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EFFECT OF SYMPATHETIC DENERVATION ON THE CHOLINESTERASE IN THE NICTITATING MEMBRANE AND THE IRIS

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The work to be described in this paper is a continuation of observations already made in this laboratory on the effect of nerve degeneration on the enzymes in various tissues. Robinson (1952) found that amine oxidase was present in the nictitating membrane and in the iris of the cat, and Burn and Robinson (1952) studied the effect of removing the superior cervical ganglion on the amount present. In the nictitating membrane they found that the degeneration of the sympathetic nerves caused a fall which reached a minimum about the 10th–12th day after extirpation of the ganglion. The amount of amine oxidase then rose and returned to normal by the 35th day. The restoration of the enzyme was difficult to explain except by supposing that regeneration of nerve fibres in the nictitating membrane had occurred, and in a further investigation (Burn and Robinson, 1953) it was found that when the stellate ganglion was removed as well as the superior cervical ganglion the fall in amine oxidase was greater and there was no recovery. In addition to the observations on the nictitating membrane which were made in large numbers of cats, a few observations were made on the effect of degeneration of the sympathetic fibres on the amine oxidase in the iris and in the arteries of the cat's foreleg. These indicated that a fall occurred in the iris and in the blood vessels also, but they require confirmation on account of their small number, especially those in the blood vessels in which only three manometric experiments were performed.

An investigation was also undertaken by Schofield (1952) on the cholinesterase present in the iris of the cat. It is innervated by fibres coming from the ciliary ganglion. After extirpation of the ganglion it was found that there was a fall in cholinesterase to about 40% of the amount present in the corresponding normal iris, when acetyl-β-methylcholine was used as a substrate, and a fall to about 80% when benzoylcholine was used as a substrate.

These observations are of interest because they contribute to an explanation of the increased sensitivity of denervated structures. Thus the fall in amine oxidase in the nictitating membrane was found to be correlated with the increased sensitivity of the membrane to noradrenaline in a series of 25 cats. However, the fall in amine oxidase did not explain all the changes in the denervated membrane, since this becomes hypersensitive not only to noradrenaline and adrenaline but also to acetylcholine (Rosenblueth, 1932). In 1935 Bacq and Fredericq described experiments which indicated that some of the fibres in the sympathetic pathway to the nictitating membrane were cholinergic ; they found that stimulation of the cervical sympathetic had a greater effect when eserine was instilled into the conjunctival sac, and that it had a smaller effect after the administration of atropine. This result suggested to us that cholinesterase might be present in the nictitating membrane, and that the hypersensitivity to acetylcholine observed after degeneration of the sympathetic fibres might be due to a fall in its amount. The present experiments were therefore undertaken to see if cholinesterase was present in the nictitating membrane and to discover if the amount was modified by degeneration of the sympathetic nerves.

A further question arose out of the observations which had been thus far made. Experiments had shown that removal of the sympathetic fibres from the nictitating membrane led to a fall in amine oxidase, and that removal of the parasympathetic fibres from the iris led to a fall in cholinesterase. Would removal of sympathetic fibres from the iris affect cholinesterase? The experiments which were planned to investigate cholinesterase in the nictitating membrane afforded an opportunity of answering this question as well. Observations

were therefore made in the iris as well as in the nictitating membrane.

METHODS

Manometric Observations.—The nictitating membrane, after removal from the eyeball, was pinned out and dissected free from striated muscle, fat, the Harderian gland, and cartilage. The remaining tissue was placed in a mortar, chopped as finely as possible with scissors, frozen to −10° C. with 2 ml. bicarbonate Ringer solution, ground up until it thawed, and made up to 6.5 ml. with bicarbonate Ringer.

The iris, after cutting away the cornea, was gently lifted out with forceps. It was chopped and ground as described for the nictitating membrane and suspended in 3 ml. bicarbonate Ringer.

Cholinesterase was estimated by the manometric method, using acetylcholine, acetyl-β-methylcholine, and butyrylcholine as substrates, the final concentrations being 0.02M, 0.03M, and 0.02M respectively. The following quantities were used: substrate 0.3 ml., nictitating membrane suspension 1.5 ml., iris suspension 0.5 ml. with acetylcholine and acetyl-β-methylcholine as substrates, 1 ml. with butyrylcholine as substrate. The volume was brought to 3 ml. with bicarbonate Ringer. The gas mixture was nitrogen with 5% carbon dioxide. The course of the reaction was followed for 30 min. at 37.5° C.

Removal of the Ganglion.—A series of 15 cats were prepared by removal of the right superior cervical ganglion under ether anaesthesia using full aseptic precautions. Between 6–24 days later the cats were killed by coal gas and then bled from the carotid arteries.

RESULTS

Nictitating Membrane.—Cholinesterase was found to be present in the nictitating membrane, and its amount was estimated by the use of all three substrates. Figures for the right and left membranes of normal eyes were obtained for 6 cats in order to ensure that the method of estimation was satisfactory. On account of the difficulty of distinguishing between smooth muscle and the surrounding connective tissue in dissecting the membranes, the results were expressed, not per gramme of tissue, but per nictitating membrane. The results are shown in Table I, and the mean figures for the right and left eyes agreed closely for each of the three substrates. Thus when a group of 6 cats was studied reasonable uniformity was observed in the two eyes.

TABLE I

CHOLINESTERASE IN NORMAL NICTITATING MEMBRANES

μl. CO_2 per nictitating membrane per hr.

Acetylcholine		Acetyl-β-methylcholine		Butyrylcholine	
Right	Left	Right	Left	Right	Left
181	212	86	78	379	340
515	591	174	141	1,130	815
355	276	155	171	620	532
875	1,075	168	141	1,350	1,730
285	216	82	39	393	433
428	375	134	142	507	609
Mean 440	457	133	119	730	743

Results were then obtained in 14 cats in which the right superior cervical ganglion had been removed previously in a sterile operation. They are shown in Table II.

TABLE II

CHOLINESTERASE IN DENERVATED NICTITATING MEMBRANES

μl. CO_2 per nictitating membrane per hr.

Days Since Operation	Acetylcholine		Acetyl-β-methylcholine		Butyrylcholine	
	Denervated Right	Normal Left	Denervated Right	Normal Left	Denervated Right	Normal Left
6	367	698	90	163	723	1,210
8	647	560	125	169	1,650	1,180
8	227	261	0	45	710	796
9	311	193	100	88	555	536
10	394	397	60	54	1,015	872
11	286	391	81	91	679	857
13	662	645	98	117	1,368	1,095
13	379	388	56	87	858	955
14	247	450	17	65	804	933
14	587	702	155	291	1,010	890
14	449	289	208	161	774	863
19	480	352	103	140	1,110	1,130
21	266	167	0	37	805	725
24	825	722	242	220	980	1,390
Mean	445	444	95	123	931	959

The mean figures for the normal left membranes in Table II were close to the mean figures in Table I for the corresponding substrates except for butyrylcholine, for which the mean figures in Table II were higher. However, the results show that, while denervation did not modify the figures for acetylcholine and for butyrylcholine, it reduced the figures for acetyl-β-methylcholine. The mean difference between the 14 pairs of membranes when acetyl-β-methylcholine was the substrate was 28.1, and the standard error was 11.97. Hence $t=2.35$ and $P=0.037$.

Thus degeneration of the sympathetic fibres to the nictitating membrane caused a fall in true cholinesterase, but not in pseudocholinesterase.

Iris.—Estimates of the cholinesterase present in the normal irises of 8 cats are given in Table III. With each substrate the figures for the right eye were rather lower than the figures for the left eye.

The results of examining 15 cats in which the right iris was denervated by removal of the superior cervical ganglion are shown in Table IV.

TABLE III
CHOLINESTERASE IN THE IRIS
μl. CO_2/g./hr.

Acetylcholine		Acetyl-β-methylcholine		Butyrylcholine	
Normal Right	Normal Left	Normal Right	Normal Left	Normal Right	Normal Left
5,670	3,350	3,150	4,240	—	—
10,500	13,000	6,040	6,560	985	1,720
5,500	8,200	2,030	2,620	1,580	1,565
7,950	11,800	6,480	8,430	3,750	4,320
7,860	8,075	5,700	5,160	1,715	1,550
5,875	7,530	3,715	4,760	3,120	3,750
9,480	8,430	6,920	6,940	1,030	988
6,360	6,830	3,940	4,360	652	586
Mean 7,400	8,402	4,747	5,383	1,833	2,068

TABLE IV
CHOLINESTERASE IN THE IRIS
μl. CO_2/g./hr.

Days Denervated	Acetylcholine		Acetyl-β-methylcholine		Butyrylcholine	
	Denervated Right	Normal Left	Denervated Right	Normal Left	Denervated Right	Normal Left
6	4,180	4,420	2,840	3,400	881	1,438
8	5,520	6,080	3,290	4,280	2,510	3,325
8	6,920	11,900	4,595	7,500	1,360	3,490
9	8,980	8,525	5,170	3,960	1,050	1,355
10	8,350	9,725	3,265	7,250	1,700	2,175
11	3,030	6,095	1,795	3,790	1,230	925
12	5,330	6,750	3,570	4,670	—	—
13	8,100	10,150	5,480	6,840	1,550	2,480
13	7,670	8,090	4,730	6,550	2,380	3,420
14	4,930	6,715	2,510	2,220	1,190	1,550
14	2,840	9,400	1,940	7,090	873	1,970
14	8,060	8,700	5,780	6,100	1,430	1,625
19	6,405	5,730	4,050	4,170	1,340	2,770
21	6,420	6,430	5,640	5,360	220	1,322
24	8,530	8,440	5,590	6,430	3,080	4,805
Mean	6,351	7,810	4,016	5,370	1,485	2,332

Again the mean values for the right eye were found to be lower than those for the left eye with all three substrates, but whereas the difference was not much greater than the difference for normal right and normal left eyes with acetylcholine and acetyl-β-methylcholine, it was considerably greater with butyrylcholine, indicating that there was a fall in pseudocholinesterase as a result of denervation. Because of the difference in the results for normal right and normal left irises, the figure for each right iris was calculated as a percentage of the figure for the corresponding left iris. The mean results are given in Table V. They show that the cholinesterase was less in the denervated right iris than in the normal right iris with all three substrates. The difference was, however, not significant either for acetylcholine or for acetyl-β-methylcholine, but it was significant for butyrylcholine. The percentage was 93.5 for the normal right iris and it was 66.8 for the denervated right iris. The standard errors, calculated from the formula

$$\sqrt{\frac{Sd^2}{n(n-1)}}$$

were 7.31 and 7.21 respectively. Hence, using the formula

$$t=\frac{m_1-m_2}{\sqrt{\varepsilon_1^2+\varepsilon_2^2}},$$

$t=2.61$ and $P=0.017$. The value of t was also calculated as described by Burn, Finney, and Goodwin (1950), p. 42, and found to be 2.33, from which $P=0.032$. Thus removal of the sympathetic fibres to the iris was found to cause a fall in the pseudocholinesterase which was present.

TABLE V
MEAN VALUES FOR CHOLINESTERASE IN RIGHT IRIS AS PERCENTAGE OF LEFT

	Acetylcholine	Acetyl-β-methylcholine	Butyrylcholine
Normal right ..	95·6 (8)	87·4 (8)	93·5 (7)
Denervated right	83·5 (15)	79·9 (15)	66·8 (14)

Number of observations in brackets.

DISCUSSION

The finding that degeneration of the sympathetic fibres to the nictitating membrane was followed by a fall in amine oxidase (Burn and Robinson, 1952, 1953) served to explain the hypersensitivity which developed to noradrenaline and adrenaline, but it did not account for the hypersensitivity to acetylcholine observed by Rosenblueth (1932). We have looked, therefore, for the presence of cholinesterase in the nictitating membrane and, having found it, have studied the effect of degeneration of the sympathetic fibres upon its amount. We have observed a fall in true cholinesterase without a change in pseudocholinesterase. It is probable that the hypersensitivity of the denervated membrane to acetylcholine is due to this fall.

The observations which were made upon the iris were unexpected. The work of Schofield (1952) showed that degeneration of the ciliary nerves caused a fall in true cholinesterase to about 40% of the normal amount, and a slight fall in pseudocholinesterase. Since degeneration of the sympathetic fibres caused a fall in amine oxidase (Burn and Robinson, 1952) it seemed improbable that it would affect the cholinesterase. It was therefore of great interest to observe that a fall in pseudocholinesterase occurred.

In the denervated nictitating membrane the evidence is strong that the hypersensitivity to noradrenaline and adrenaline is due to a fall in amine

oxidase. By inference the hypersensitivity of the iris to these amines is also due to the fall in amine oxidase which occurs; it is, however, not a large fall, and we now have evidence that there is also a fall in pseudocholinesterase. The question therefore arises whether the fall in pseudocholinesterase plays any part in causing the hypersensitivity of the iris. Now Bülbring and Burn (1942) showed that the constrictor action of adrenaline in the vessels of the dog's hindleg when perfused with blood was augmented by the presence of neostigmine in the blood. This suggested that the constrictor action of adrenaline depended on the amount of cholinesterase, and that if this was reduced by the presence of an inhibitor the constrictor action was then greater. It is therefore possible that the hypersensitivity of the iris to adrenaline after degeneration of the sympathetic supply is due either wholly or in part to the fall in pseudocholinesterase. The dilatation of the iris by cocaine is generally explained as due to a potentiation of the action of adrenaline by cocaine. Burn and Robinson (1952), in discussing the action of cocaine on the nictitating membrane, suggested that its effects were due to an inhibition of amine oxidase which was demonstrated to occur *in vitro* by Philpot (1940). We must now remember that Blaschko, Chou, and Wajda (1947) found that cocaine inhibited the hydrolysis of benzoylcholine by horse serum, and that this inhibition was greater than the inhibition of true cholinesterase. Thus the possibility arises that in dilating the iris cocaine acts by inhibiting pseudocholinesterase. We may then go further and point out that cocaine also causes blanching of the conjunctiva, which is also said to be due to potentiation of the effect of adrenaline. Can this also be due to an inhibition of pseudocholinesterase?

Summary

1. Cholinesterase is present in the nictitating membrane of the cat both in the "true" and in the "pseudo" forms.

2. Removal of the superior cervical ganglion results in a fall in the amount of "true" cholinesterase, and this fall may explain the hypersensitivity of the denervated nictitating membrane to acetylcholine.

3. Removal of the superior cervical ganglion results in a fall in the pseudocholinesterase present in the iris.

4. The question is raised whether the hypersensitivity of the iris to adrenaline after removal of the superior cervical ganglion is due to the fall in pseudocholinesterase.

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References

Bacq, Z. M., and Fredericq, H. (1935). *Arch. int. Physiol.*, **40**, 297.

Blaschko, H., Chou, T. C., and Wajda, I. (1947). *Brit. J. Pharmacol.*, **2**, 108.

Bülbring, E., and Burn, J. H. (1942). *J. Physiol.*, **101**, 224.

Burn, J. H., Finney, D. J., and Goodwin, L. G. (1950). *Biological Standardization*, 2nd ed., Oxford University Press.

—— and Robinson, J. (1952). *Brit. J. Pharmacol.*, **7**, 304.

—— —— (1953). *J. Physiol.*, **120**, 224.

Philpot, F. J. (1940). Ibid., **97**, 301.

Robinson, J. (1952). *Brit. J. Pharmacol.*, **7**, 99.

Rosenblueth, A. (1932). *Amer. J. Physiol.*, **100**, 443.

Schofield, B. M. (1952). *Brit. J. Pharmacol.*, **7**, 670.