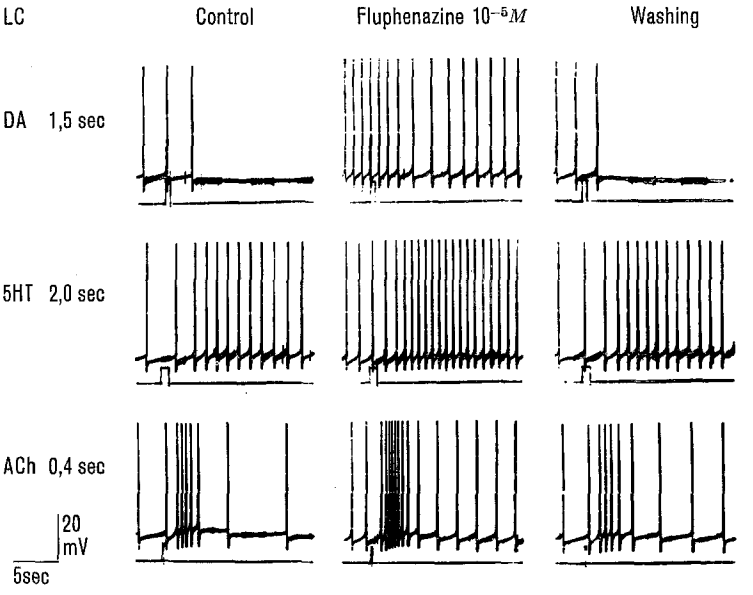

Fig. 2. Effects of 10 μM fluphenazine on responses to microelectrophoretic application of dopamine (400 nA for 1.5 sec), 5-hydroxytryptamine (250 nA for 2.0 sec) and acetylcholine (250 nA for 0.4 sec) in a spontaneously active cell type LC. The response to dopamine is blocked; this effect is reversible by washing in drug-free seawater. Iontophoretic currents from electrode to ground, measured as potential differences across a 2 MOhm resistance, are shown in lower trace of each recording.

and completely blocked within 15 min by 100 μM fluphenazine. In these neurones 10 μM mepazine had no effect (Figure 1c) and 100 μM mepazine did not impair dopamine response after 15 min perfusion. When perfusion with 100 μM mepazine was continued for further 20 min, response to dopamine started to decrease. However, this decrease did not seem to be specific for dopamine and was accompanied by impairment of the acetylcholine response which was also observed after long-lasting perfusion with 100 μM fluphenazine.

Discussion. The results provide a direct neurophysiological evidence for the blocking effect of 2 antipsychotic drugs on dopamine receptors and agree with neurochemical, pharmacological and indirect physiological observations. Poor solubility and the long latency of effect may have been responsible for an earlier finding that haloperidol was not an antagonist of dopamine on molluscan neurones[30]. D- and H-responses to dopamine involve different ionic mechanisms and are blocked selectively by tubocurarine and strychnine (D)[26,29] or ergotamine (H)[26]. Thus, it has been concluded that the effects of dopamine are mediated by 2 distinct receptors[26]. The reversible, probably competitive blocking effect of antipsychotic drugs on both types of responses in the present study might indicate that the receptors are structurally similar.

Zusammenfassung. In *Aplysia* Ganglienzellen blockierten Fluphenazine und Haloperidol in Konzentrationen zwischen 10 und 100 μM hyperpolarisierende und depolarisierende Antworten auf mikroelektrophoretisch appliziertes Dopamin. Antworten auf Acetylcholin und 5-Hydroxytryptamin blieben unbeeinflusst. Mepazine, ein Phenothiazin ohne antipsychotische Wirkung, hatte wenig Effekt. Die Ergebnisse sind ein direkter neurophysiologischer Beweis für die spezifische Blockade von Dopaminrezeptoren durch Neuroleptika.

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Effect of 6-Hydroxydopamine on Retinal Development in the Chick[1]

Attempts have been made to correlate the ontogenesis of retinal neurotransmitter systems with the development of optic function. The role of various neurotransmitter systems, particularly the cholinergic and monoaminergic systems in the retina is largely obscure, although it is well established that both cholinergic and adrenergic innervations are found in the layers and cells of the retina in most mammalian and avian species[2–6]. For example, in the developing chick retina, SHEN et al.[7] have demonstrated histochemically the presence of cholinesterase (ChE) in the prospective ganglion cells as early as the 4th day of incubation, and acetylcholinesterase was shown in the innermost layer and the amacrine cells on the 6th day. LINDEMAN[8] reported the presence of acetylcholine (Ach) and ChE activity in the retina on the 8th day. The peak rate of increment in ChE activity corresponds to the appearance of the constrictor reflex on the 19th day.

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EHINGER[9] described the presence of adrenergic nerves in the middle and inner layers of the avian retina as comparable to those of mammalian species. Adrenergic neurons possibly represent the displaced amacrine cells; eremite and alloganglionic cells are also reported. More recent studies[10–12] revealed that a system of intraretinal dopamine-containing neurons exists at the junction of the inner plexiform and inner nuclear layers and a plexus of norepinephrine-containing nerve terminals are present in the choroid of several mammalian species. Functionally, it was suggested that dopamine (DA) may act as an inhibitory neurotransmitter on the basis that ^{3}H-DA was taken up and released from the retina and that in the dark-adapted animals, the rate of release can be increased by the flash of light.

To the best of our knowledge, no studies have appeared in the literature on the role of catecholamines in the development of the chick retina. In this study we used 6-hydroxydopamine, an agent which is known to produce prolonged depletion of catecholamines and degeneration of catecholamine-containing neurons and nerve terminals[13–15], in order to elucidate the possible role of neurotransmitter systems, particularly the catecholamines, on retinal development in the chicken.

Materials and methods. 120 fertile chicken eggs were obtained from a nearby farm and placed in an incubator under culture conditions. 6-OHDA (supplied by Regis Chemical Co., Chicago, Illinois) was dissolved under sterile conditions in chilled, normal saline containing 0.1% ascorbic acid. The embryos were divided into 3 groups: the 1st group was treated with 6-OHDA, the 2nd group was treated with the vehicle solution, and the 3rd group (control) received no treatment. The embryos were further divided into sub-groups for treatment on the 3rd and 8th days of incubation, respectively. 2 doses of 6-OHDA were used: a high dose of 0.2 mg/0.1 ml and a low dose of 0.1 mg/0.1 ml. Under culture conditions a small window was opened on the shell in a sterilized culture chamber, and the drug or vehicle solutions were injected directly into the yolk-sac. The window was closed with molten paraffin, and the eggs were returned to the incubator immediately.

After a period of incubation, eggs with 19-day-old chick embryos were removed from the incubator, opened, and the embryos were examined for gross abnormalities. The eyes from these embryos were then removed and cut into anterior and posterior halves at the equators. The posterior halves, with the vitreous removed, were fixed in neutral buffered formalin (10%), processed and embedded in paraffin. H and E was used as a routine histological stain.

Results. The histological appearance of a normal 19-day chick embryonic retina is shown in Figure 1. Treatment with 6-OHDA in either 0.1 mg or 0.2 mg doses showed no significant reduction in the survival rate of the embryos incubated for 19 days. A survival rate of approximately 70% was obtained. Histological examination of the retina showed that all groups of embryos treated with 6-OHDA had different degrees of photoreceptor malformation which appeared to be related to dosage and time.

In embryos injected on the 3rd day with a 0.2 mg dose, the stratification of the retina appeared normal except for the photoreceptor layer, and to a lesser degree the outer plexiform layer. The layer of rods and cones was devoid of inner and outer segments (Figure 2). The pigment epithelium appeared in either close or loose attachment to the neural retina which contained a thin eosinophilic layer of acellular material adjacent to it. The outer nuclear layer (nuclei of photoreceptor cells) possessed the normal 2 to 3 layers of cell bodies whereas the outer plexiform layer appeared abnormally thin. The inner nuclear layer appeared to be normal. The inner plexiform layer was thicker in appearance and contained more displaced ganglion cells (arrow, Figure 2) than the control retinas. The ganglion cell layer, on the other hand, appeared to be morphologically comparable to the control, although in some specimens conically-shaped cells with dark, condensed oval nuclei were evident in this

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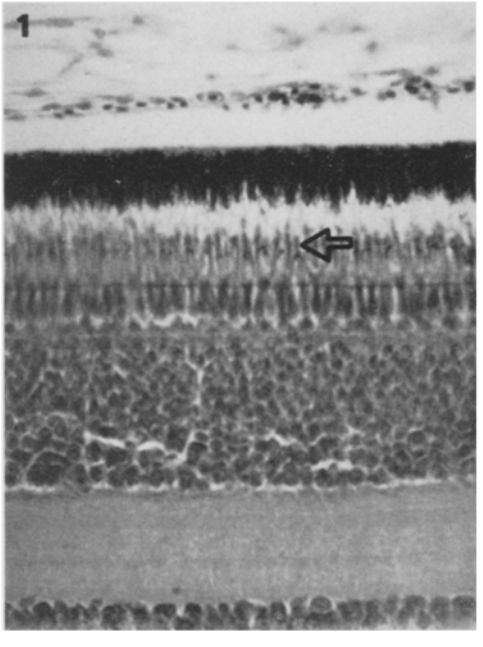

Fig. 1. 19-day-old chick embryonic retina. Note layer of photoreceptor (arrow). Fast green stain. ×600.

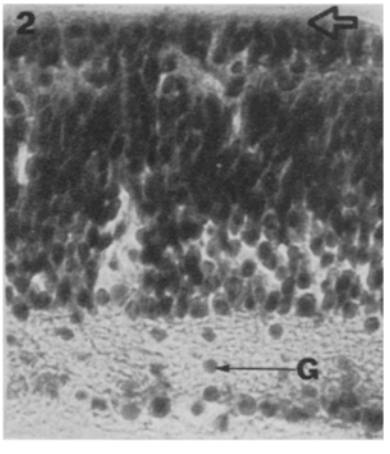

Fig. 2. 19-day chick embryonic retina treated on 3rd day with 6 hydroxydopamine (0.2 mg/egg). Note absence of photoreceptors (arrow) and presence of displaced ganglion cells (G) in the inner plexiform layer. H & E. ×900.

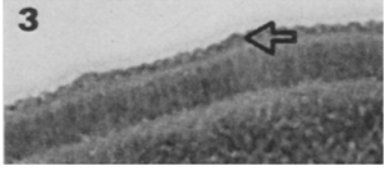

Fig. 3. 19-day chick embryonic retina treated with 6 hydroxydopamine (0.1 mg/egg) on the 3rd day. Note presence of small inner segment buddings (arrow). H & E. ×900.

Effects of 6-hydroxydopamine treatment on the development of the retina of the 19-day chick embryo

Layers	Control	Treatment		
		0.2 mg/egg (3rd day)	0.1 mg/egg (3rd day)	0.2 mg/egg, 0.1 mg/egg (8th day)
Pigment epithelium	+	+	+	+
Photoreceptors				
Outer segments	+	−	−	−
Inner segments	+	−	+ (buds)	+ (buds)
Outer nuclear layer	+	+	+	+
Outer plexiform layer	+	+ (thin)	+ (thicker)	+ (thicker)
Inner nuclear layer	+	+	+	+
Inner plexiform layer	+	+	+	+
Ganglion cell layer	+	+	+	+
Nerve fibre layer	+	+	+	+
Internal limiting membrane	+	+	+	+

layer. The identity of these cells has yet to be established. The nerve fibre layer and the internal limiting membrane were present and normal..

The embryos given a lower dose of 6-OHDA (0.1 mg) on the 3rd day showed similar developmental defects. The photoreceptor outer segments were absent. The inner segments, although not completely missing, appeared as small buds (Figure 3) which apparently failed to develop further. The buddings were of varying sizes and were usually seen in close association with the pigment epithelium. A layer of eosinophilic material was also observed on the surface of the buddings. Whether this layer of eosinophilic material was secreted by the pigment epithelium or was a part of a basement membrane could not be determined. The outer nuclear layer contained nuclei with karyorrhexis or early karyolysis, a condition not seen with a higher dosage of 6-OHDA. Likewise, the outer plexiform layer appeared significantly thicker, and the inner nuclear layer showed no apparent difference from the control. The inner plexiform layer was thick, and displaced ganglion cells were rarely observed. The ganglion cell layer, the nerve fiber layer and the internal limiting membrane were all present.

In embryos treated with both 0.1 mg and 0.2 mg doses of 6-OHDA on the 8th day, the results were somewhat similar to the 0.1 mg dose on the 3rd day: outer segments still failed to develop; the inner segments were present only as buds. No apparent dosage difference was observed between these 2 groups. All the other retinal layers appeared normal, and the displaced ganglion cells were rarely seen. The pigment epithelium extended as far internally as neural retina through their long projecting microvilli. The Table summarizes the effects of 6-OHDA treatment on retinal development in the chick.

Discussion. The results obtained in this study reveal that 6-OHDA can undoubtedly induce developmental defects in the photoreceptors of the chicken retina. The defective development of the inner segments and the failure of the outer segments to develop at all under the experimental conditions used are indicative that the normal development of the outer segments is an event which takes place only after the inner segment development has reached a certain stage.

6-OHDA is a compound known to produce selective degeneration of the sympathetic nerves of the autonomic nervous system in newborn and adult animals. However, the possibility exists that destruction of the adrenergic elements by 6-OHDA may lead to proteolytic and inflammatory processes which produce direct cytotoxic effects on the autonomic nervous system. During normal chick development the sympathetic nervous system begins to develop around the 3rd day[16]. For this reason, 6-OHDA was administered at this critical period of development. Recent studies by Angeletti et al.[17] indicate that the nerve growth factor (NGF) influences the development of sympathetic ganglia and that 6-OHDA reduces the accumulation of NGF. It is tempting to speculate, therefore, that the trophic influence on the autonomic nervous system, particularly the dopaminergic neurons which are known to be present at the junction of the inner nuclear and plexiform layers, and the norepinephrine-containing nerve terminals in the choroid, may affect the development of the photoreceptors. Treatment with 6-OHDA on the 3rd day may block the development of the catecholaminergic system, which in turn may block the development of the inner and outer segments. Embryos injected with 6-OHDA on the 8th day of development also exhibit defective inner segments and lack outer segments, a phenomenon resulting from probably the same chemical basis. There is no apparent dosage effect perhaps owing to the fact that the inner segments have already started to develop by the 8th day, at the time when 6-OHDA was injected.

Further studies are now in progress to elucidate the role of neurohormones on the development and function of the chicken retina.

Zusammenfassung. Hühnerembryonen wurden am 3. und 8. Inkubationstag mit 6-Hydroxydopamin behandelt. Die histologische Analyse der Retina ergab am 19. Tag eine charakteristische Hemmung der Photorezeptoren (Stäbchen und Zapfen).

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