJÖRKMAN[3] found that increased sympathetic nerve activity had no influence on the noradrenalin content of cat spleen, heart, liver or kidney. This suggested that in these tissues noradrenalin synthesis in the adrenergic nerves keeps pace with release also during sustained hyperactivity.

Recent studies have shown that the noradrenalin content of certain sympathetically innervated tissues can be more easily altered by changes in sympathetic impulse activity than hitherto expected. Prolonged electrical stimulation of the sympathetic nerves to cat skeletal muscle[4] and rat salivary gland[5], with frequencies considered to be within the physiological range, results in depletion of the noradrenalin content of these tissues.

The present study was undertaken to investigate whether an increased sympathetic activity induced by inhalation of high concentrations of CO_2 could affect the noradrenalin content of the adrenergic nerves in cat skeletal muscle.

Cats weighing 2.1–3.4 kg were anaesthetized with sodium pentobarbital i.v. and maintained on artificial respiration via a tracheal cannula. Gallamine HCl (50 mg/kg) was given to obtain complete muscle relaxation. One of the lumbar sympathetic chains was isolated by an abdominal approach and transected at the level of L4-L5. Gas mixtures containing 100% oxygen or 25% CO_2 in oxygen were administered by means of the respiration pump for a period of 2 h, after which the gastrocnemius and tibialis anterior muscles from both legs were removed and placed in ice-cold 0.4 *N* perchloric acid. The muscles were homogenized with an Ultra-Turrax homogenizer. The noradrenalin content of the extracts was determined essentially according to HÄGGENDAL[6].

Results and discussion. The presence of 25% CO_2 in the inhalation mixture did not significantly change the noradrenalin content in sympathetically decentralized gastrocnemius muscle (90 ± 7 ng/g in animals breathing 100% oxygen versus 97 ± 14 in animals given 25% CO_2 in oxygen). On the other hand, there was a marked depletion of the noradrenalin content in the muscles with intact sympathetic outflow (Table). When compared on a % basis with the content in the contralateral decentralized muscles from the same animal, the difference was significant ($p < 0.01$). Although the number of experiments on the tibialis anterior muscles is small, the data indicate the same changes as found in the gastrocnemius muscles. Also in animals breathing 100% oxygen, the noradrenalin values in the innervated muscle tended to be low, but the difference was not statistically significant.

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