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*NOR*ADRENALINE AND ADRENALINE IN VESSELS OF THE RABBIT EAR IN RELATION TO THE ACTION OF AMINE OXIDASE

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There is much variation in the activity of *nor*adrenaline compared with that of adrenaline. Barger and Dale (1910) observed that, whereas in the beginning of an experiment the pressor action of *nor*adrenaline was greater than that of adrenaline, at the end it was often less than that of adrenaline. Burn and Hutcheon (1949) recorded a similar change in the relative contracting action on the spleen. Since the cause of this varying relation is difficult to determine in the intact animal, we have sought to investigate it first in a simpler preparation, namely, the vessels of the rabbit ear perfused with Locke's solution, in which comparisons have already been made by different workers. There is fair agreement about the facts; Luduena, Ananenko, Siegmund, and Miller (1949) found that the ratio between amounts of *l-nor*adrenaline and *l*-adrenaline which produced the same constriction was 2.5 to 1.5; Gaddum, Peart, and Vogt (1949) found the ratio was 3.0 to 1.0; Burn and Hutcheon (1949) found the ratio was 4.0 to 1.0. Thus occasionally *l-nor*adrenaline is as active as *l*-adrenaline, but usually it is found to be weaker.

Experiments with perfused rabbit ears

Change in ratio with duration of perfusion.—The first point we investigated was whether the ratio varied according to the length of time the ear was perfused. The central artery of the ear was cannulated at the base and the outflow was collected in a funnel which took it to a recorder of the kind described by Stephenson (1948). The perfusion was at room temperature, the composition of the Locke solution being NaCl 9.0 g., KCl 0.42 g., $CaCl_2$ 0.24 g., $NaHCO_3$ 0.5 g., dextrose 1.0 g., glass distilled water 1 litre. In the later experiments oxygen was bubbled through the solution beforehand, though no difference was observed. We used *l-nor*adrenaline which was very kindly given to us by Dr. M. L. Tainter of the Sterling-Winthrop Research Institute. Solutions of *nor*adrenaline and adrenaline were prepared to contain 10 μg./ml., and were acidified. From these a dilution in the perfusion fluid was freshly prepared before each injection into the artery cannula.

We found that when the ear was freshly perfused the action of *nor*adrenaline was weak compared with that of adrenaline, so that the ratio N/A of amounts of *nor*adrenaline and adrenaline producing the same constriction was high. After the perfusion had been continued overnight adrenaline was usually found to be more active and *nor*adrenaline still more active, so that the ratio was nearer to unity. Fig. 1

shows the similar vasoconstrictor action of 0.02 μg. *l-nor*adrenaline and 0.005 μg. *l*-adrenaline during the early stage of the perfusion; the ratio N/A is therefore = 4. In the lower part of the figure observations made the next morning, the perfusion

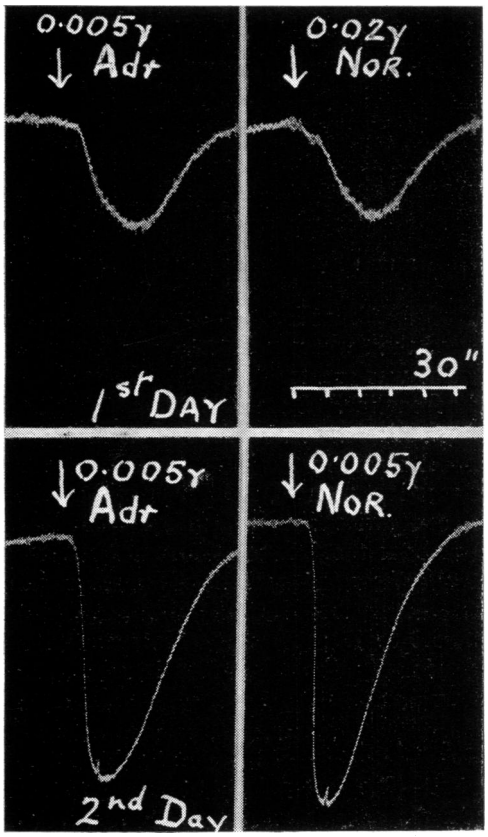

FIG. 1.—Records of the outflow from the vessels of the rabbit ear perfused with Locke's solution. A drop in the record indicates vasoconstriction. The two upper records were obtained during perfusion soon after the preparation had been set up. The two lower records were obtained about 24 hr. later. Note that on the first day 0.02 μg. *nor*adrenaline was needed to produce constriction equal to that of 0.005 μg. adrenaline. On the second day the vessels were more sensitive to adrenaline, and *nor*adrenaline was as active as adrenaline.

having continued overnight, showed that, while adrenaline was more active, *nor*adrenaline was much more active and the ratio N/A = 1. A series of 29 experiments of this kind was performed, and the results of the first 13 are given in detail in Table I, the mean ratio on the first day being 7.16 and on the second day 2.47. The mean ratio of all 29 experiments on the first day was 5.9 and on the second day 2.7, which are similar figures to those for the first 13 experiments. The actual amounts injected on the first day cannot in all cases be compared with the amounts injected on the second day, and these amounts give no precise guide to the change in sensitiveness. The point we wish to stress in the results is the change from the first day to the second in the relative amounts of *nor*adrenaline and adrenaline which have the same constrictor action.

The effect of ephedrine.—The presence of amine oxidase in the rabbit ear was first shown by Schapira (1945); it has recently been demonstrated by R. H. S. Thompson in the rabbit ear vessels (unpublished). It seemed possible that the fall in the N/A

TABLE I

AMOUNTS OF *l-nor*ADRENALINE AND *l*-ADRENALINE PRODUCING THE SAME VASOCONSTRICTION IN THE RABBIT EAR VESSELS DURING PERFUSION ON (*a*) THE FIRST DAY, (*b*) THE SECOND DAY

Exp.	1st day			2nd day		
	*Nor*adrenaline μg.	Adrenaline μg.	Ratio N/A	*Nor*adrenaline μg.	Adrenaline μg.	Ratio N/A
1	0.02	0.005	4	0.005	0.005	1
2	0.002	0.001	2	0.02	0.005	4
3	0.005	0.001	5	0.0125	0.005	2.5
4	0.1	0.01	10	0.02	0.015	1.3
5	0.06	0.01	6	0.15	0.05	3
6	0.01	0.001	10	0.15	0.05	3
7	0.02	0.0075	2.7	0.015	0.005	3
8	0.2	0.01	20	0.015	0.005	3
9	0.075	0.005	15	0.025	0.005	5
10	0.02	0.01	2	0.01	0.005	2
11	0.015	0.005	3	0.01	0.005	2
12	0.023	0.005	4.5	0.0075	0.005	1.5
13	0.02	0.005	4	0.015	0.015	1
Mean ratio of 13 experiments			7.16			2.47
Mean ,, ,, 29 ,, ...			5.9			2.7

ratio on the second day might be related to a diminution either in the amount or in the activity of the amine oxidase present. Burn and Hutcheon (1949) have already suggested that the inactivity of *nor*adrenaline on the normally innervated pupil and on the normally innervated nictitating membrane might be explained by the presence at the nerve ending of a high concentration of an enzyme which destroys *nor*adrenaline more easily than it destroys adrenaline.

To test the idea that the high N/A ratio on the first day is due to the activity of amine oxidase, we made use of the observations of Blaschko, Richter, and Schlossmann (1937) that ephedrine inhibits the action of amine oxidase. We carried out experiments in which the N/A ratio in the rabbit ear vessels was determined (*a*) when the preparation was fresh, (*b*) during perfusion with Locke's solution containing ephedrine hydrochloride, and (*c*) during a further period of perfusion with Locke's solution alone. If the high ratio for N/A in the fresh preparation was due to the more rapid destruction of *nor*adrenaline, then during perfusion with Locke containing ephedrine the enzyme responsible for the destruction might be inhibited, and the ratio N/A might fall to a value nearer unity. When the ephedrine was removed, the ratio might then rise again.

All experiments were completed within a few hours of setting up the preparation, so that the results could not be affected by oedema. Ephedrine hydrochloride was added to the Locke's solution in the amount of 10 μg. per ml., which was the concentration used by Gaddum and Kwiatkowski (1938) when they first demonstrated the increased sensitiveness of the vessels to adrenaline and to sympathetic stimulation in the presence of ephedrine. The details of the results in 7 experiments are given in Table II, though 13 experiments were made in all, and the mean ratios N/A for all 13 experiments are also given. Satisfactory agreement was obtained between the mean ratio 5.5 in Table II obtained when the preparation was first perfused and the similar ratio 5.9 obtained

TABLE II

CHANGES IN THE RATIO OF AMOUNTS OF *nor*ADRENALINE AND ADRENALINE PRODUCING THE SAME CONSTRICTION IN RABBIT EAR VESSELS BEFORE, DURING, AND AFTER THE ADDITION OF EPHEDRINE TO THE PERFUSING FLUID

Exp.	At first			During ephedrine perfusion			After ephedrine perfusion		
	Nor-adrenaline μg.	Adrenaline μg.	N/A	*Nor*-adrenaline μg.	Adrenaline μg.	N/A	*Nor*-adrenaline μg.	Adrenaline μg.	N/A
14	0.05	0.015	3.3	0.015	0.01	1.5	0.015	0.005	3.0
15	0.04	0.01	4.0	0.01	0.005	2.0	0.015	0.005	3.0
16	0.04	0.01	4.0	0.005	0.0025	2.0	0.003	0.001	3.0
17	0.03	0.005	6.0	0.005	0.0025	2.0	0.01	0.0025	4.0
18	0.06	0.01	6.0	0.01	0.005	2.0	0.01	0.0025	4.0
19	0.06	0.01	6.0	0.0025	0.0013	2.0	0.02	0.005	4.0
20	0.02	0.005	4.0	0.0075	0.005	1.5	0.003	0.001	3.0
Mean of 7 experiments ..			4.8			1.9			3.4
Mean of 13 experiments ..			5.5			1.7			4.1

on the first day for 29 experiments in Table I. During perfusion with ephedrine the ratio fell to 1.7, and later when the ephedrine perfusion was discontinued the ratio rose again to 4.1. Thus the observations were consistent with the suggestion that the high ratio for N/A observed at first might be due to more rapid destruction of *nor*adrenaline than of adrenaline.

Comparison of Corbasil with adrenaline.—The results, however, did not necessarily indicate that the enzyme involved was amine oxidase. It seemed probable that the addition of ephedrine to the perfusion fluid would be likely to reduce the action of whatever enzyme was present, since the structure of ephedrine would enable it to compete with *nor*adrenaline or adrenaline for any adrenolytic enzyme.

We therefore attempted to carry the investigation further by using Corbasil, which differs from adrenaline simply by the attachment of a methyl group to the α carbon atom. This substance, like ephedrine, can combine with and inhibit the action of amine oxidase, but it is not destroyed by it. We compared Corbasil with adrenaline, just as we had compared *nor*adrenaline with adrenaline, by determining amounts which produced the same vasoconstriction before, during, and after perfusion with ephedrine.

When Corbasil was compared with adrenaline it was found that in the fresh preparation Corbasil was often without vasoconstrictor action except in relatively large amounts. When the perfusion had continued for 1.5 hr., the vessels were then more sensitive. The change is shown in Table III in which the results in 9 experiments where the ratio was determined at once are shown side by side with 7 experiments in which the ratio was determined after 1.5 hr. In both groups the mean amount of adrenaline was almost the same, but in the second group the mean amount of Corbasil was about a quarter of that in the first, showing that the difference in the C/A ratio in the two groups was due to the increase in sensitiveness to Corbasil after 1.5 hr. perfusion.

The effect of adding ephedrine to the perfusion fluid was therefore determined in those experiments in which the ratio between the amounts of Corbasil and adrenaline having equal constrictor effects was observed at the end of 1.5 hr. perfusion. The

TABLE III

AMOUNTS OF CORBASIL AND ADRENALINE PRODUCING THE SAME VASOCONSTRICTION IN RABBIT EAR VESSELS

Experiments in which C/A ratio was determined as soon as perfusion began				Experiments in which C/A ratio was determined after 1.5 hr. perfusion			
Exp.	Corbasil μg.	Adrenaline μg.	C/A	Exp.	Corbasil μg.	Adrenaline μg.	C/A
21	0.6	0.02	30	30	0.025	0.0025	10
22	0.8	0.02	40	31	0.04	0.01	4
23	0.01	0.001	10	32	0.2	0.02	10
24	0.02	0.001	20	33	0.025	0.0025	10
25	0.005	0.00075	6.6	34	0.03	0.005	6
26	0.2	0.005	40	35	0.015	0.002	8
27	0.026	0.002	13	36	0.03	0.004	7.5
28	0.03	0.002	15				
29	0.045	0.005	9				
Mean	0.193	0.0063	20.4		0.052	0.0066	8.0

TABLE IV

AMOUNTS OF CORBASIL AND ADRENALINE PRODUCING THE SAME VASOCONSTRICTION; OBSERVATIONS BEFORE PERFUSION WITH EPHEDRINE WERE MADE ONLY WHEN THE PERFUSION WITH LOCKE'S SOLUTION HAD CONTINUED FOR 1.5 HR.

Exp.	Before perfusion with ephedrine			During perfusion with ephedrine (10 μg./ml.)			After perfusion with ephedrine		
	Corbasil μg.	Adrenaline μg.	C/A	Corbasil μg.	Adrenaline μg.	C/A	Corbasil μg.	Adrenaline μg.	C/A
37	0.04	0.003	13	0.06	0.006	10	0.02	0.002	10
38	0.04	0.003	9	0.05	0.0045	9	0.06	0.0075	8.5
39	0.03	0.009	3.5	0.045	0.015	3	0.03	0.0075	4
40	0.01	0.001	10	0.03	0.01	3	0.013	0.0025	5
41	0.015	0.002	7	0.02	0.002	10	0.02	0.003	6
42	0.05	0.0045	11	0.05	0.004	12	0.02	0.002	10
43	0.0025	0.0005	5	0.018	0.003	6	0.056	0.008	7
Mean			8.3			6.9			6.9
Mean of 14 experiments			8.15			7.6			8.1

results are shown in Table IV, and are difficult to interpret. They differ from those in Table II in that during perfusion with ephedrine the vessels did not become more sensitive to adrenaline. The results in Table II were in full confirmation of the finding of Gaddum and Kwiatkowski that in the presence of ephedrine the vessels become more sensitive to adrenaline, for in each experiment a smaller amount of adrenaline was injected during the ephedrine perfusion. In Table IV, such an increased sensitiveness to adrenaline during perfusion with ephedrine was never observed. The mean ratio of amounts of Corbasil and of adrenaline which caused the same constriction did not alter appreciably; it was 8.3 before ephedrine was added to the perfusing fluid, and 6.9 during perfusion with ephedrine; it remained at 6.9 after

perfusion with ephedrine ceased. This result was clearly unlike the result in Table II, which showed that ephedrine reduced the ratio between the amount of *nor*adrenaline and the amount of adrenaline which produced the same constriction.

The failure of ephedrine to modify the Corbasil-adrenaline ratio supported the explanation of the modification of the *nor*adrenaline ratio. It remained, however, to obtain evidence that amine oxidase destroyed *nor*adrenaline more rapidly than it destroyed adrenaline.

Experiments with amine oxidase

Oxygen uptake.—When adrenaline is incubated with amine oxidase, oxygen is consumed and adrenaline disappears at a rate which is about the same as that when *nor*adrenaline is used instead of adrenaline. Results obtained in this way do not suggest any appreciable difference in the reactions of adrenaline and *nor*adrenaline with amine oxidase.

There is, however, the possibility that if both substrates are present together amine oxidase may then display a greater affinity for one of them, and this may be oxidized more rapidly than the other. If a mixture of equal parts of adrenaline and *nor*adrenaline is incubated, it may then happen that the relative proportion of the two will change, and ultimately only one may still be present. A method for determining the relative proportion of adrenaline and *nor*adrenaline in a mixture has been described by Burn, Hutcheon, and Parker (1950).

In the following experiments a washed suspension of acetone-dried liver was used; such suspensions have high amine-oxidase activity (Blaschko and Hawkins, 1950). The suspensions were incubated in an atmosphere of oxygen at 37° in manometer flasks with adrenaline or *nor*adrenaline, or a mixture of equal parts of both amines, and the oxygen uptake was recorded. Each flask contained 1.4 ml. of the liver suspension, containing 140 mg. of liver powder, and 0.2 ml. of 0.1 M-semicarbazide, in order to prevent further oxidation of the primary products of oxidation. In each flask, the total volume of fluid was brought up to 2.0 ml. by adding 0.4 ml. of a 0.025 M-solution of adrenaline or of *nor*adrenaline, or 0.2 ml. of each of these solutions. The amine concentration at the beginning of the incubation was therefore 0.005 M; the adrenaline-*nor*adrenaline mixtures contained 211 μg. of *nor*adrenaline plus 229 μg. of adrenaline per ml.

The amount of oxygen expected to have been taken up at the end of the oxidation reaction was 112 μl. O_2. Actually, the oxygen uptake did not come to a complete standstill, but continued very slowly, probably owing to non-enzymic oxidation. The course of the O_2 uptake is shown in Fig. 2. In Fig. 2 the initial rate of uptake with *nor*adrenaline is slightly greater than that of adrenaline, but this difference was not observed in all experiments.

Fig. 2 also shows the O_2 uptake in three flasks which contained *nor*adrenaline-adrenaline mixtures. The incubation of these mixtures was not followed to completion. The incubation of the first of these samples was only continued until about a quarter of the theoretical amount of oxygen had been taken up. The enzyme was then rapidly removed by centrifugation; the supernatant was acidified and boiled. The second sample was incubated until about a half, and the third sample until about three-quarters, of the theoretical oxygen uptake was observed; the samples were likewise centrifuged, acidified, and boiled when incubation was terminated.

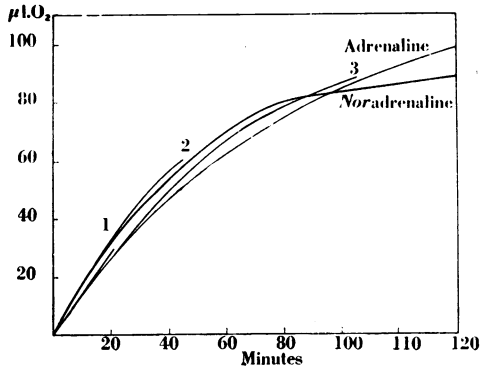

FIG. 2.—This is a record showing oxygen uptake during the incubation with rabbit liver powder of (*a*) adrenaline, (*b*) *nor*-adrenaline, (*c*) mixtures of equal parts of adrenaline and *nor*adrenaline; the incubation of these mixtures was interrupted at the points indicated, (1), (2), and (3). The mixtures were then examined on the blood pressure and nictitating membrane of the spinal cat. We are indebted to Dr. H. Blaschko for this Figure.

More rapid disappearance of nor*adrenaline*.—The proportion of adrenaline and *nor*adrenaline in the incubated samples was then determined by observations in the spinal cat. The method depends on the observation that, while adrenaline causes a contraction of the nictitating membrane, *nor*adrenaline has little or no action upon it. The intravenous injection of a mixture of *nor*adrenaline and adrenaline causes a rise of blood pressure and a contraction of the nictitating membrane, and, from the ratio of the contraction of the membrane to the rise of blood pressure, the proportion of the two amines can be determined by matching with known mixtures. In each experiment on a spinal cat the effect of the incubated samples was compared with that of known mixtures of the two solutions from which the mixtures for incubation were prepared.

The results showed that in the course of incubation, while the total pressor activity fell in proportion to the oxygen consumed, the percentage of adrenaline in the mixture rose and the percentage of *nor*adrenaline declined. The observations made are given in Table V.

TABLE V

INCUBATION OF A MIXTURE CONTAINING EQUAL PARTS OF ADRENALINE AND *nor*ADRENALINE WITH AN ACETONE-DRIED POWDER OF LIVER

Exp.	Liver from	Percentage of adrenaline in mixture at different stages of incubation; incubation arrested when O_2 consumption was		
		¼ complete	½ complete	¾ complete
44	Rabbit	58	88	100
45	,,	48	77	79
46	,,	—	71	90
47	,,	52	72	88
48	,,	59	72	88
49	Cat	64	70	76

In experiment 44, before incubation began, each mixture contained, as already stated, about 440 μg. active base per ml. The oxygen consumption was a quarter complete after 30 min. in one mixture; incubation was arrested and the mixture then had a pressor action equivalent to 320 μg. adrenaline per ml., and of this pressor action 58 per cent was due to adrenaline. In a second mixture the oxygen consumption was half complete after 60 min.; the mixture then had a pressor action equivalent to 200 μg. adrenaline per ml., and of this pressor action 88 per cent was due to adrenaline.

Finally, in a third mixture, oxygen consumption was about three-quarters complete after 155 min.; the mixture then had a pressor action equivalent to 61 μg. adrenaline per ml., and of this pressor action 100 per cent was due to adrenaline.

Exps. 44 to 48 were carried out with an acetone powder from rabbit liver, Exp. 49 with a similar powder from cat liver. Thus the results were consistent in showing that under the conditions of the experiment *nor*adrenaline was destroyed more rapidly than adrenaline, presumably because of a greater affinity of the enzyme for the former substance.

Discussion

The experiments described throw some light in the first place on the varying figures which have been obtained by several workers for the relative vasoconstrictor action of *nor*adrenaline and adrenaline in the vessels of the rabbit ear. When the ear is freshly perfused *nor*adrenaline has relatively little constrictor action compared with adrenaline; when the perfusion has continued overnight its constrictor action is much closer to that of adrenaline, though still less.

Since it has been found by Schapira (1945) that amine oxidase is present in the rabbit ear and by Thompson (unpublished) that it is present in the ear vessels, it seemed possible that the increased constrictor action of *nor*adrenaline after 24 hr. was due to diminished destruction by amine oxidase. Now ephedrine is known to inhibit the action of amine oxidase (Blaschko, Richter, and Schlossmann, 1937), and we therefore compared the ratio of amounts of *nor*adrenaline and adrenaline which produced the same constriction, before, during, and after perfusion with ephedrine. We observed that ephedrine caused a drop in the ratio, and that the ratio rose again when the ephedrine was discontinued. This result is consistent with the explanation that inhibition of amine oxidase by ephedrine renders the vessels more sensitive to adrenaline, but still more sensitive to *nor*adrenaline.

Now Corbasil is a substance similar to adrenaline with a methyl group attached to the α carbon atom of the side chain. It is therefore not destroyed by amine oxidase, but it inhibits the action of this enzyme. We determined the effect of ephedrine perfusion on the ratio of the amounts of Corbasil and adrenaline which produced equal constriction. We found that ephedrine had no effect on this ratio, and indeed in these experiments ephedrine appeared to have no effect at all since it failed to sensitize the vessels to adrenaline. The failure of ephedrine to exert an action could be explained by supposing that the enzyme was already inhibited by the successive injections of Corbasil which were previously given. However this may be, the observations showed that, while ephedrine lowered the ratio of amounts of *nor*adrenaline and adrenaline which produced equal constriction, it did not modify the corresponding ratio of amounts of Corbasil and adrenaline.

The fall in the ratio of amounts of *nor*adrenaline and adrenaline which caused equal vasoconstriction, produced by including ephedrine in the perfusion fluid, suggested that amine oxidase destroyed *nor*adrenaline more readily than it destroyed adrenaline. Evidence for this was lacking. When preparations containing amine oxidase act on adrenaline or on *nor*adrenaline, the rate at which oxidation proceeds is similar for the two substances, as was observed by Blaschko, Richter, and Schlossmann (1937). We have confirmed this finding. If, however, the two substances are placed together in the same manometer flask in the presence of an acetone-dried

preparation of rabbit liver, or of cat liver, the *nor*adrenaline disappears at a faster rate than the adrenaline. Mixtures of equal amounts in the course of incubation were found to decline in percentage of *nor*adrenaline present and to increase in percentage of adrenaline.

All these observations combine in favour of the supposition that, in the rabbit ear vessels, adrenaline and *nor*adrenaline are destroyed by amine oxidase, which is known to be present there, and that the relative weakness of *nor*adrenaline in the freshly perfused ear is due to its more rapid destruction by this enzyme. The observations, however, cannot be said to prove this point since an increased sensitivity of the vessels need not be due to the action of amine oxidase. For example, the increased sensitivity to Corbasil during the first hour's perfusion (Table III), and the increased sensitivity to adrenaline after cessation of perfusion with ephedrine (Table II), cannot be explained by the action of amine oxidase.

SUMMARY

1. Amine oxidase, in the form of an acetone-dried preparation of rabbit or cat liver, has a greater affinity for *nor*adrenaline than for adrenaline, since, when it is allowed to act upon a mixture of equal parts of adrenaline and *nor*adrenaline, the percentage of adrenaline rises during the fall of the total quantity of pressor amines.

2. When a rabbit ear is perfused with Locke's solution, and when constrictor effects are obtained by injecting small quantities of *nor*adrenaline and of adrenaline into the perfusing fluid, the ratio of amounts of the two substances which produce the same constriction is high, indicating that *nor*adrenaline is relatively weak. When the perfusion has continued for 24 hr., *nor*adrenaline more nearly approaches adrenaline in constrictor action.

3. Since amine oxidase is present in rabbit ear vessels, these observations could be explained by supposing that at first amine oxidase is very active, and its greater affinity for *nor*adrenaline is responsible for the weak constrictor action of this substance, but that after 24 hr. amine oxidase becomes less active.

4. Support is given to this explanation by the fact that the addition of ephedrine, which inhibits amine oxidase, to the perfusing fluid increases the constrictor action of *nor*adrenaline more than it increases that of adrenaline.

5. Ephedrine, when added to the perfusing fluid, does not modify the relative constrictor action of Corbasil (which is not destroyed by amine oxidase) and adrenaline.

It is a pleasure to thank Dr. H. Blaschko for arranging the experiments in which the pressor amines were incubated with amine oxidase, and for Fig. 2.

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