Contrary to our observations, ABRAMS et al.[10] have found an increased sensitivity to intra-arterially administered epinephrine and norepinephrine in human forearm following an arterial occlusion. This vascular bed, however, is complicated by parallel coupling of muscular and cutaneous circulations. A redistribution of blood flow from one region to another could be responsible for this finding[11].

The remarkable similarity of the results obtained with both drugs used in our experiments indicates that the mechanism responsible for the depression of pressor respones during the reactive hyperemia is probably nonspecific in nature. If the decrease in sensitivity of vascular bed to vasoconstrictor stimuli during the local hypoxia is a general phenomenon, it can be an important regulating mechanism helping to improve the local oxygen supply of tissues by diminishing the effect of any vasoconstrictor substance coincidentally present in the arterial wall.

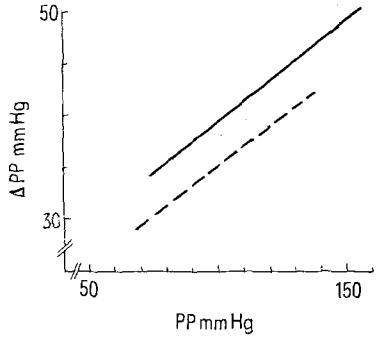

Fig. 3. The relationship between vasoconstrictor responses of canine mesenteric vascular bed (△ PP) to single doses (ED_{40}) of angiotensin (full line) or norepinephrine (broken line) and initial perfusion pressure (PP). Changes in the initial perfusion pressure were induced by preceeding arterial occlusion.

Zusammenfassung. Nachweis, dass die Reaktion der mesenterialen Blutgefässe beim Hund nach einmaliger intraarterieller Injektion von Angiotensin oder Noradrenalin während der durch die 2,5 min andauernden arteriellen Oklusion ausgelösten reaktiven Hyperämie herabgesetzt wird. Diese Verminderung der vasokonstriktorischen Reaktion dürfte insbesondere auf eine Sensibilitätsverminderung der Gefässmuskeln auf die Pharmaka und dessen bei der reaktiven Hyperämie verminderten pO_2 in Blut und Gewebe zurückzuführen sein.

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Cytochemical Localization of 5-Hydroxydopamine in Adrenergic Elements of Cat Adrenal Medulla

A number of experiments by different researchers have demonstrated atypical nerve fibres in the adrenal medulla that do not conform to the widely held principle of preganglionic cholinergic innervation[1–3]. Denervation of the adrenal gland results in the persistent presence of nerve fibres that do not degenerate, even after sectioning of the splanchnic nerve as proximally as the coeliac ganglion[1] or after sectioning the thoracic and first two lumbar spinal nerves[2]. Light microscopy clearly demonstrates bipolar or multipolar nerve cells in the adrenal medulla[3,4] while adjunct cholinesterase reactions reveal additional fibres that either are cholinesterase negative[5] or demonstrate pseudocholinesterase activity following denervation[3]. In addition with electron microscopy postganglionic nerve ending morphology has been described formerly in dogfish[6] and in toad adrenal medulla[7].

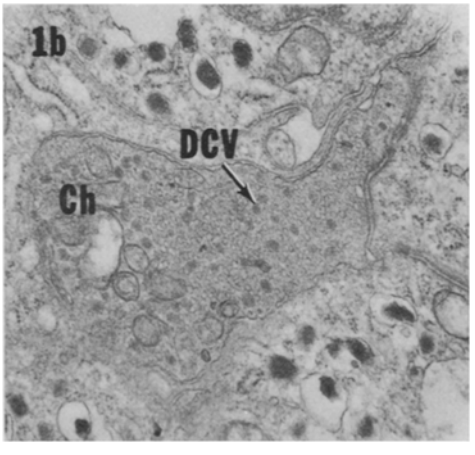

Fig. 1. b) Cholinergic (Ch) nerve ending of control cat adrenal medulla. A typical synapse at the chromaffin cell surface is seen; there are dense cored (DCV) and hollow vesicles neither demonstrating positive reaction for NE or 5-OHDA. ×20,332.

The question of whether or not adrenergic nerves are present in the adrenal medulla still remains due to the lack of a technique that distinguishes histochemically adrenergic from the greater number of cholinergic nerve fibres and endings. 5-Hydroxydopamine (5-OHDA) provides a precise labelling of adrenergic nerves as determined in earlier experiments examining other organ structures[8,9].

5-Hydroxydopamine has been shown to be selectively taken up by adrenergic nerve fibres and accumulated in nerve ending vesicles. Qualitative procedures done in this laboratory have demonstrated a definite reaction of precipitate formation between 5-OHDA and combination

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glutaraldehyde and molybdate solution. Therefore, thsi study demonstrates a localization of biogenic amine storage sites in nerve elements of the adrenal medulla using 5-OHDA, a 'false' adrenergic transmitter, as a marker. No attempt is made to examine the problem of adrenergic vs. cholinergic innervation of the adrenal medulla. This is a highly involved physiological problem, and the subject of further investigations which are in progress.

Methods and materials. Test animals used were mature male cats each of which was kept in a light dark regulated room and sacrificed at the same time each day to avoid diurnal effects[10]. The experimental group was given 3 injections of 75 mg/kg 5-OHDA i.p. over a period of 36 h at 12 h intervals[11]. 4 h following the final injection experimental and control animals were anesthetized with sodium pentobarbital and bilateral adrenalectomies were performed. Following rapid removal the tissue was fixed by immersion in 4% glutaraldehyde, 1% sodium molybdate in 0.2 *M* sodium cacodylate buffer at pH 7.2 for 2 h[12]. Following a 2-h molybdate treatment small blocks of tissue were treated with 1% osmium tetroxide in cacodylate buffer for 1 h, dehydrated in scaled alcohol solutions, and embedded in maraglass. Preparation of experimental and control tissues followed identical procedures. Thick and thin sections were made on a LKB Ultratome III, and electron microscopic photography done with a JEOL 100-B4 electron microscope. On grid staining was with lead citrate.

Observations. Control animal tissue was examined on stained and unstained grids. There is a positive reaction for norepinephrine (NE) containing granules; this positive reaction is not seen outside of NE containing cells. Sections demonstrate typical cholinergic nerve endings[13] evident at chromaffin cell surfaces; there is no positive reaction for NE (Figure 1b). In addition fibre bundles observed in cell-cell interstices demonstrate no distinguishing morphological characteristics that might indicate nerve fibres that are of different origin than those that surrounding. These include myelinated, unmyelinated, and denuded nerve fibres; all contain no positive reaction for NE. Examination of the experimental tissue reveals a comparable number of cholinergic nerve endings at chromaffin cell surfaces; they are indistinguishable from their control counterparts. There are, however, atypical nerve fibres in the chromaffin cell interstices (Figure 1a) unlike any observed in the control.

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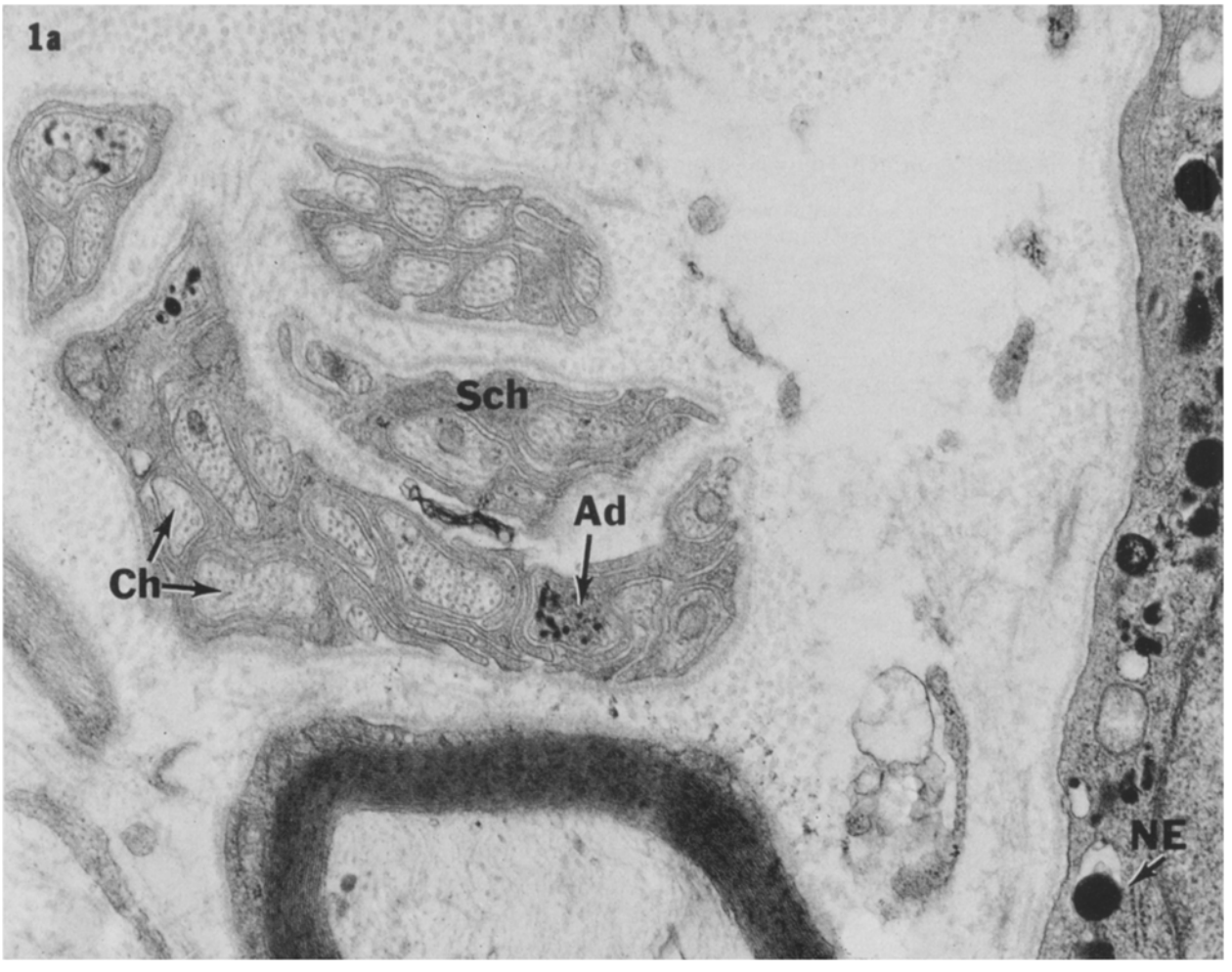

Fig. 1. a) Numerous unmyelinated nerve fibres enclosed in Schwann (Sch) cells. 3 of the nerve fibres are adrenergic (Ad) and contain positive reaction for 5-OHDA. The reaction product is as dense as that of norepinephrine (NE) containing granules. ×14,000.

Reaction positive for 5-OHDA is apparent in many but not all nerve fibres in the adrenal medulla. These 5-OHDA positive fibres may be characterized by their unique 1. morphology, 2. geographic location relative to NE containing cells and 3. their uptake of 5-OHDA. The reaction product is equivalent to the density of NE containing granules in both stained and unstained specimens. The nerve fibres are primarily of the 'bouton en passage'[3] variety containing large vesicles completely filled with positive material (Figure 2a). These are distinct from the dense cored vesicles typical of cholinergic nerve endings both in terms of 1. the density of the reaction product (equivalent to density of epinephrine granule density) and 2. the amount of dense material the granule contains. (The cholinergic dense cored vesicle contains less dense staining material than a comparable vesicle in an adrenergic nerve ending[8, 11]). Adrenergic nerve fibres also contain fewer vesicles that are empty or unfilled with positive reacting material. Some of the nerve elements appear more as a 'terminal bouton' or, more likely, axon varicosity. They contain, in addition to some mitochondria, the larger filled vesicles and fewer, usually filled, smaller vesicles (Figure 2b). These axon varicosities are not observed in the synaptic configuration of the classical cholinergic nerve ending at the chromaffin cell surface. Dotted vesicles are also not seen[14].

Areas in the adrenal medulla exhibiting bundles of 5-OHDA labelled nerve fibres are consistent and can be specifically characterized. Free nerve fibres are apparent only in the collagenous interstices of NE containing cell and perivascular areas. In these interstitial areas there are numerous instances of unmyelinated nerve fibres with positive reaction for 5-OHDA. In the majority of cases the 'boutons en passage' are enveloped in a Schwann cell (Figure 2a). Up to now, 5-OHDA labelled nerve endings have not been observed adjacent to NE cells. Often the axons and varicosities are located within approximately

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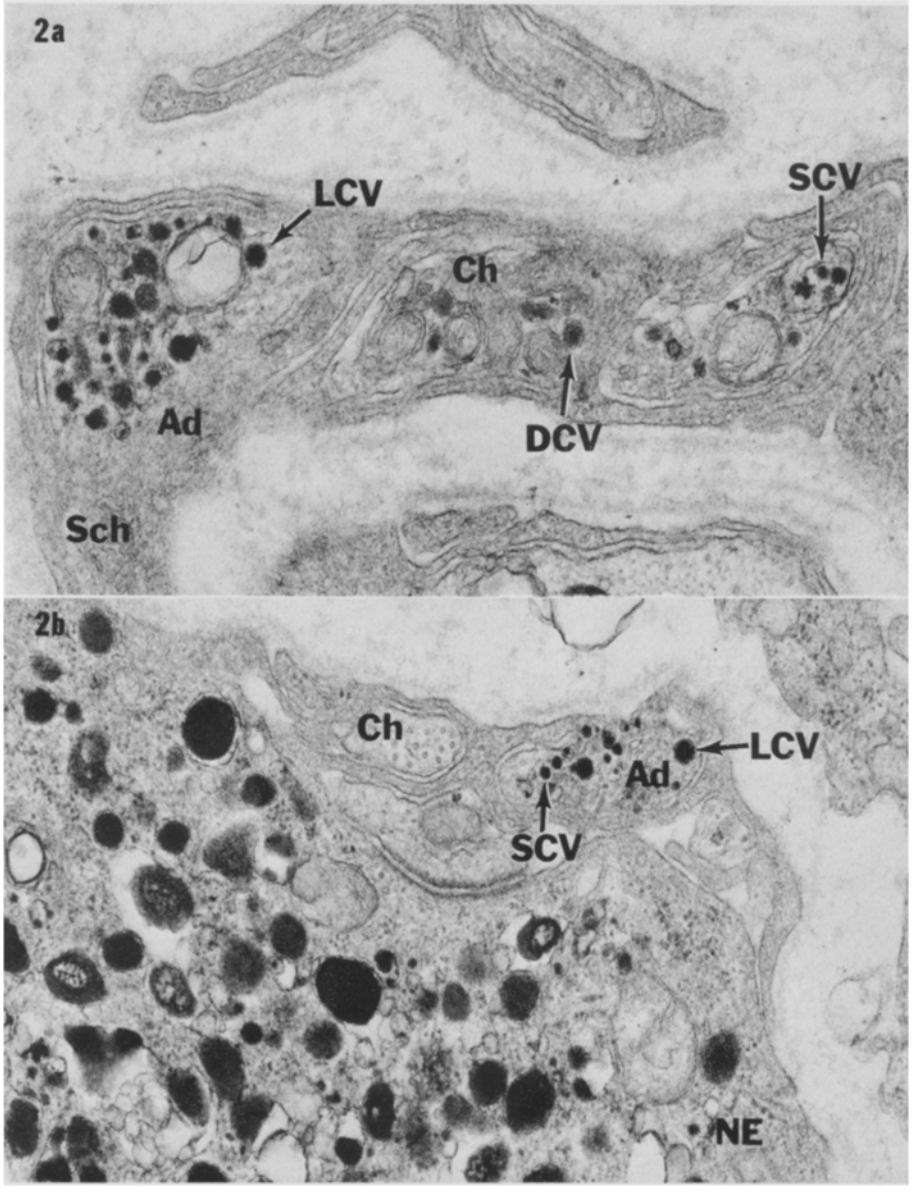

Fig. 2. a) Two probable adrenergic (Ad) axon varicosities containing large completely filled vesicles (LCV), small completely filled (SCV) and empty vesicles. Mitochondria are also present. Note, the cholinergic (Ch) nerve fibre with dense cored vesicles (DCV), mitochondria, and no positive reaction for 5-OHDA. The 3 nerve fibres are enveloped in a Schwann cell and unmyelinated. × 30,000. b) Adrenergic (Ad) 'bouton en passage' adjacent to a norepinephrine (NE) containing cell. Note that an adrenergic fibre contains large completely filled vesicles (LCV) and numerous small completely filled vesicles (SCV) with positive reaction for 5-OHDA. There is no corresponding positive reaction in the cholinergic (Ch) axon. × 13,500.

300 Å of the chromaffin cell membrane, and at these times they are devoid of a Schwann cell sheath.

Discussion. TRANZER et al.[15] and CHIBA[11] have established the reliability of the efficiency of 5-OHDA as a marker of amine storage vesicles in adrenergic nerves. Though the presence of noncholinergic neural elements in the adrenal medulla has been alluded to by many experiments, a precise demonstration has not been possible. Evidence from this experiment reveals no morphological change in cholinergic nerves in the adrenal medulla following 5-OHDA administration; however, a marked accumulation of electron dense material in other nerve fibres is obvious indicating adrenergic properties.

The different vesicle types seen in the 5-OHDA labelled adrenergic fibres are of 3 types, e.g. 1. large completely filled vesicles, 2. smaller dense cored and 3. empty vesicles. When present these represent morphologically distinct structures as opposed to probable stages of filling as previously reported by CHIBA[11].

The localization of labelled fibres is distinctly and significantly associated with NE containing cell areas. Though the presence of labelled fibres in epinephrine areas is a possibility such a situation has not been observed. The association of the adrenergic fibres exclusively to NE cells indicates a highly specific functional activity.

The significance of adrenergic function in the adrenal medulla is beyond the scope of this experiment. The demonstration of adrenergic nerves does, however, readily supply a plausible explanation of heretofore unexplained findings particularly the incomplete degeneration of adrenal medullary nerve fibres following denervation[1,2] and continued pseudocholinesterase activity after denervation of the adrenal gland[3]. Postganglionic adrenergic nerves do not degenerate following sectioning of preganglionic axons and demonstrate a different nerve fibre and ending morphology similar to that described by PIEZZI[7]. Further substantiation of adrenergic nerve presence in the adrenal medulla is ongoing in this laboratory involving monoamine oxidase inhibition as in the pineal[15], in addition to 6-hydroxydopamine degeneration of these same adrenergic medullary nerve fibres. These results concommitant with the 5-OHDA demonstrations establish adrenergic nerve fibre presence in the adrenal medulla. This is an imposing reality when viewed in terms of the implied nerve control mechanisms of adrenal medullary secretion and this selective cytochemical study which demonstrates an adrenergic nerve presence illuminates the significance of adrenergic function in the adrenal medulla. The intricacies of such a functional specificity must await the results of further detailed physiological investigations.

Zusammenfassung. Nachweis von mit 5-Hydroxydopamin markierten Noradrenalin-Bläschen in postganglionären Nerven, wodurch die Bläschen eine elektronendichte, mit dem Elektronenmikroskop sichtbare Substanz enthalten. Solche Nerven mit positiver Reaktion für 5-OHDA werden im Nebennierenmark erwachsener Kater und nur im Gebiet noradrenalinhaltiger Zellen nachgewiesen.

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10 October 1973.

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[16] The authors wish to express their sincere thanks to Mrs. JANE CRICK for her competent technical assistance.

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Ergebnisse des Makrophagen-Migrations-Testes während der Frühphase der Rauscher-Leukämie

In letzter Zeit wurden immunologische Vorgänge während der Infektion mit leukämogenen Viren beschrieben. Es wurde zuvor gezeigt, dass sich die zelluläre, wie auch die humorale immunologische Antwort im Verlauf experimentell erzeugter Leukämien verändert (CEGLOWSKI et al.[1], SALAMON[2], WEDDERBURN et al.[3], PETERSON et al.[4] und DENT et al.[5]). Bislang blieb allerdings die Pathodynamik der Immunreaktion während der Leukämogenese ungeklärt. Die von RAUSCHER[6] beschriebene Leukämie unterscheidet sich aufgrund ihres phasischen Verlaufes von anderen experimentellen Leukämien. Während einer Frühphase unmittelbar nach der Verimpfung proliferieren erythropoetische Elemente in der Milz. Die dabei gleichgeschalteten morphologischen Veränderungen lassen nach eigenen Beobachtungen (KOMITOWSKI et al.[7]) Zusammenhänge mit einem immunologischen Geschehen vermuten.

Der von GEORGE und VAUGHAN[8] entwickelte Hemmtest der Makrophagen-Migration bietet die Möglichkeit, zelluläre immunologische Prozesse zu erfassen und zu quantifizieren. Es erschien uns interessant, diesen Test zur Prüfung auf mögliche Änderungen im immunologischen Verhalten während der Frühphase der RAUSCHER-Leukämie einzusetzen.

Als Versuchstiere dienten 78 weibliche 8–10 Wochen alte NMRI-Mäuse. 39 Tiere wurden mit RAUSCHER-Virus infiziert, die verbliebenen Tiere dienten als Kontrolle, denen i.p. physiologische NaCl-Lösung verimpft wurde. Die RAUSCHER-Leukämie wurde durch i.p. Verimpfung von 0,2 ml eines zellfreien Überstandes aus homogenisierten leukämischen Milzen des gleichen Stammes erzeugt. Aus den Milzen der Veruschs- und Kontrolltiere stellten wir mit Hanks BSS-Lösung Suspensionen her. Wir hämolysierten die Erythrozyten nach der Methode von SHORTMANN et al.[9] und resuspendierten die Zellen in «Minimal Essential Medium» (MEM). Dem Nährmedium der Kontrollgruppe und der Rauschergruppe wurde in je einer Versuchsreihe Rauschervirusantigen zugesetzt. So erhielten wir von jeder Gruppe zwei Versuchsreihen: eine Reihe ohne Antigen und eine Reihe mit zusätzlichem Antigen.

Das gewonnene Material zogen wir in Mikrokapillaren auf und zentrifugierten 3 min bei 500 *g*. Die Kapillaren wurden über dem entstandenen Pellet abgetrennt, in sterile Plastikkammern eingebracht und in einer mit Luft und CO_2 angereicherten Atmosphäre über 24 h bei 37 °C inkubiert. Danach haben wir die Areale der aus den Kapillaren ausgewanderten Zellen mit einem elektronischen Bildanalysator (Zeiss Mikrovideomat) nach einer neu entwickelten Methode (KURKA et al.[10]) ausgemessen.

Bereits 24 h nach Verimpfung des RAUSCHER-Virus ist die Makrophagenwanderung deutlich gehemmt. Dieser Effekt ist nach Zugabe von Antigen noch ausgeprägter