

CHOLINESTERASE IN THE IRIS AFTER REMOVAL OF THE CILIARY GANGLION

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When tissues are denervated, they often become more sensitive to chemical stimuli, though to some substances they become less sensitive; thus the cat's iris becomes more sensitive to adrenaline, but does not respond to tyramine at all (Burn and Tainter, 1931). The increase in sensitivity has not yet been explained; it is probably due to several factors. One factor may be a fall in the amount of enzyme near the nerve ending, but until recently the only evidence of such a fall in the autonomic system was the fall in cholinesterase observed by v. Brücke (1937) in the superior cervical ganglion after section of the preganglionic fibres. It has now been shown (Burn and Robinson, 1952) that when the superior cervical ganglion is removed in the cat there is a fall in the amount of amine oxidase in the nictitating membrane, and likewise that when the stellate ganglion is removed there is a fall in the amount of amine oxidase in the arteries of the cat's foreleg. In view of the evidence of Shen and Cannon (1936) that the iris of the cat's eye becomes more sensitive to acetylcholine after removal of the ciliary ganglion, experiments have been carried out to see if there is a fall in the amount of cholinesterase in the iris after this operation.

METHOD

The right ciliary ganglion was removed in a series of 14 cats under pentobarbitone anaesthesia. In extirpating the ganglion an incision was made through the skin from the auditory meatus almost to the outer junction of the eyelids. Portions of the zygoma were removed, and access to the post-orbital space was then readily obtained. The external eye muscles were retracted, and after some practice the ganglion was found without difficulty. The cats were killed at 2, 6-8, 21, or 35 days later with coal gas. Both irises were removed immediately and stored at -15° C. until estimations were carried out. Quantitative determinations of cholinesterase were made manometrically by Warburg's technique, using 0.2 M-acetylcholine, 0.3 M-acetyl- β -methylcholine, and 0.06 M-benzoylcholine as substrates. Each iris was weighed and a suspension was made by cutting the tissue with scissors, freezing in a mortar at -15° C., and then pounding; 3 ml. of bicarbonate Ringer was added to each iris. For the manometric experiments conical flasks with one side bulb were used, and in each experiment a "substrate blank" was set up. When benzoylcholine was the substrate 1.3 ml. extract and 1.1 ml. bicarbonate Ringer were put into the flask. With the other substrates 0.5 ml. extract and 1.9 ml. bicarbonate Ringer were used, as the enzyme activity was greater; 0.6 ml. substrate was put into the side tube. The flasks were filled with nitrogen and carbon dioxide and incubated at 37.5° C. Under these conditions the carbon dioxide evolution of the substrate without enzyme was small. Readings were taken every 5 min. for 30 min., starting 3 min. after

tipping. The carbon dioxide evolution was plotted on a graph against the time, and the enzyme activity calculated from the reading at 20 min. and expressed as ml. CO₂ evolved/g. of tissue/hr.

RESULTS

The general result of the observations was that the removal of the ganglion was found to cause a fall in the cholinesterase in the iris. Details of the estimations with the three substrates are given in Table I. In all observations except two the figures for cholinesterase in the denervated iris were less than those for the corresponding

TABLE I
ESTIMATION OF CHOLINESTERASE IN CAT IRIS

Time in days after denervation	Cholinesterase activities expressed as ml. CO ₂ /g./hr. for different substrates					
	Acetylcholine		Acetyl- β -methylcholine		Benzoylcholine	
	Normal	Denervated	Normal	Denervated	Normal	Denervated
2	4.85	4.08	4.15	3.69	0.20	0.19
	12.8	10.0	11.9	8.5	0.66	0.49
	12.2	13.4	9.72	8.63	0.61	0.58
6-8	—	—	5.66	3.17	0.46	0.32
	5.71	3.28	4.04	2.49	0.21	0.24
	9.03	5.52	8.27	4.65	0.58	0.52
21	—	—	5.2	2.17	0.33	0.29
	5.79	2.75	5.01	1.98	0.39	0.33
	14.9	6.93	14.9	5.87	0.76	0.55
35	—	—	2.00	1.26	0.47	0.37
	—	—	5.28	2.27	0.44	0.37
	9.67	3.21	6.51	2.00	0.50	0.31
	7.90	2.74	6.62	1.28	0.37	0.31
	8.14	3.46	7.15	2.33	0.66	0.46

normal iris. The exceptions were the third figures when acetylcholine was used as substrate on the second day after denervation, and the second figures when benzoylcholine was used as substrate 6-8 days after denervation.

The result in each denervated iris was calculated as a percentage of the result in the corresponding normal iris, and a mean figure for each substrate at a given time after denervation was then obtained. The mean percentages have been plotted in Fig. 1, from which it appears that the percentage fall when acetylcholine was used as a substrate was almost identical with that when acetyl- β -methylcholine was used. The percentage fall when benzoylcholine was used was much less.

DISCUSSION

The results obtained furnish another illustration, in addition to those already mentioned, that when an autonomic nerve fibre degenerates there is a decrease in the amount of the enzyme which is normally engaged in destroying the transmitter. It seems very probable that when this decrease in the enzyme is demonstrable the increase in sensitivity of the tissue to the transmitter is, at least in part, due to the decrease. An increase in the sensitivity of the cat's iris to acetylcholine has been

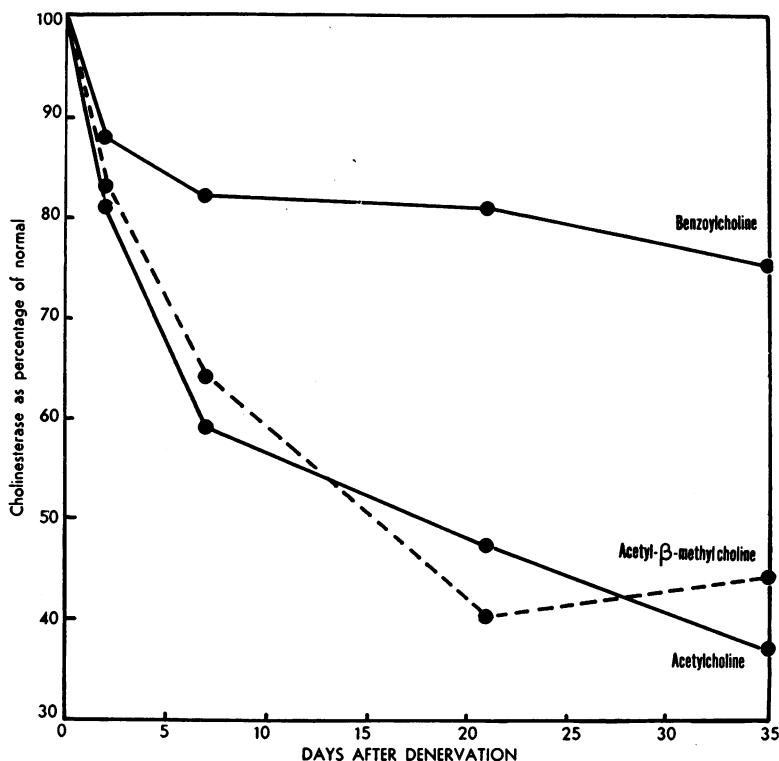


FIG. 1.—Cholinesterase in the denervated iris of the cat some days after removal of the ciliary ganglion. The ordinate is the amount in the denervated iris expressed as a percentage of that in the corresponding normal iris. Each point is the mean value for 2–5 cats.

described by Shen and Cannon (1936) and also by Keil and Root (1941) to follow the extirpation of the ciliary ganglion. (The observations of Shen and Cannon have been described by Cannon and Rosenblueth, 1949.) Shen and Cannon made their observations in cats in which one ciliary ganglion was removed two weeks previously and the remaining ciliary ganglion and both superior cervical ganglia were removed acutely. Eserine was instilled into the conjunctival sac of both eyes followed by acetylcholine applied in the same way. The pupil of the acutely denervated eye gradually constricted in the course of one hour, while the pupil of the eye from which the ciliary ganglion was removed two weeks previously passed through a phase of extreme constriction, maximal in thirty minutes, and diminishing to equal the constriction in the other eye at the end of one hour. Presumably the constriction in the acutely denervated eye was due to eserine prolonging the action of the transmitter, and that in the eye denervated two weeks before was due to hypersensitivity to the acetylcholine instilled. Keil and Root described increased sensitivity after section of the preganglionic fibres to the ciliary ganglion or extirpation of the ganglion itself. They determined the reaction of the iris by intravenous injection into the unanaesthetized cat of a dose of 5 mg./kg. of acetylcholine, and observed that the sensitivity remained at a maximum from 5 to 18 days, after which it declined,

reaching a steady level at about 35 days. The amount of acetylcholine given was very large and the sympathetic pathways to both eyes were intact. The records in their paper clearly suggest that the hypersensitivity declined after 18 days, but it is difficult to suppose that the method they used was reliable for detecting changes in the degree of hypersensitivity. Keil and Root suggested that the initial increase in sensitivity might be due to a loss of cholinesterase, and that the later fall in sensitivity might be due to a partial restoration of it. The present observations show that the greater part of the cholinesterase in the iris is true or specific cholinesterase ; after denervation the amount of this enzyme drops to about 40 per cent, and there is no restoration at the end of thirty-five days.

SUMMARY

When the ciliary ganglion is removed from a cat, the amount of cholinesterase, which is chiefly true or specific cholinesterase, in the iris declines. The amount in the denervated iris expressed in terms of that in the normal iris falls to about 40 per cent ; it reaches this level at about 21 days and remains there, showing no return up to 35 days. This fall in cholinesterase is the same whether the substrate used in the manometric estimation is acetylcholine or acetyl- β -methylcholine. When benzoylcholine is used as a substrate the fall is much less ; the enzyme in the iris has, however, little action on benzoylcholine.

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