

Fig. 4. Nuclei and enlarged nucleoli of 60-hour-old explants on basal medium. $\times 1250$. (a) Nucleolus with regions of differing light transmission properties. (b, c) Nucleoli each with highly refractile inclusions. (d) Nucleolus with a single large refractile inclusion occupying the majority of the nucleolus (Compare with Figure 15 of reference¹¹). Note the starch granules (S) adjacent to the nucleus. (e) 2 nucleoli with the upper one showing regions with differing light transmission properties; lower nucleolus contains numerous highly refractile inclusions. Overlapping and juxtaposition of one inclusion with another obscures their individuality (see reference⁵)¹².

volume and synthetic activities in cells⁸⁻¹⁰. By withholding nutrients it has been possible to separate nucleolar enlargement, an activity associated with non-growing and growing lemon fruit explants, from the formation of refractile nucleolar inclusions, an activity associated with growing tissue. Cytochemical and cytomorphological investigations of these 2 nucleolar phenomena are under way^{12,13}.

Résumé. L'expansion des nucléoles et la formation de corps réfractaires nucléolaires sont deux étapes dans l'activité cytologique observée dans la croissance de tissus de citron in vitro. En privant les tissus de substances nutritives, on a réussi à séparer ces deux étapes.

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⁹ M. BIRNSTIEL, *A. Rev. Plant Physiol.* 78, 25 (1967).

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¹² It should be pointed out that in the unstained material the contrast between the refractile nucleolar inclusions and the nucleus is so high that the background invariably becomes dark as the refractile inclusions are brought out during printing.

¹³ Addendum. (a) Dehydration and paraffin infiltration here and in previous investigations were done with isopropanol. (b) Occasionally stalks from some lemons showed 1-2% nucleoli with refractile inclusions after 48-96 h on distilled water. Physiological age differences between the fruits used, especially with respect to available endogenous nutrients, could account for the infrequent occurrence of this nucleolar morphology on distilled water. Another possibility is contamination of the stalks with nutrients from the sac juice during excision from the fruit.

Chemical Sympathectomy: Histochemical and Submicroscopical Consequences of 6-Hydroxy-Dopamine Treatment in the Rat Iris

TRANZER and THOENEN¹ recently reported that systemic administration of 6-Hydroxydopamine (6-HODA) results in a degeneration of postganglionic autonomic nerve fibers in the cat. In this present study, the effects of this drug upon catecholamine fluorescence and electron microscopic structure of postganglionic adrenergic fibers in the rat iris will be reported, as compared with the alterations seen after a surgical removal of the superior cervical sympathetic ganglion.

Investigations were carried out on male albino rats. The animals were injected 4 times with 20 mg/kg 6-HODA with 12 h intervals and sacrificed 24, 48 and 72 h after the first injection. Both irides were dissected either in physiological saline (for fluorescence microscopy) or in Karnovsky's aldehyde fixative (for electron microscopy). Pineal gland and vas deferens was excised for electron microscopy only. The standard formaldehyde-condensation technique of FALCK was used to locate catecholamines^{2,3}; the usual electron microscopic embedding and sectioning procedures were employed for ultrastructural studies, using a Reichert Ultratome with glass knives, Reynold's lead citrate staining and a Tesla 242 D table electron microscope.

Twenty-four hours after the first injection, no changes in the peripheral autonomic innervation apparatus could be observed, either by means of fluorescence microscopy or with electron microscopy. 48 h after the first injection, the terminal branches of the adrenergic plexus exhibited signs of depletion, yet no characteristics of degeneration could be seen. 72 h after the first injection, however, fluorescence of the terminal arborization of the adrenergic neurons completely ceased, and only large (pre-terminal) branches of the adrenergic axons exerted fluorescence, that, however, appeared to be completely normal. At the same time, well-defined degenerative signs became apparent in the terminal adrenergic axons, characterized by the appearance of large vacuoles and in a complete disappearance of axoplasmic material from the axolemmal sheath. This kind of 'osmiophobic' degeneration pattern is characteristic for the postganglionic adrenergic fibers

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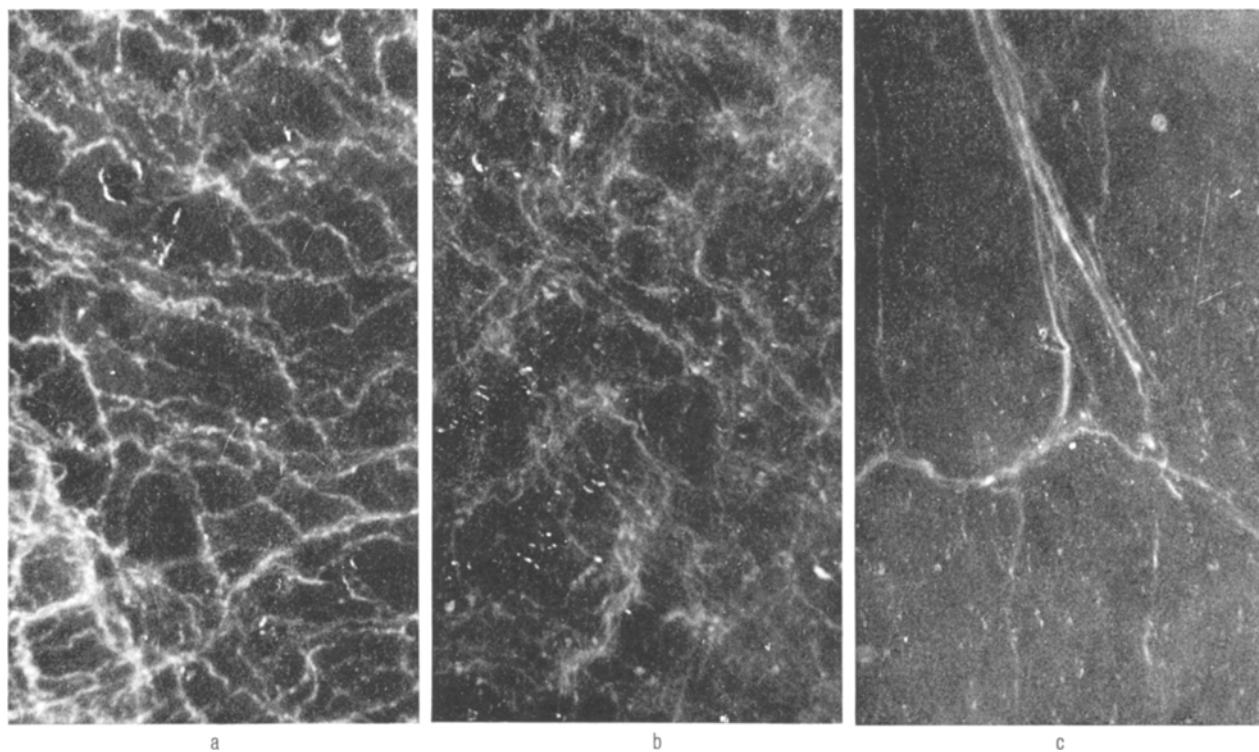


Fig. 1. Rat iris; stretch preparations ($\times 240$). Catecholamine reaction according to FALCK. a, control specimen; b, 48 h after 6-HODA administration; c, 72 h after 6-HODA administration. Only preterminal (non-varicose) branches exhibit fluorescence.

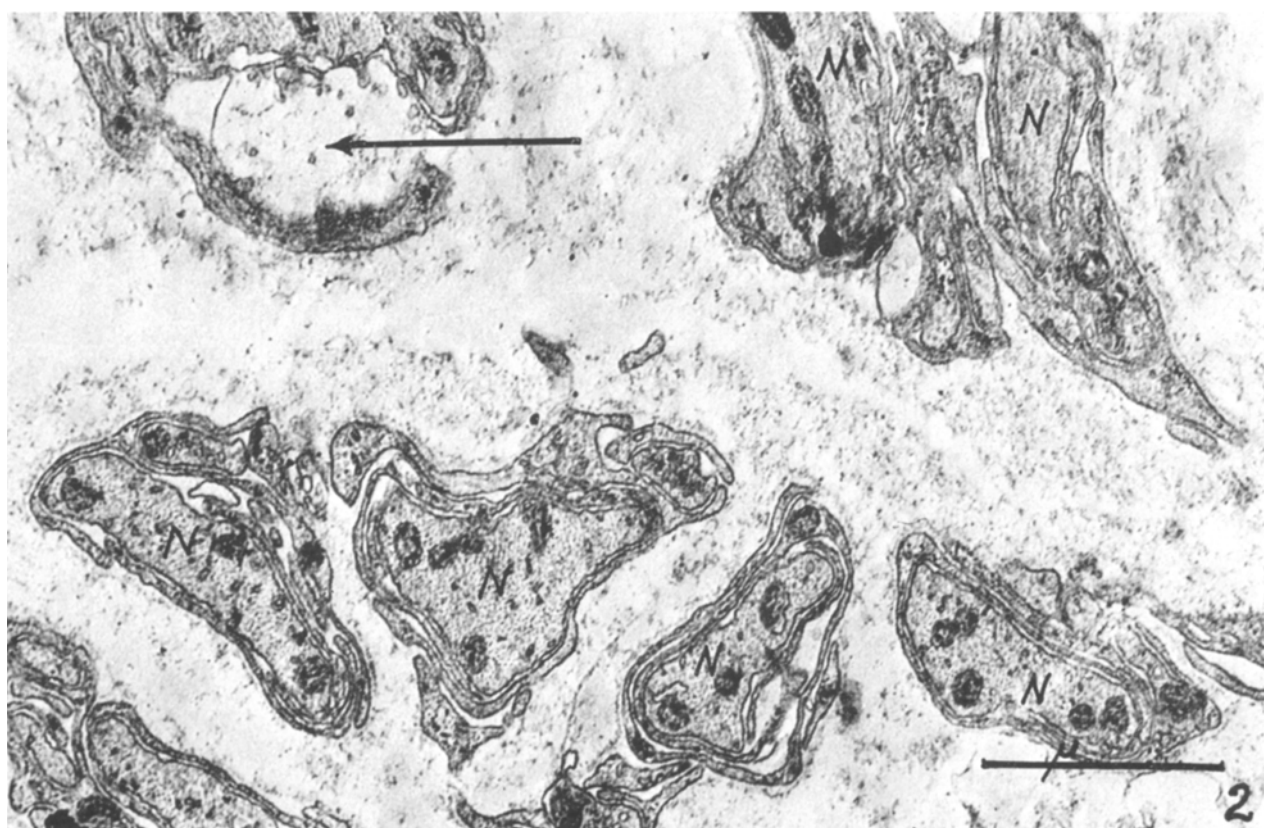


Fig. 2. Rat iris; electron microgram 72 h after 6-HODA administration. The majority of the nerve fibers exhibit a normal appearance (N), though in most cases the gap between axolemma and Schwann cell membrane is dilated. Arrow points at a nerve fibre that has undergone complete (osmiophobic) degeneration. $\times 32,500$.

of the rat iris, being in striking contrast to the osmophilic (cytolysosomal) degeneration patterns observed elsewhere.

When comparing the effects of this 'chemical sympathectomy' with those after the surgical removal of the superior cervical sympathetic ganglion, 2 main differential features are conspicuous. First, the time course is different. 36 h after surgery virtually no fluorescent (norepinephrine-containing) nerve fiber can be seen in the iris as shown already by one of us³. Secondly, even after a considerably longer period after 6-HODA administration (72 h), catecholamines disappear from the very terminal apparatus only, whereas the reaction of pre-terminal (non-varicose) axons remains unchanged. In striking contrast to this, after surgery catecholamines disappear from both terminal and pre-terminal axons virtually at the same time.

Preferential degeneration of adrenergic terminals (versus pre-terminals) may be due to the preferential uptake of the 'false transmitter' (6-HODA) by the terminals (versus pre-terminals), possibly this drug inducing a neuronectrotizing effect. On the other hand, the possibility cannot be excluded that it is the supporting element of the terminal nerve plexus, i.e. the enveloping interstitial cells, that are subjected to alterations by 6-HODA treatment. The interstitial cell of Cajal (similar to the lemmoblastic Schwann cells) can be regarded as a nutritive element of the terminal autonomic nerve

fiber. Thus any damage to these cells will inevitably result in the destruction of the (terminal) nerve fibers 'nursed' by them, not apparently affecting structure or chemistry of the pre-terminal axon, and being in a real 'direct' trophic (nutritive) relation with the cell of its origin, i.e. with the non-attacked sympathetic ganglion cell⁴.

Zusammenfassung. Eine systematische Verabreichung von 6-Hydroxydopamin bewirkt eine Noradrenalin-Depletion aus terminalen Sympathikusfasern der Ratteniris. Die adrenergischen Nervenfasern werden von einer sekundären Degeneration ergriffen («osmiophobe» Degenerationsform), entsprechend der Degeneration der Nervenfasern nach Exstirpation des Ganglion cervicale superius.

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⁴ Our thanks are due to the Hoffmann-La Roche Factory, Basel (Switzerland) for furnishing us with a sample of 6-HODA.

Foetal Blood Vessels on the Chorial Surface of the Human Placenta in Abnormal Pregnancy and Development

Involvement of the placenta as a whole in abnormal states of pregnancy and development has been reported by many workers¹⁻⁵.

Modifications in anatomy of foetal blood vessels of placenta have been shown in congenital anomalies⁶, multiple pregnancies⁷, hydramnios⁸ and ante partum haemorrhage⁹. While elaborating their earlier reports, BHARGAVA and RAJA¹⁰ have described the 'Parameters of stress', in the anatomy of foetal blood vessels of the chorial surface of the placenta, namely tortuosity of veins and arteries, arterio-venous dissociation, reversal of normal arteriovenous relationship of artery crossing superficially to vein and reversal of primary division ratio of veins and arteries. They have graded these parameters in relation to their level of occurrence (primary, secondary, terminal and singly and in combinations) and degree of severity (mild, moderate, marked and severe). These parameters develop in response to functional demands in later part of first trimester of pregnancy (BHARGAVA¹¹).

Injection of corrosion preparations, using buterite acetate cellulose in acetone, have been made on 463 placentae (prematurity 53; placenta previa 27; hydramnios 116; multiple pregnancy 149; and developmental defects 118). Anatomical features in relation to the incidence, level of occurrence and degree of severity of all the parameters in individual groups of these placentae have been tested for association with similar observations on 167 placentae from normal cases by means of chi-

square test. Interpretations of these analyses in relation to their significance are presented in Table I.

Attempts at ranking of these clinical entities by parameters or vice versa show a significant value of coefficient of concordance, thereby showing a high degree of agreement between the ranking and ranked entities. The above inferences are strongly suggestive of involvement of foetal vascular bed, resulting in marked exaggeration of the venous component as such in hydramnios and placenta previa, jointly with the arterial one, in developmental defects and multiple pregnancies, and mildly in cases of prematurity.

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⁶ I. BHARGAVA and M. B. INDURKAR, *J. Anat. Soc. India* 14, 43 (1965).

⁷ I. BHARGAVA and P. T. K. RAJA, *J. Anat. Soc. India* 16, 36 (1967).

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