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EFFECT OF DENERVATION ON AMINE OXIDASE IN STRUCTURES INNERVATED BY THE SYMPATHETIC

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The presence of amine oxidase in the blood vessels of the rabbit has been described by Thompson and Tickner (1951). Its presence in the nictitating membrane and the iris of the cat has been described by one of us (Robinson, 1952), who has also observed its presence in the blood vessels of the cat. Experiments have now been performed to see whether the amount of amine oxidase present is modified by denervation, for if amine oxidase is concerned with the destruction of the sympathetic transmitter noradrenaline, and if the degeneration of the sympathetic fibres reduced its amount, the increased sensitivity of denervated structures to noradrenaline and adrenaline might be explained.

METHODS

Observations have been made on the nictitating membrane and on the iris of the cat's eye and on vessels of the cat's foreleg. The nictitating membrane and the iris of the right side were denervated by extirpation of the superior cervical ganglion in an aseptic operation ; the vessels of the right foreleg were denervated in other cats by a similar removal of the stellate ganglion. At varying periods after the operation the amine oxidase in the organs was determined manometrically, a comparison being made of the amount present in the normal and in the denervated structures.

Control observations on all three tissues were first made to compare the two normal sides. For observations on the nictitating membrane at first two membranes were used for one experiment ; that is to say, the membranes from two right eyes were compared with the membranes from two left eyes ; later it was found possible to compare the membrane from one right eye with that from one left eye. For observations on the iris, the irises from three to seven right eyes were compared with the corresponding number from left eyes. For observations on the blood vessels, the vessels from four to six right legs were compared with vessels from the corresponding number of left legs.

Each nictitating membrane was removed immediately after death by cutting round the orbit with a scalpel, removing the eyeball from the orbit, and dissecting the membrane off the eyeball. The tissue having been weighed, a suspension was prepared by alternately cutting the tissue with scissors and pounding it in an ice-cold mortar ; 4 ml. of 0.067M-sodium monohydrogen phosphate buffer of *p*H 7.4 was added for each membrane. For further details the paper by Robinson (1952) should be consulted. Tyramine hydrochloride was used as a substrate, and the incubation was carried out in the presence of KCN in order to exclude oxidation reactions other than those due to the amine oxidase system.

Amine oxidase activity was expressed as the additional oxygen uptake in μl. per g. per hr., and this figure was determined in two ways. It was calculated from the rate of

additional oxygen uptake during the first 20 or 30 min. of the incubation, and it was also determined from the additional oxygen uptake measured at the end of one hour. Except where otherwise stated, all the results given were calculated by the first method. In the early experiments the cats were killed by gassing with coal gas and cutting the neck vessels when the cats were unconscious. In the later experiments the cats were killed by giving an intraperitoneal injection of sodium amytal (for a supply of which we are indebted to Messrs. Eli Lilly & Co. Ltd.), and when the cats were anaesthetized the abdomen was opened and the aorta was incised. The results were the same by the two methods of killing.

The arteries of each foreleg of the cat were dissected immediately after death, dried on filter paper, weighed, and at once stored at −10° C. For the manometric experiments they were prepared as has been described for the nictitating membranes. Approximately 0.3 g. tissue was suspended in 4 ml. phosphate buffer, and 1.6 ml. of this suspension was placed in each manometer flask. Tyramine was used as the substrate and observations were made in the presence of KCN. In one experiment shown in Table V, performed nine days after denervation, the tissue was prepared as a homogenate instead of by alternate chopping and pounding; as a result the O_2 uptake in the normal tissue was much greater than in the other experiments.

RESULTS

The nictitating membrane.—Table I gives details of eight experiments in which the normal right nictitating membranes were compared with the normal left membranes, and also of eight other experiments in which the denervated right membranes were compared with the normal left membranes, denervation having been performed nine to twelve days previously.

TABLE I

AMINE OXIDASE IN NICTITATING MEMBRANES EXPRESSED AS μl. O_2/g./hr.

Exp.	Normal left	Normal right	Denervated right	Days denervated	Right as % of left
41	147	168	—	—	114
42	126	133	—	—	105
43	200	223	—	—	112
44	69	81	—	—	117
45	64	60	—	—	94
46	102	143	—	—	140
47	72	85	—	—	118
48	95	114	—	—	120
3	125	—	111	9	88
4	152	—	65	9	42
32	273	—	122	9	45
5	114	—	34	10	30
6	115	—	66	10	57
24	118	—	68	10	58
25	32	—	31.5	10	98
10	116	—	52	12	44

The mean figure for the normal right membranes was 115 per cent of the normal left membranes, whereas the mean figure for the denervated right membranes was 58 per cent. The difference between these figures is highly significant ($P<0.01$), and the conclusion can be drawn that in the period of nine to twelve days after

denervation the amine oxidase in the membrane fell to not much more than half its initial value. The higher mean value for amine oxidase in normal right membranes than in normal left membranes may be a true finding, since the standard error of the value 115 was not more than 4.65. On the other hand, the normal controls were examined first of all, and it may be that there was some difference in the dissection of the nictitating membranes of the two eyes which was eliminated by practice.

Variation in fall in amine oxidase.—The figures in Table I for amine oxidase in denervated membranes show much variation. Two are as high as 98 and 88 per cent, while one is as low as 30 per cent. This variation at first made it seem unlikely that the fall in amine oxidase could account for the increased sensitivity of the membrane to the action of noradrenaline and adrenaline. We knew, however, that there are differences between cats in the response of the normally innervated nictitating membrane to noradrenaline. In some cats even large doses of noradrenaline produce almost no effect on the normal membrane, whereas in others smaller doses produce an easily measured response. We therefore decided to measure the degree of sensitization produced by denervation in order to see whether it was correlated with the change in the amount of amine oxidase.

Twenty-one cats were then prepared by removal of the right superior cervical ganglion, and after varying intervals each cat was anaesthetized, the cervical sympathetic chain was divided on the left side, and a spinal preparation was made. The nictitating membranes were threaded so that the contractions in them could be recorded. A series of injections of noradrenaline and of adrenaline was given to determine the ratio of the contractions in the two membranes. When sufficient observations had been made, the nictitating membranes were removed from the cat and were at once stored at $-15°$ C. until manometric observations could be carried out.

Results in three cats are shown in Fig. 1, from which it will be apparent that there was a great variation in the degree of sensitization produced by denervation. In Fig. 1,A the sensitization was extreme, since the injection of 7 μg. noradrenaline caused a scarcely perceptible movement of the normal nictitating membrane, but a large contraction of the denervated membrane. In Fig. 1,B there was no sensitization at all. Both the normal and the denervated membranes contracted to the same extent. Yet the cats used to obtain the records in Fig. 1,A and in Fig. 1,B were prepared in the same way by the same worker and the operation for removal of the superior cervical ganglion was performed on each cat 9 and 8 days respectively before the final experiment. Finally, in Fig. 1,C is shown a result intermediate between those in sections A and B of the same Figure ; in response to the injection of 7 μg. noradrenaline there was a small contraction of the normal nictitating membrane and a larger contraction of the denervated membrane.

Relation between sensitization and amine oxidase.—In the experiment on each cat we gave a series of injections of noradrenaline and a series of injections of adrenaline, and we measured the contractions of the membranes so produced. The result of each injection was expressed as the ratio of the contraction in the denervated membrane to that in the normal membrane (D/N ratio). The mean ratios for the different injections of noradrenaline, and for the different injections of adren-

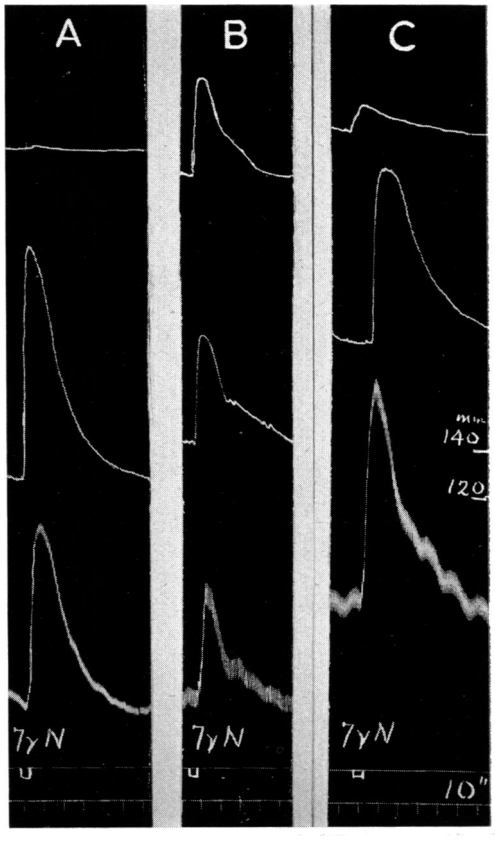

FIG. 1.—Responses to 7 μg. noradrenaline in 3 cats. Top record is normal nictitating membrane. Middle record is denervated membrane. Bottom record is arterial blood pressure. Note that the ratio of the contraction of the denervated to the normal membrane (D/N) is very high in A, is about unity in B, and has an intermediate value in C. The amine oxidase content of the membranes was determined, and the amount in the denervated membrane expressed as a percentage of that in the normal membrane.

	A	B	C
Mean D/N ratio	48.5	1.7	11.1
Amine oxidase %	43	95	66

aline, were calculated for each cat. The different ratios for the three experiments illustrated in Fig. 1 are set out in Table II. The range of doses used was from 5–10 μg. in all experiments. Although there was some variation in the ratios for noradrenaline and for adrenaline in each experiment, it will be seen from Table II that this variation was not great.

A determination of the amount of amine oxidase in the nictitating membranes of each cat was also made. In the first cat of Fig. 1, where denervation produced great sensitization, there was a large fall in amine oxidase in the denervated membrane to 43 per cent of that in the normal membrane. In the second cat of Fig. 1, where denervation failed to sensitize, there was no appreciable fall in amine oxidase, the figure obtained being 95 per cent. In the third cat, where denervation produced a moderate degree of sensitization, there was a fall in amine oxidase to 66 per cent of that in the normal membrane. Thus there was correspondence between the degree of sensitization and the fall in amine oxidase in the three cats.

Similar observations were made in seven other cats during the period 8–12 days after denervation and the results are given in Table III. In order to determine

TABLE II

Calculation of the D/N ratio, this being the mean figure for the ratio of the contractions in the denervated and normal membranes, from the results of the experiments illustrated in Fig. 1

	Exp. A		Exp. B		Exp. C	
	Dose μg.	D/N ratio	Dose μg.	D/N ratio	Dose μg.	D/N ratio
Noradrenaline ..	5	52	8	2.1	8	7.0
	7	52	6	1.4	4	12.5
	5	45	5	2.1	5	10.8
	6	45	6	1.5	6	14.3
			7	1.1	5	11.0
			10	2.3		
Mean ..	5.75	48.5	8.4	1.75	5.6	11.1
Adrenaline	7	4.5	10	0.7	10	1.6
	10	3.4	10	0.5	10	1.4
	10	4.0	10	0.8	12	1.5
	12	5.9	10	1.0	12	1.5
	10	7.25	10	1.0	10	1.5
Mean ..	10	5.0	10	0.82	10.4	1.5

TABLE III

Increased sensitivity to noradrenaline and adrenaline of denervated membrane, measured by the D/N ratio, and the percentage of amine oxidase in the denervated membrane

Exp.	Days denervated	μl. O_2/g./hr. in normal membrane	Amine oxidase percentage in denervated		D/N ratio		
					Noradrenaline		Adrenaline
A	9	113	43		48.5		5.0
B	8	89	95.		1.75		0.82
C	9	112	66		11.1		1.5
4	8	78.5	57		17.7		1.81
5	10	100	29		28.3		3.75
6	10	96	49	62.4	29.1	15.9	4.45
7	8	86	98		4.4		1.7
8	8	107	75		9.8		1.4
9	8	76	71		4.65		1.0
10	12	100	41.5		4.0		1.1
11	19	65	44		26.4		5.2
12	19	54	70		0.9		0.4
14	25	108	66		10.2		0.5
15	25	114	85		31		6.1
16	26	53	85		8.4		2.26
17	26	82	64	83.3	2.6	12.1	0.57
18	26	102	86		15.6		2.67
19	28	62	141		6.5		1.66
20	28	96	59		0.8		0.41
21	33	68	100		10		2.0
22	33	82	117		20.6		4.4

whether the percentage of amine oxidase in the denervated membrane was correlated with the D/N ratio the results for amine oxidase were plotted as abscissae in Fig. 2 against the logarithm of the D/N ratios (× 10) for noradrenaline as ordinates and in Fig. 3 against the corresponding figures for adrenaline as ordinates. The values for amine oxidase were calculated from the extra oxygen uptake at the end of 1 hr. These values were rather more closely correlated with the nictitating membrane contractions than those calculated from the rate of oxygen uptake in the first period of 20–30 min.

We are indebted to Dr. D. J. Finney for Figs. 2 and 3 and for the following statements : " The correlation coefficients for log D/N ratio with amine oxidase were –0.709 for noradrenaline and –0.634 for adrenaline as compared with –0.632 for the 5 per cent significance level. The figures for the D/N ratios for noradrenaline were themselves closely correlated with the figures for the D/N ratios for adrenaline, the correlation coefficient being –0.919. The regression line of log D/N ratio for noradrenaline (Y_1) on amine oxidase percentage x was

$$Y_1 = 2.895 - 0.0143x$$

and the corresponding regression line for adrenaline (Z_1) was

$$Z_1 = 1.747 - 0.0076x$$

Significance of the correlation coefficients implies that these regressions (which are drawn in Fig. 2 and in Fig. 3 respectively) are significant."

These results can be criticized on the ground that the relative size of the contractions of the denervated and normal nictitating membranes depends on the dose,

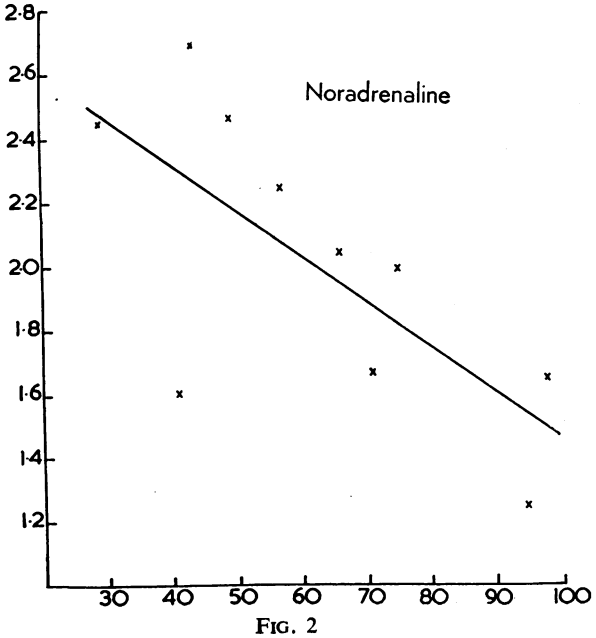

FIG. 2

FIG. 2.—Showing relation between logarithm of D/N ratio of nictitating membranes to noradrenaline (ordinate) and percentage of amine oxidase in denervated membrane (abscissa), in 10 cats examined between 8–12 days after denervation. The equation to the regression line is given in the text.

FIG. 3.—A similar relation to that in Fig. 2, but obtained by injecting adrenaline.

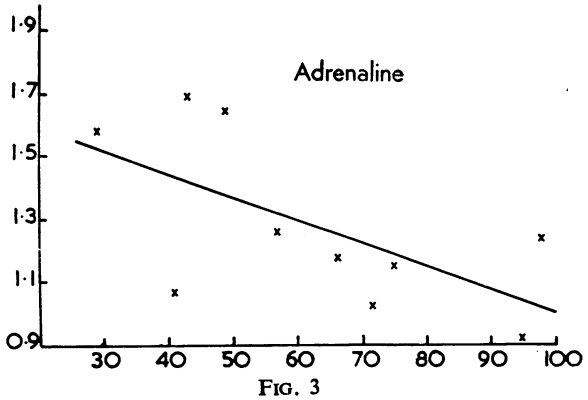

FIG. 3

the D/N ratio becoming smaller as the dose increases. However, the doses used in the different experiments were similar, ranging for noradrenaline from 4 to 10 μg., the mean being 7.2 μg., and ranging for adrenaline from 9 to 13 μg., except in Exp. 5, in which 3 doses of 20 μg. were used, thereby probably giving too low a ratio. So far as noradrenaline was concerned the D/N ratios varied greatly in different experiments, and errors in estimating each, due to difference in dosage, could not have changed them appreciably. So far as adrenaline was concerned the dose used was very nearly constant throughout. Since the normal membrane contracts fairly well in response to adrenaline, the ratios could be determined more accurately than for noradrenaline. Since, however, they did not greatly differ, the significant correlation of these ratios with the amine oxidase percentage in the denervated membrane was quite unexpected and gives strong support to the evidence.

Denervation for longer periods.—The effects of denervation so far described, the fall in amine oxidase correlated with the increased sensitivity of the membrane, were observed in the period 8–12 days after removing the ganglion. Further experiments were then carried out at longer intervals, from 19–33 days, and in these there was no correlation between the fall in amine oxidase and the increase in sensitivity. The results of these experiments are given in Table III, together with those which appear graphically in Fig. 2.

Table III shows that the mean amine oxidase percentage was higher in the experiments in which the period of denervation was longer. Whereas the mean percentage for cats denervated 8–12 days was 62.4, the mean percentage for cats denervated 19–33 days was 83.3. The sensitivity of the membrane to noradrenaline moved in accordance with this, the mean D/N ratio falling from 15.9 to 12.1. Thus if the deficiency of amine oxidase in the denervated membrane at 8–12 days was taken as 100, then the deficiency in the period 19–33 days was $\frac{62.4}{83.3} \times 100 = 75$ per cent of this deficiency ; similarly, if the supersensitivity of the denervated membrane to noradrenaline at 8–12 days was taken as 100, then in the period 19–33 days it fell to $\frac{12.1}{15.9} \times 100 = 76$ per cent. Thus the mean change in amine oxidase agreed closely with the mean change in sensitivity.

The rise in the amine oxidase at the longer period of denervation was a very surprising and wholly unexpected observation. In addition to the results in Table III, 18 other experiments had been carried out at different intervals after denervation in which the change in amine oxidase alone was measured. In each of these experiments two cats were used, so that we had information on the changes in amine oxidase in the denervated membrane in a total of 57 cats (including those in Table III). The results were arranged in groups according to the time after denervation at which they were obtained, and they appear in Fig. 4.

Fig. 4 shows that the amine oxidase percentage was lowest 10–12 days after denervation, and then steadily rose to the normal value which it reached about four or five weeks after the removal of the ganglion. From these results it was clear that denervation initiated two changes, a fall presumably due to the removal of nervous control reaching a low point after 10–12 days, and also a recovery process whereby the deficiency of amine oxidase was gradually made good perhaps

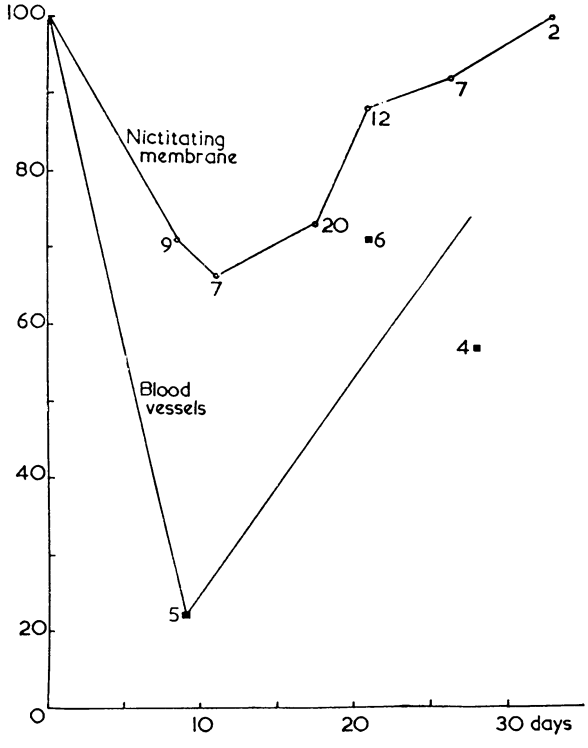

FIG. 4.—The percentage of amine oxidase in the denervated organ, compared with that in the normal organ, is recorded as ordinate, and the number of days after denervation is recorded as abscissa. The upper curve is drawn from observations on the nictitating membrane in which 39 manometric experiments were performed. The lower curve is drawn from observations on the foreleg arteries in which only 3 manometric experiments were performed. The figure beside each point is the number of cats used.

from some source outside the membrane. The failure to observe a correlation between the sensitivity of the membrane and the amine oxidase percentage in the later period thus received a possible explanation.

Effect of cocaine.—The evidence which has been given that the increased sensitivity of the denervated membrane to noradrenaline and adrenaline is caused by the decline in the amount of amine oxidase is supported by the effect of cocaine. Fröhlich and Loewi (1910) first showed that cocaine increased the action of adrenaline on the blood pressure ; later Rosenblueth and Cannon (1932) showed that it increased the action of adrenaline on the nictitating membrane. In 1940 Philpot demonstrated that cocaine inhibited the action of amine oxidase *in vitro*.

Cocaine hydrochloride was injected intramuscularly in 4 doses each of 2 mg. in different sites in the spinal cat. Two changes were often seen which are illustrated in Fig. 5. There was a rise of blood pressure, which was maintained, and there was an increase in the tone of the normally innervated nictitating membrane. Both these changes can be explained by inhibition of amine oxidase, since the denervated membrane did not increase in tone. We presume that in the normal membrane and in the blood vessels the existing tone represented the balance between the production of noradrenaline at the nerve endings and its destruction by amine oxidase. When cocaine was given and the enzyme was inhibited, the balance was altered in favour of increased tone.

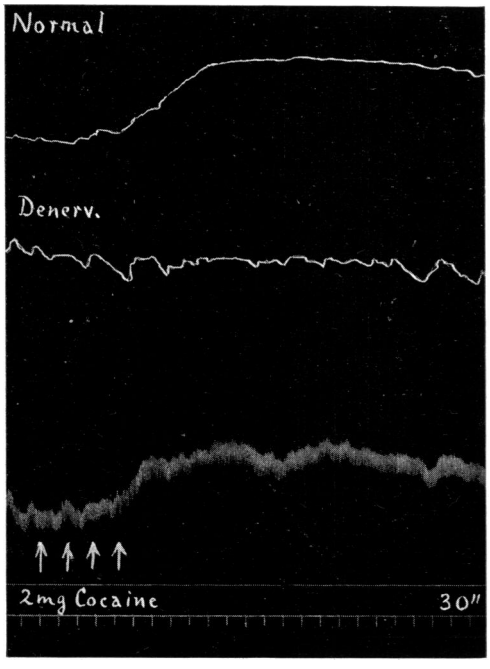

FIG. 5.—To show that the injection of cocaine in four doses of 2 mg. each into the muscles of a cat caused a rise of blood pressure (bottom record), a contraction of the normal membrane (top record), but no contraction of the denervated membrane (middle record).

The response of the normally innervated nictitating membrane to noradrenaline was greatly increased by cocaine, while the response of the denervated membrane was little affected, being increased occasionally. The ratio of the contraction of the denervated membrane to that of the normal membrane was reduced so as to be nearly equal to unity. In five experiments in which the contractions of the normal and denervated membranes were recorded, the mean D/N ratios for all injections of noradrenaline given before cocaine were consecutively 18.0, 3.0, 32, >50, and 1.0. After cocaine they became 1.4, 0.8, 1.3, 1.3 and 0.7, the mean figure being 1.1. Table IV shows the change produced by cocaine on the contractions caused by one dose of noradrenaline in each of these experiments.

TABLE IV

EFFECTS OF COCAINE ON CONTRACTIONS OF NORMAL AND DENERVATED NICTITATING MEMBRANES

Exp.	Dose: μg. Noradrenaline	Before cocaine			After cocaine		
		Contraction (mm.)		D/N	Contraction (mm.)		D/N
		Normal	Denerv.		Normal	Denerv.	
1	9	2	24	12	12	23	1.9
2	7	5	10	2	12	9	0.75
3	10	0	20	>20	11	15	1.36
4	7.5	0	28.5	>28.5	26.5	34	1.28
5	8	11	11	1			
5	15				24	17.5	0.73

The blood vessels.—The results for the blood vessels in a series of cats are shown in Table V. Three experiments were performed, each on a group of 4–6 cats in which the right foreleg arteries, not previously denervated, were compared

TABLE V

AMINE OXIDASE IN ARTERIES OF FORELEG OF CAT EXPRESSED AS μl. O_2/g./hr.

Number of cats	Normal left	Normal right	Denervated right	Days denervated	Right as % of left
6	360	370	—	—	103
4	365	315	—	—	86
5	202	213	—	—	105
5	1,380	—	395	9	22
6	375	—	265	21	71
4	272	—	154	28	57

with the left foreleg arteries. When the amine oxidase activity of the right leg vessels was expressed as a percentage of that in the left, the figures were 103, 86, and 105. In three other experiments in which the right foreleg was previously denervated, the figures were, after 9 days, 22 and, after 21 and 28 days, 71 and 57 respectively. Thus in the arteries as well as in the nictitating membrane the amine oxidase fell after denervation, the fall being greatest at 9 days and less after 3–4 weeks, suggesting that the amine oxidase was being restored. The results are shown in Fig. 4. We would emphasize, however, that these are results from three manometric experiments only.

The iris.—The results with the iris on the whole corresponded with the results on the nictitating membranes and the arteries. Two experiments in which the normal iris of one side was compared with the normal iris of the other, the number of cats used being six in the one experiment and five in the other, gave good agreement, as shown in Table VI. The amine oxidase in the denervated iris was lowest compared with the normal iris when the observations were made 7–14 days after denervation. The remaining observations were made from 15–26 days after removal of the ganglion and are consistent with a return towards the normal value in this period.

TABLE VI

AMINE OXIDASE IN IRIS OF CAT EXPRESSED AS μl. O_2/g./hr.

Number of cats	Normal left	Normal right	Denervated right	Days denervated	Right as % of left
6	162	165	—	—	102
5	205	195	—	—	95
4	451	—	100	7–14	22
5	336	—	256	15	76
6	352	—	306	16	87
6	214	—	235	20	110
7	241	—	230	21	95
6	192	—	157	21	82
3	197	—	136	26	69

Discussion

The evidence, that after degeneration of the sympathetic fibres in the nictitating membrane, in the arteries, and in the iris of the cat there is a fall in the amount of amine oxidase, indicates that there is a close relation in function between the nerves and this enzyme. The fall in the amount of enzyme in the nictitating membrane is greatest between 8 and 12 days after extirpation of the ganglion, but its extent varies greatly. The residual enzyme was observed to be as little as 29 per cent of that in the normal membrane or as much as 98 per cent, but whatever it was the fall in enzyme was correlated with the magnitude of the increased sensitivity of the denervated membrane to noradrenaline and also to adrenaline. This correlation affords strong evidence that when noradrenaline or adrenaline is injected the effect it exerts on the nictitating membrane is determined by the amount of amine oxidase present and not by the amount of any other enzyme. The further conclusion seems justified that noradrenaline liberated as the sympathetic transmitter is also destroyed by amine oxidase.

In the blood vessels of the cat's foreleg and in the iris of the cat's eye there was also a fall in amine oxidase after denervation which in the vessels was greatest at 9 days. These results suggest that amine oxidase destroys noradrenaline both in the blood vessels and in the iris. The conclusion is supported by the effect of cocaine, which is a substance known to inhibit amine oxidase. When injected into the spinal cat cocaine usually caused the blood pressure to rise and the tone of the innervated nictitating membrane to increase ; there was no change in the tone of the denervated membrane. Presumably there was a continuous release of noradrenaline at the nerve endings in the membrane and in the vessels, corresponding perhaps to the continuous release of acetylcholine at the motor endplate, described by Fatt and Katz (1950) and called by them " endplate noise." When the rate of destruction of this continuously released noradrenaline was reduced by the injection of cocaine, the tone in the nictitating membrane and the vessels was correspondingly raised.

The injection of cocaine likewise increased the response of the innervated nictitating membrane to noradrenaline so that it became practically the same as the response of the denervated membrane. This was true whether the difference in sensitivity of the two membranes was large or small. Such a change should occur if the increased sensitivity of the denervated structure is due to the fall in amine oxidase.

Four arguments against amine oxidase as the agent which destroys adrenaline or noradrenaline in the nictitating membrane require consideration. Bacq (1949) has pointed out that, when it was shown by Philpot (1940) that cocaine inhibits amine oxidase *in vitro,* she found that other local anaesthetics were equally strong or stronger inhibitors, but, he says, " there is no parallelism between the degree of enzyme inhibition and the sensitizing power." For example, cinchocaine (percaine or nupercaine) was the best inhibitor *in vitro,* yet it had only a slight potentiating action compared with that of cocaine (Bacq and Lefèbvre, 1934). If two substances act similarly *in vitro,* it is easily possible that they may act differently *in vivo,* since their action *in vivo* will depend on the rate of absorption when instilled into the eye, and will depend on both having a structure with the same adsorption and partition properties when injected intravenously during transit to the site and at the site itself (Albert, 1951).

Bacq (1949) further states that the view that cocaine causes sensitization by inhibition of amine oxidase does not explain why cocaine abolishes the action of tyramine instead of increasing it. There is now evidence which is fairly complete, and which will be discussed in another paper, that the action of tyramine in the body (excepting perhaps on the heart) is due to its attraction for amine oxidase, which destroys tyramine in preference to destroying other substrates. Tyramine has very little action on denervated structures (Burn and Tainter, 1931 ; Burn, 1932 ; Bülbring and Burn, 1938). Consequently when cocaine is first given and amine oxidase is inhibited, tyramine is without appreciable effect. Tyramine is thus similar in action to ephedrine ; both substances lead to the deviation of amine oxidase from its normal task ; tyramine is, however, destroyed and its action is of short duration, while ephedrine is not destroyed.

A third argument which has been put forward against the view that the sensitization of the nictitating membrane is due to the fall in amine oxidase is that the substance Corbasil (or Cobefrin) which has a $-CH_3$ group on the α carbon atom of the side chain, and which is not destroyed by amine oxidase, causes a greater contraction of the denervated membrane than of the normal membrane. It should be said in the first place that the difference in the sensitivity of the two membranes is far less for these substances than it is for noradrenaline. The maximum D/N ratio observed by Bülbring and Burn (1949) for Corbasil was 2.4, whereas for noradrenaline the ratio may be as great as 50. In the second place substances with a $-CH_3$ group on the α carbon atom, which inhibit the action of amine oxidase on adrenaline, must do so by combining with the enzyme, although they are not destroyed by it. When Corbasil reaches the nictitating membrane in the body, some of the molecules will therefore combine with the enzyme, and the remaining molecules will cause the membrane to contract. In the denervated membrane, where there is less enzyme, there will be more molecules free to cause the contraction. The contraction will therefore be greater.

Finally Rosenblueth (1932) has found that the denervated membrane is supersensitive to acetylcholine, pilocarpine, eserine, and histamine, and (Bacq and Rosenblueth, 1934) to calcium and potassium salts. Cannon and Rosenblueth (1949) therefore argue that " the supersensitivity cannot reasonably be attributed to a decrease in amine oxidase." Without discussing this argument in detail we would point out that it is unlikely that all these agents have their own specific receptors on the nictitating membrane ; it is more likely that they have some small power of attaching themselves to the receptors on which noradrenaline and adrenaline act, and therefore to the receptors on the enzyme as well. For this reason they will have a greater action on the denervated membrane, where there is less enzyme, just as Corbasil has. More work is certainly required on this point, but it is clear that the objection of Cannon and Rosenblueth is not unanswerable.

On the basis of our observations, that in the first 12 days after removal of the ganglion the increased sensitivity of the denervated membrane is correlated with a fall in amine oxidase, we consider that the supersensitivity of denervated structures (sometimes referred to as Cannon's law) is explained in the nictitating membrane by the fall in the enzyme.

Our results have shown that after reaching a low point about the 10th–12th day the amine oxidase in the denervated membrane slowly returns to the normal at about

4–5 weeks. This return was surprising, since the superior cervical ganglion was extirpated and there seemed to be no possibility of regeneration of the nerves. Although our evidence in denervated arteries is less complete, in them also we found the fall in amine oxidase greatest at 9 days and much less later. There are no other records of such a restoration of enzyme in course of time after denervation, but two other observations may be relevant. Keil and Root (1941) found that after extirpation of the ciliary ganglion the sensitivity of the cat's pupil was greatly increased in six days, and then in the next four weeks it steadily declined. Examination of the figures of v. Euler and Purkhold (1951) shows that the amount of noradrenaline in the spleen and the kidney fell very low 8–15 days after denervation, but was then partially restored after 3–4 months. In the latter experiments regeneration of the nerves might have occurred.

The restoration of amine oxidase in the nictitating membrane, and probably in the blood vessels, may be due to the transference of the enzyme from the liver. The restoration in the membrane was accompanied by a decline in the mean sensitivity to the action of noradrenaline, but there was no correlation in individual cats between the sensitivity and the amount of enzyme such as was observed at 8–12 days. For this reason the suggestion arose that the amine oxidase accumulating once more in the membrane might not always reach those points where it was effective in controlling the reaction of the membrane to noradrenaline. There were two other considerations in favour of the hypothesis that amine oxidase can be present, not only in the denervated but also in the normal membrane at two sites, namely, at points where it is effective and also at points where it is ineffective.

The first of these can be appreciated by examining Fig. 1, Exp. B. In this experiment there was practically no fall in the enzyme after denervation, and the contractions in the two membranes were almost the same. Both, however, were large. In experiments such as this, where the fall of enzyme after denervation was slight or absent, the sensitivity of the normal membrane to noradrenaline was always unusually great. The amount of amine oxidase in the normal membrane would therefore be expected to be unusually small. Actually the amount of enzyme was not unusually small, as Table III shows. There was, however, very little loss of enzyme on denervation. The inverse relation between the size of the response of the normal membrane and the extent of the fall in amine oxidase in the denervated membrane, well shown in the three parts of Fig. 1, are, however, explained on the hypothesis that the amine oxidase exists at (*a*) effective sites, and (*b*) ineffective sites, and that after denervation a fall occurs only in the amount present at the effective sites. In Fig. 1, Exp. B, there was little enzyme at effective sites and therefore the response of the normal nictitating membrane was large ; further, since the amount of enzyme at effective sites was small, the fall in amine oxidase on denervation was also small.

The action of cocaine can also be clarified on this hypothesis. Denervation in no experiment reduced the amine oxidase to zero, and therefore cocaine, by inhibiting the residue of amine oxidase, should have increased the response of the denervated membrane to noradrenaline. But this was not observed. Cocaine rarely increased the response of the denervated membrane ; its effect was to increase the response of the normal membrane until it was closely similar to that of the denervated membrane. This result is explained if it is supposed that the amine oxidase

still present is at an ineffective site, and that its inhibition by cocaine makes no difference to the response of the membrane to noradrenaline.

The hypothesis that amine oxidase exists at effective and ineffective sites can now be applied to the restoration of the enzyme in the denervated membrane. If we assume that the accumulating enzyme is deposited at both sites we then understand why there is a return of the sensitivity to noradrenaline towards normal, but no correlation in individual membranes between the sensitivity and the total amount of enzyme.

In conclusion it may be said that the evidence presented in this and an earlier paper (Burn and Robinson, 1951) supports the hypothesis of Burn and Hutcheon (1949) that the relative weakness of noradrenaline on the normally innervated nictitating membrane, iris, and blood vessels is explained by the presence of an enzyme which destroys noradrenaline more rapidly than it destroys adrenaline. In these organs we consider that amine oxidase plays the same role in destroying noradrenaline which cholinesterase plays in destroying the humoral transmitter of parasympathetic impulses.

SUMMARY

1. The nictitating membrane, the iris, and the blood vessels of the cat have been shown to contain amine oxidase. A study has been made of the effect of extirpating the ganglion, with consequent degeneration of the postganglionic fibres, upon the amount of amine oxidase present.

2. Denervation results in a fall in amine oxidase in all three tissues, the amount of enzyme in the denervated side being compared with the amount on the opposite side. The vessels of one foreleg were denervated by removing the stellate ganglion.

3. In the nictitating membrane the fall is very variable, and it is greatest at 8–10 days after denervation. The increase in the sensitivity to noradrenaline of the denervated membrane was measured, and at 8–10 days after denervation it was found to be significantly correlated with the fall in the amount of the enzyme. Similarly, the increase in the sensitivity to adrenaline was also measured, and it was also significantly correlated with the fall in the amount of the enzyme.

4. About ten days after denervation the amount of amine oxidase in the denervated membrane was found to rise again and by 33 days to be equal to the amount in the normal membrane. The course of the fall and rise was studied in a total of 57 cats.

5. In the blood vessels and in the iris also there appears to be an early fall in the amine oxidase, greatest at about 10 days, with a subsequent return towards normal.

6. The effect of cocaine on the nictitating membrane is consistent with the view that its action is due to an inhibition of amine oxidase.

7. The results indicate that amine oxidase at the postganglionic terminations in the nictitating membrane, the blood vessels, and the iris plays a similar part to cholinesterase at cholinergic nerve endings.

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