precise determination of the time of the initial appearance of the dye. Consequently, this time is estimated by extrapolation. This presents no problem in our method, as the noise associated with the detector is almost completely suppressed by electronic filtering. However, even our method has not as yet overcome some basic problems involved in the detection of fluorescence from vessels containing whole blood. Red blood cells strongly absorb both the blue excitation light and the green fluorescent light. As the red cells flow in the center of the vessel, fluorescein molecules in deeper layers of the blood contribute less to the emission of fluorescence than do those in the superficial layers. This surface effect is further amplified by the fact that in high concentrations the dye itself acts as a barrier to the excitation light. This last effect is minimized in our technique by the use of small amounts of fluorescein.

Finally, one should remember that since fluorescein is largely associated with plasma, its transit time through a vessel is not necessarily identical with that of whole blood. We feel that solving the problems discussed above is essential, if meaningful conclusions regarding ocular hemodynamics are to be drawn from fluorescein dilution curves.

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REFERENCES


Hypochromia of the rabbit iris induced by 6-hydroxydopamine. ALFRED BRINI.

Subconjunctival injection of 6-hydroxydopamine, which provokes extensive damage to the sympathetic nerve terminals of the iris, was used in an effort to stimulate the clinical syndrome of heterochromia. Subconjunctival injection of this drug in young, pigmented rabbits provoked a slight miosis and, after some weeks, a hypochromia of the iris of the treated eye.

Peripheral sympathetic lesions often result in a change in pigmentation of the iris on the affected side; a condition termed "sympathetic heterochromia." Clinically, sympathetic heterochromia has been observed in children suffering from congenital Horner's syndrome and less frequently in adults after trauma of the neck.

Experimentally, excision of the superior cervical sympathetic nerve or ganglion provokes Horner's syndrome which is often accompanied by hypochromia of the iris on the same side.1-10

6-Hydroxydopamine (6HODA) is known to cause chemical sympathectomy in the eye.11-15 Severe damage to the adrenergic nerve terminals is observed with electron microscopy and histofluorescence following 6HODA administration.7-8

Using the above observations, a new approach to the problem of the relationship between sympathetic innervation and iris pigmentation seemed possible. We hoped that through the use of 6HODA we would produce a sympathectomy which would be accompanied by "sympathetic heterochromia."

Black and white Dutch rabbits were used for these experiments. Their iris is dark-brown most of the time. The peripheral half, when the pupil is in a state of moderate miosis, is a slate-brown shade, which makes it appear darker than the proximal half. Along the pupillary rim the color again becomes slate-brown; thus the anterior surface of the iris can be divided into three concentric areas. The middle one is slightly prominent, and protrudes a little above the other two. With the slit-lamp, it gives a velvety appearance, and is powdered with mottled pigment on a brownish-orange background.

The 6HODA was prepared according to Monte G. Holland and others.9-11 One tenth cubic centimeter of a 1.0 per cent solution was injected under
Fig. 1. Hypopigmentation of the right, treated eye (to the right). Normal pigmentation of the left, control eye (to the left).

the right conjunctiva of six Dutch rabbits, 3 days old. These injections were repeated three weeks later.

Hypochromia was first observed four weeks after the initial injection (Fig. 1). Since the stretching of the iris in the contracted pupil has a tendency to lighten its color, the illumination of the eyes was fixed so that the treated and untreated eyes had approximately equal pupil size. The rabbits were examined with the naked eye and with the slit lamp. The general impression that the iris was lighter on the side of the injection was confirmed with the slit lamp. The depigmentation was located in the middle prominent zone of the iris. The mottled pigment was still visible in this area, but the background was definitely lighter.

Surgical sympathectomy and damage to the sympathetic nerve terminals by 6HODA have thus a similar effect on the color of the iris. This might be related to the direct innervation of the branching pigmented cells of the iris stroma in mammals or to a change in the density of the stroma by the vascular effect of the sympathetic lesion. Further studies of the iris morphology are planned in order to more precisely elucidate the mechanism of this hypochromia.

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REFERENCES