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THE HYPERSENSITIVITY OF THE DENERVATED NICTITATING MEMBRANE TO VARIOUS SUBSTANCES

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The hypersensitivity to adrenaline which develops in the nictitating membrane after removal of the superior cervical ganglion has long been known. In 1932 Rosenblueth showed that the change in sensitivity was not restricted to adrenaline, but also occurred when ACh, pilocarpine, eserine, or histamine were used to stimulate the membrane. In 1934 Bacq and Rosenblueth added to these calcium chloride and potassium chloride, which they said stimulated the denervated membrane after the adrenal glands were excluded from the circulation. Since the innervation of the nictitating membrane is sympathetic it seemed clear that the phenomenon of hypersensitivity could have no relation to the humoral transmitter of sympathetic impulses.

In the following year, however, Bacq and Fredericq (1935) published evidence that there were some cholinergic fibres present; they found that supramaximal stimulation of the cervical sympathetic chain caused a greater contraction of the nictitating membrane when eserine was instilled into the eye, and that when atropine was given later by intravenous injection the contraction following stimulation of the chain was diminished below the initial size. These observations made it possible that the hypersensitivity of the denervated membrane to ACh might be related to this cholinergic innervation. In 1936 Ross showed that in cats under allobarbitone (" Dial "), in which the adrenal glands were excluded from the circulation, the contractions of the denervated nictitating membrane caused by adrenaline, ACh, $CaCl_2$, or KCl were reduced by 933F and by yohimbine, which are ordinarily regarded as specific antagonists for adrenaline. It may be noted that the action of ACh was diminished less than that of the other three substances.

Gaddum and Goodwin (1947) in the course of other work investigated the behaviour of histamine in cats under allobarbitone to which cocaine was given and in which the adrenal glands were excluded. They found that to histamine " the membrane was usually about as sensitive as it was to adrenaline." Denervation " sensitized the membrane to small doses, but when the dose was increased the difference between the two effects disappeared and even became reversed for a very large dose."

Most of the foregoing observers have had in mind that the substances ACh, histamine, KCl, and pilocarpine liberate the amines of the adrenal medulla, and have excluded the adrenal glands. Recent work by Bein and Meier (1952) has suggested that histamine may release substances with an adrenaline-like action from other places. They removed the adrenal glands in the spinal cat and waited until an injection of histamine no longer produced a secondary rise of blood pressure after the primary fall. They then injected L-cysteine and observed a restoration of the secondary rise 45–60 min. later, the rise being much greater than that seen before adrenalectomy. They found that this secondary rise was absent if the renal vessels were clamped on both sides. Their observations did not prove that the secondary rise was due to the release of a pressor substance, but this seems the most likely explanation. If that is so, the action of ACh, histamine, pilocarpine, eserine, KCl, and $CaCl_2$ on the nictitating membranes may perhaps be indirect and due to the release of an adrenaline-like substance. Such an explanation would fit in well with the evidence that their action is augmented by cocaine and diminished by 933F and yohimbine.

A further investigation became necessary after the work of Burn and Robinson (1952, 1953), who found that the removal of the superior cervical ganglion in the cat led to a fall in amine oxidase in the nictitating membrane, and suggested that this fall was responsible for the increased action of adrenaline and noradrenaline. They supported

this view by evidence of a correlation between the extent of the fall in amine oxidase and the increase of hypersensitivity to noradrenaline which was obtained in a series of 25 cats. Thus they believed that the hypersensitivity was explained by the fall in the enzyme. But the statement of Cannon and Rosenblueth (1949) that " the supersensitivity of the denervated nictitating membrane to acetylcholine, pilocarpine, eserine, and histamine, as well as that to calcium and potassium salts cannot reasonably be attributed to a decrease of amine oxidase or of phenolases " remained unanswered.

The position of ACh was clarified by the evidence of Burn and Philpot (1953), who recorded the presence of both true and pseudo-cholinesterase in the nictitating membrane, and found that after removal of the superior cervical ganglion there was a fall in the amount of true cholinesterase. This fall would explain the hypersensitivity to ACh.

Accordingly, a study has been made of the action of histamine and other substances on the normal and denervated membranes of the cat both with the ordinary circulation and when the head was perfused with Locke's solution containing dextran. The results in the perfused preparation enabled us to see how far the action of the different substances was exerted directly on the membranes. The observations of Bacq and Fredericq (1935) have also been repeated.

Methods

Observations were made on cats under chloralose anaesthesia, under allobarbitone (" Dial ") anaesthesia and in spinal preparations. In most experiments the cats were prepared by excision of the right superior cervical ganglion 4–10 days previously. The contractions of the nictitating membrane were recorded by attaching them to isotonic levers fitted with frontal writing points; the contractions were magnified 7.5 times.

The perfusion experiments were carried out by dissecting the two external jugular veins and the two carotid arteries, and passing two ligatures around each of these vessels. The next steps were taken as rapidly as possible. A clip was put on one jugular vein, and a cannula was inserted below the clip so as to lead away from the head. The other vein was treated similarly. Artery cannulae pointing to the head were then placed in the two carotids. A Dale–Schuster pump was used to pump the perfusion fluid to a Y-piece, the ends of which were connected to the two carotid cannulae by polythene tubing. Two polythene tubes were joined to the vein cannulae and led the effluent to a collecting jar. A 4-way cannula was placed on the path from the pump to the Y-piece, immediately before the Y-piece, so that a thermometer could be inserted, and the pressure recorded. The time from the interruption of the natural circulation to the beginning of the perfusion was 10–15 min. The perfusion fluid, which was well oxygenated beforehand, was a mixture of 1 part of a 6% dextran solution and 3 parts of Locke's solution. The temperature was 37°. Care was taken that the rate of outflow from the tubes leading from the veins was the same on the two sides; this depended on the position of the cannulae. Injections were made into the perfusion fluid just before it reached the Y-piece, so that the injected material was distributed to the two sides evenly. Observations were almost always completed in 10–20 min. from the start of the perfusion.

When experiments were carried out in cats in which the cervical sympathetic chain was stimulated, the chain was laid on the two prongs of shielded electrodes and covered with liquid paraffin. In two experiments a unipolar electrode was gently hooked round the postganglionic fibres. Stimulation was provided by using an induction coil, using Lewis's rotary contact breaker in the primary circuit.

Results

Experiments in the Whole Animal

The results of 18 experiments which were carried out in the spinal cat may be illustrated from two of them. For ACh, KCl, and $CaCl_2$ the results were fairly consistent, but for histamine and pilocarpine they varied greatly. As shown in Fig. 1, 0.1 mg. pilocarpine nitrate was without effect on the denervated membrane or on the normal membrane, but as shown in Fig. 2 it caused both to contract, the denervated less than the normal. The effect of 40 mg. $CaCl_2$ was the same in both experiments; both membranes contracted, the denervated more than the normal. The effect of 0.1 mg. histamine was to cause contraction of the normal membrane as shown in both Fig. 1 and Fig. 2. In Fig. 1 the denervated membrane contracted less and more slowly, while in Fig. 2 it did not contract; it relaxed slightly. The effect of 30 mg. KCl was to cause contraction of the denervated membrane in both experiments, but it caused contraction of the normal membrane only in the second. The effect of ACh was to cause both membranes to contract, the denervated more than the normal. Very little attention was given to eserine, but in the dose of 0.3 mg. used in the experiment of Fig. 1, it had no appreciable effect.

Action of Histamine.—Since the action of histamine appeared to be of great importance in deciding how far the hypersensitivity of the denervated membrane was specific, in 18 other experiments a study of the action of histamine alone was made. The normal membrane contracted in response to histamine in 10 out of 14

experiments in spinal cats, but not in two experiments in cats under chloralose or in two experiments in cats under allobarbitone. The normal membrane contracted in each of seven experiments on spinal cats in which the adrenal glands were first excluded from the circulation. The normal membrane contracted in each of eight experiments after the cat received 8 mg. cocaine by intramuscular injection ; half of these experiments were in spinal cats and half in cats anaesthetized with chloralose or allobarbitone. The effect of cocaine is shown in Fig. 3, in which 20 μg. histamine was without effect before cocaine was given, but caused a contraction after ; in this experiment the exclusion of the adrenal glands abolished the effect of histamine.

The results with the denervated membrane were more difficult to understand. Thus only in 8 out of 18 experiments did the denervated membrane contract more than the normal membrane. In two others the denervated contracted to the same extent as the normal membrane ; in four others the denervated contracted, but less than the normal membrane ; in two others the denervated membrane did not contract although the normal membrane did, and finally in two others the denervated membrane relaxed, while the normal membrane contracted.

We have no satisfactory explanation to offer of the relaxation of the denervated membrane by histamine ; it was observed very often as a prelude to contraction. In two experiments we injected 1 mg. decamethonium to stop reflex movements of the cat, and in each of these we observed contraction of the normal membrane accompanied by apparent relaxation of the denervated membrane. We supposed that the effects of decamethonium were exerted on the external ocular

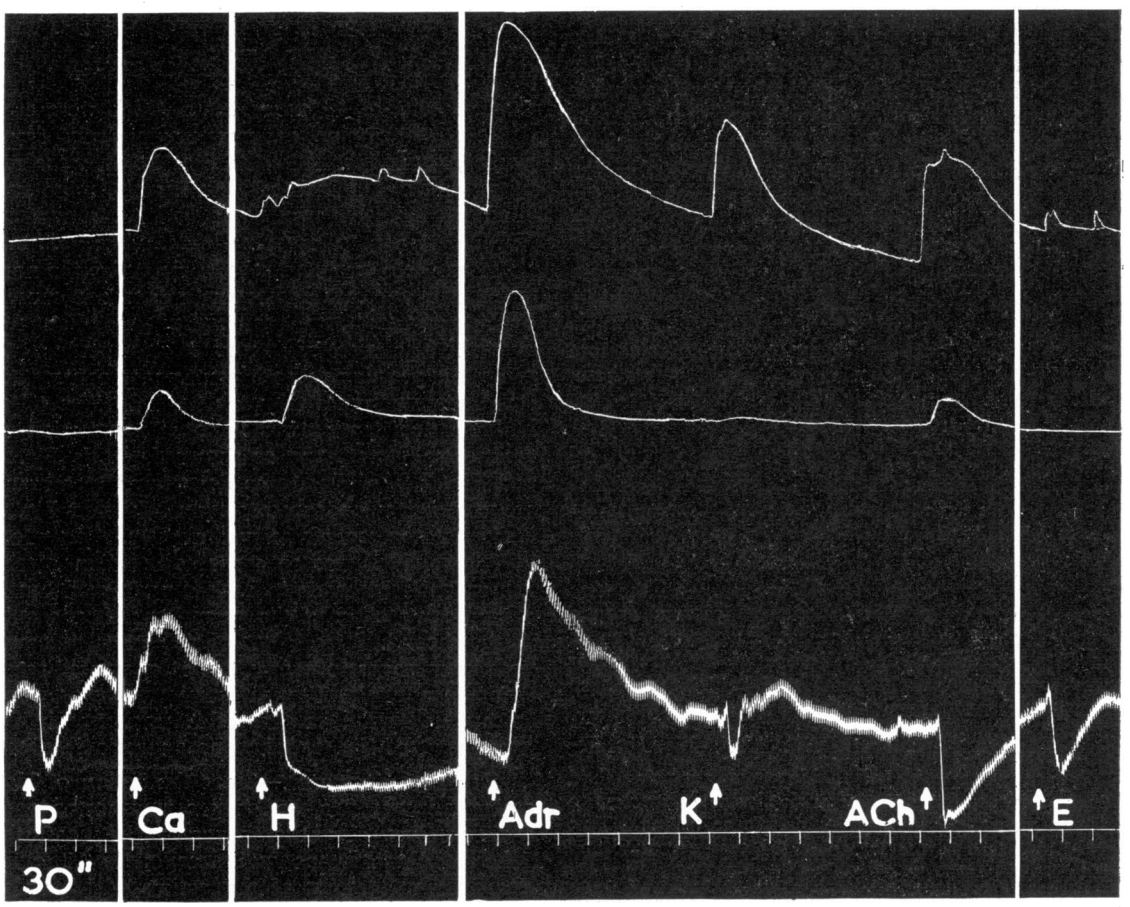

FIG. 1.—Spinal cat. Records, from above down, are: denervated nictitating membrane; normal nictitating membrane; arterial blood pressure. Intravenous injections were: P, 0.1 mg. pilocarpine nitrate; Ca, 40 mg. $CaCl_2$; H, 0.1 mg. histamine (base); Adr, 10 μg. adrenaline; K, 30 mg. KCl; ACh, 40 μg. acetylcholine; E, 0.3 mg. eserine sulphate.

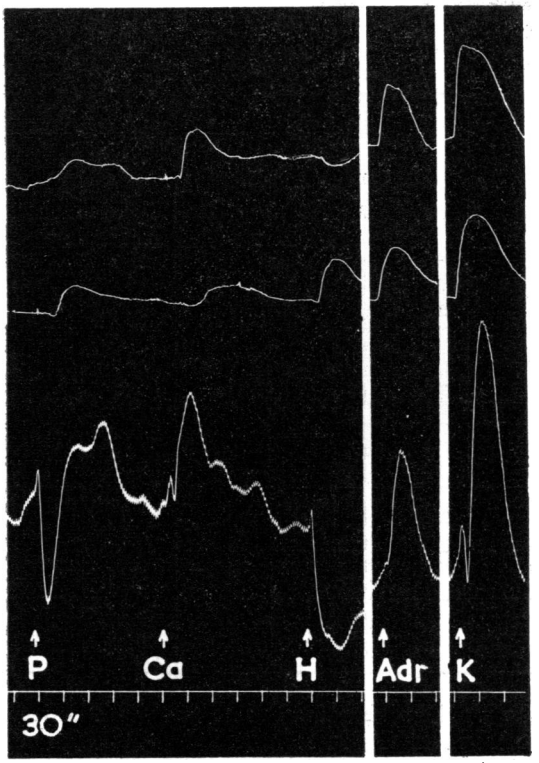

FIG. 2.—Spinal cat; records as in Fig. 1. Intravenous injections as follows: P, 0.1 mg. pilocarpine nitrate; Ca, 40 mg. $CaCl_2$; H, 0.1 mg. histamine (base); Adr, 10 μg. adrenaline; K, 30 mg. KCl.

muscles, movements of which Rosenblueth and Bard (1932) and Paton and Thompson (unpublished) have shown to affect the record of the movements of the nictitating membrane. It may be that some of the effects of histamine seen after exclusion of the adrenals are also due to action on these muscles.

In all these experiments the effect of adrenaline was observed as well as that of histamine. The effect of 5–10 μg. adrenaline was nearly always greater, usually much greater, on the denervated than the normal membrane. Moreover, the effect of adrenaline increased as the dose increased. This did not seem to be true for histamine, though different doses were tested in only four experiments. In one of these 7 μg. histamine caused the denervated membrane to contract 4 mm. (as recorded on the drum), 33 μg. caused a contraction of 6.5 mm., and 100 μg. caused a contraction also of 6.5 mm. In a second, 20 μg. histamine caused the denervated membrane to contract 7 mm.; 100 μg. caused it to contract 6 mm., and 300 μg. caused it to contract 12 mm. In a

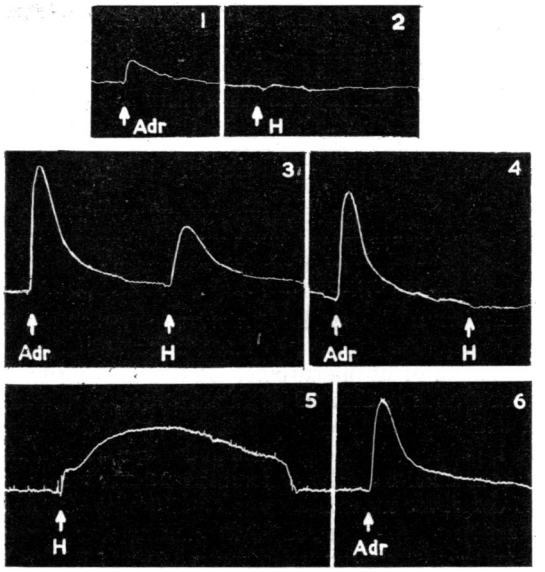

FIG. 3.—Cat under allobarbitone. Normal nictitating membrane. Contractions due to: (1) 5 μg. adrenaline. (2) 20 μg. histamine (base). (8 mg. cocaine was then injected intramuscularly.) (3) 5 μg. adrenaline and 20 μg. histamine. (The adrenals were then tied.) (4) 5 μg. adrenaline and 20 μg. histamine (40 mg./kg. cysteine was injected intravenously). (5) One hour later, 5 μg. histamine. (6) 5 μg. adrenaline.

third, 20 μg., 100 μg., and 300 μg. all caused the denervated membrane to contract 3 mm. In these three experiments the denervated membrane contracted more than the normal membrane throughout. These effects are all small, not graded according to dose, and do not confirm that the membrane was usually as sensitive to histamine as to adrenaline (Gaddum and Goodwin, 1947). We tested the effect of injecting cysteine hydrochloride, 30 mg./kg., to see if we could observe on the nictitating membrane what Bein and Meier (1952) observed on the blood pressure. Five experiments were carried out in the spinal preparation, a positive result being obtained in only one; this is shown in Fig. 3. When the effect of histamine on the nictitating membrane was abolished by adrenalectomy, the injection of cysteine caused a restoration of the stimulant action one hour later; the effect was observed after the injection of 5 μg. and 20 μg. histamine.

Action of Pilocarpine.—As Fig. 1 and Fig. 2 show, the action of pilocarpine was also variable. Out of 18 experiments in which injections of pilocarpine were made, the effect on the denervated membrane was greater than that on the normal membrane in 11, it was equal to that on the normal membrane in four, and less than that on the normal membrane in three. In the 18 experiments, 28 injections were made of doses from

75 μg. to 0.5 mg. In only 9 of the 28 trials was the contraction of one of the membranes greater than 7 mm., whereas the injections of 5 μg. adrenaline in the same experiments caused a contraction greater than 7 mm. 12 out of 13 times. Thus, as with histamine, the effects of pilocarpine were small. In one experiment in which pilocarpine had no effect on the normal membranes, after the injection of cocaine it caused a contraction ; when the adrenal glands were excluded, the effect of pilocarpine was abolished.

Action of Potassium Chloride.—The action of KCl was less difficult to summarize than that of histamine or of pilocarpine. It was injected intravenously in a dose of 30 mg. in 16 experiments ; it caused a contraction of the normal membrane in 11 of these, the mean height being 8.6 mm., and a contraction of the denervated membrane in 15 of these, the mean height being 13 mm. The contraction of the denervated membrane was greater than that of the normal membrane in 11 of the 16 experiments, and about the same in the others.

Action of Calcium Chloride.—The action of $CaCl_2$ was very similar to that of KCl. A dose of 30 mg. was used in 13 experiments. The normal membrane contracted in nine of these, the mean contraction being 7.3 mm. ; the denervated membrane contracted in all experiments, the mean contraction being 14.1 mm.

Action of Acetylcholine.—ACh was studied in seven experiments only in the whole animal, for our perfusion experiments showed that it had a direct action on the nictitating membranes. When given in a dose of 40 μg. it caused contraction of the normal nictitating membrane in four out of six experiments, the mean contraction being 4 mm. ; it caused contraction of the denervated membrane in all experiments, the mean contraction being 13.8 mm.

The results so far described are summarized in Table I, which includes results for adrenaline also. They show that all substances stimulated the denervated membrane more than the normal membrane, in confirmation of earlier workers, but they also show that adrenaline produced much larger contractions in much lower doses than the rest, the effects of histamine and pilocarpine being small even when the doses used were high.

Evidence of Cholinergic Fibres.—The observations of Bacq and Fredericq (1935) were repeated in a few experiments to see if evidence of cholinergic fibres in the sympathetic supply of the nictitating membrane could be confirmed. In some experiments unipolar stimulation was applied to the ganglion itself, and eserine was applied directly to the nictitating membrane, using a solution containing 10 mg./ml. The result in one experiment is shown in Fig. 4, in which the relaxation of the membrane was slower after the application of the eserine, and then after the injection of 2 mg. atropine the height of the contraction was reduced and the relaxation was quicker.

In other experiments the cervical sympathetic chain was laid on shielded electrodes, and similar results were obtained. A positive effect of eserine was observed in four out of eight experiments, and a positive effect of atropine in seven out of

TABLE I

EFFECTS OF VARIOUS SUBSTANCES ON THE NORMAL AND ON THE DENERVATED NICTITATING MEMBRANE IN THE WHOLE ANIMAL

Substance	Dose	Normal Membrane		Denervated Membrane	
		Proportion of Expts. in which Contraction Occurred	Mean Ht. of Contraction (mm.)	Proportion of Expts. in which Contraction Occurred	Mean Ht. of Contraction (mm.)
Histamine	7–20 μg.	0/3	0	3/3	4·7
	33–50 ,,	1/2	0·8	2/2	10·3
	100 ,,	6/8	2·8	8/8	6·9
	500 ,,	1/2	2·3	2/2	7·5
Pilocarpine	75 ,,	5/7	3·3	6/7	3·6
	100 ,,	7/14	1·1	14/14	6·0
	150–200 ,,	5/5	5·8	5/5	8·4
	300 ,,	1/1	4·0	1/1	11·5
	500 ,,	2/2	2·5	2/2	16·8
KCl	30 mg.	11/16	8·6	15/16	13·1
	40 ,,	0/1	0	1/1	24
	50 ,,	0/1	0	1/1	15
$CaCl_2$	30 ,,	9/13	7·3	13/13	14·1
	40 ,,	2/2	7·5	2/2	14·3
	50 ,,	2/4	3·0	3/4	13·8
ACh	20 μg.	1/1	12·0	1/1	9·0
	40 ,,	4/6	4·0	6/6	13·8
Adrenaline	5 ,,	12/12	9·7	12/12	22
	10 ,,	10/10	15·0	10/10	29
	20 ,,	2/2	22·0	2/2	53

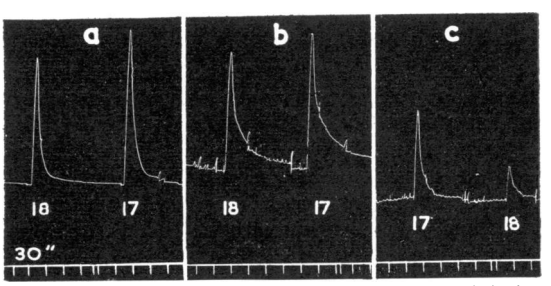

FIG. 4.—Cat under chloralose. Record of contractions of nictitating membrane when postganglionic fibres were stimulated by secondary coil (distance from primary in cm. shown under contraction). *a*, initial responses; *b*, after 1% eserine was instilled in the conjunctival sac; *c*, after 2 mg. atropine was injected intravenously. Relaxation was slower in *b*. Contractions were smaller and relaxation quicker in *c*.

seven experiments. Our results confirmed the observations of Bacq and Fredericq that in a certain proportion of cats evidence of the presence of cholinergic fibres can be obtained by this method.

Perfusion Experiments

The early perfusion experiments were carried out using Locke's solution, and were mostly unsuccessful because the tissue soon became oedematous. When, however, 25% of a 6% solution of dextran was used and the temperature was maintained at 37°, satisfactory results were obtained as shown in Figs. 5 and 6.

The general result of the perfusion experiments was that the membranes responded to the injection of adrenaline and of ACh with large contractions which increased with the dose; when the other substances were injected, in some experiments there were very small contractions, but in most experiments there were none. The results in Fig. 5 were obtained when Locke's solution only was used for perfusion. The injection of 5 μg. adrenaline caused a large contraction of the normal membrane, and a still larger contraction of the denervated membrane. The next injection was 40 mg. $CaCl_2$, which had no effect on either membrane. The next injection was 100 μg. ACh, which caused contractions similar to those caused by adrenaline. Although the preparation was capable of large responses to adrenaline and ACh, the injection of 0.2 mg. histamine produced only a tiny contraction in both membranes, and the injection of 40 mg. KCl was still less effective. In Fig. 6, when dextran–Locke was used, 25 mg. $CaCl_2$, 15 mg. KCl and 50 μg. histamine had no appreciable action, but pilocarpine caused a small contraction both in the dose of 50 μg. and of 0.5 mg. In this respect pilocarpine and histamine resembled one another and were unlike ACh and adrenaline—namely, that the injection of 10 or 20 times as much had scarcely more effect. The results of the nine best perfusion experiments are given in Table II, these being experiments in which the effects of adrenaline and ACh were reasonably large. In all, 17 perfusion experiments were carried out, and in none of these had histamine, KCl, pilocarpine, or $CaCl_2$ an effect to be compared with that of adrenaline or ACh.

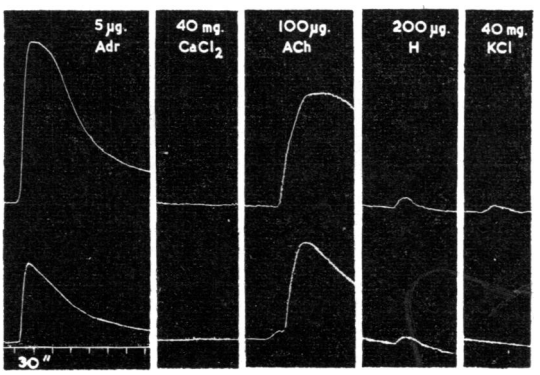

FIG. 5.—Cat's head perfused with Locke's solution. Upper record, denervated nictitating membrane; lower record, normal membrane. Successive injections of 5 μg. adrenaline; 40 mg. $CaCl_2$; 100 μg. acetylcholine; 200 μg. histamine; 40 mg. KCl.

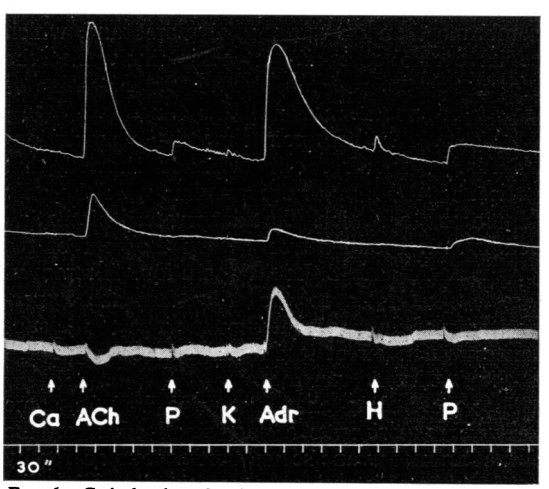

FIG. 6.—Cat's head perfused with dextran-Locke. Records as in Fig. 5, with the perfusion pressure below. Successive injections of 25 mg. $CaCl_2$; 20 μg. acetylcholine; 50 μg. pilocarpine; 15 mg. KCl; 5 μg. adrenaline; 50 μg. histamine; and 500 μg. pilocarpine.

DISCUSSION

The belief that the denervated nictitating membrane is more sensitive than the normal membrane to the action of almost any stimulant substance has been widely held since the observations of Rosenblueth (1932) and of Bacq and Rosenblueth (1934). The view is one which puts the increased sensitivity of denervated tissues almost beyond the reach of experimental investigation, since it explains the hypersensitivity as due to an increase in permeability at some undefinable point between the blood vessels and the receptors at which the chemical substance acts.

We have investigated the hypersensitivity of the denervated membrane in experiments in which the cat's head was perfused with Locke's solution containing dextran, and have found that the nictitating membranes responded to adrenaline and to ACh readily, but practically not at all to histamine, pilocarpine, $CaCl_2$, and KCl. These results appear to us to alter the situation, for they show that the

TABLE II

EFFECTS OF VARIOUS SUBSTANCES ON THE NORMAL AND ON THE DENERVATED NICTITATING MEMBRANE IN THE PERFUSED HEAD

(Figures are heights of contraction in mm. NM = normal membrane. DM = denervated membrane)

Exp.	Adrenaline			Histamine			Pilocarpine			KCl			$CaCl_2$			Acetylcholine		
	Dose μg.	NM	DM	Dose μg.	NM	DM	Dose μg.	NM	DM	Dose mg.	NM	DM	Dose mg.	NM	DM	Dose μg.	NM	DM
1	5	4	39	50	0	5	50	0	5	15	0	2	30	0	0	30	13	42
	—	—	—	500	1	5	500	4	6	—	—	—	—	—	—	—	—	—
2	5	3	40	50	0	1	50	0	0	15	0	0	15	0	0	25	6	7
	—	—	—	—	—	—	1 mg.	3	2	—	—	—	100	0	0	—	—	—
3	5	2·5	12	50	0	0	50	2	0	15	0	0	—	—	—	20	4	6
	—	—	—	200	0	1	250	0	2	60	0	1	—	—	—	50	3	9
4	5	2	19	50	0	0	50	0	0	15	0	0	15	0	0	20	1	9
	—	—	—	100	1	1	—	—	—	—	—	—	—	—	—	—	—	—
5	5	2	8	50	0	0	50	1	5	30	0	3	30	0	0	20	4	13
	—	—	—	100	0	2	—	—	—	—	—	—	—	—	—	—	—	—
6	2·5	2	10	50	0	0	50	0	0	15	0	0	—	—	—	50	3	3
				200	0	2	500	0	0	30	0	0	—	—	—	—	—	—
7	5	2	26	50	0	3	100	0	0	15	0	0	15	0	0	100	4	20
8	2	6	13	200	3	6	—	—	—	40	0	0	40	0	0	40	8	11
9	5	25	50	40	3	1	—	—	—	20	0	0	40	0	1	100	30	37
	—	—	—	200	3	3	—	—	—	40	0	2	—	—	—	—	—	—

action of histamine, pilocarpine, $CaCl_2$, and KCl which is seen in the body is almost certainly an indirect action due to the release of an adrenaline-like substance. There have been several observations previously made which ought to have suggested this. In the first place the effect of histamine, and of some others of these substances, is augmented by cocaine. There are no other effects of histamine which are augmented by cocaine. Augmentation by cocaine is, however, well recognized to occur with noradrenaline and adrenaline. In the second place, Ross (1936) showed that the contractions of the denervated nictitating membrane caused by adrenaline, $CaCl_2$, and KCl were reduced by 933F and yohimbine; the contraction caused by ACh was much less reduced. These anti-adrenaline substances do not reduce other effects of $CaCl_2$ and KCl.

That many substances release adrenaline and noradrenaline from the adrenal glands has long been known; for this reason previous observers have excluded the adrenals. We have found more than once that, after this exclusion, histamine and pilocarpine have no action on the membranes even in the cat treated with cocaine. Any direct action they may have must therefore be very variable, and unlike the regular action of adrenaline. Bein and Meier (1952) have shown that the pressor action of histamine, seen to follow the initial fall in the spinal cat, disappears after adrenalectomy, but can be restored by the injection of L-cysteine. We have observed in one experiment that, when the effect of histamine on the normal nictitating membrane was abolished by adrenalectomy, the injection of L-cysteine restored it. Now, unless we suppose that L-cysteine "sensitizes" the nictitating membrane to histamine, we seem to have evidence in this one case that histamine was acting by liberating some other substance. Since the adrenal glands were excluded, the release must have been from some other tissue. In view of the fact, demonstrated by Gaddum and Goodwin, that some doses of histamine have less effect on the denervated membrane than on the normal membrane, which we have also observed, it seems likely that the hypothetical substance released by histamine is neither adrenaline nor noradrenaline.

While putting these points mainly as suggestions for further inquiry, our evidence showed that adrenaline, ACh, and noradrenaline were the only substances to which the denervated nictitating membrane responded freely when the head of the cat was perfused with Locke's solution. It removed a serious obstacle to the view that the hypersensitivity of the denervated membrane to the substances which affect it directly can be explained by the fall in the enzymes.

The observation that histamine had in some perfusion experiments a very small effect on the nictitating membrane seems best accounted for by the fact that histamine has some adrenaline-like actions, as, for example, on the coronary vessels of the cat and dog and on the uterus of the rat and mouse, so that it may have some slight

power of attaching itself to receptors for adrenaline. Similarly, pilocarpine is a substance having properties similar to those of histamine on the one hand (Burn, 1925), and to those of ACh on the other. Hence pilocarpine may also be expected to have some slight power of stimulating receptors for adrenaline or for ACh. The fact that slight effects on the denervated membrane due to histamine, pilocarpine, or KCl were not seen on the normal membrane can be interpreted by the loss of enzyme in the denervated tissue. If these substances have some very small power of combining with adrenaline or ACh receptors, presumably they have some very small power of combining with the sites on the enzymes to which adrenaline or ACh normally become attached. In the denervated tissue, where the enzyme receptors have been shown to be fewer, more molecules of histamine, pilocarpine, or KCl will combine with the receptors and cause some stimulation.

Summary

1. The denervated nictitating membrane of the cat has been shown by others to contract more than the normal membrane in response to injections of KCl, $CaCl_2$, ACh, pilocarpine, histamine, and eserine; it has further been stated that the effects of these substances on the nictitating membranes are not dependent on the presence of the adrenal glands.

2. We have made observations by perfusing the cat's head with Locke's solution containing dextran, and have found that the only substances to which the denervated and normal nictitating membranes readily respond are adrenaline and ACh. We have never observed a response to $CaCl_2$, and responses to KCl, histamine, and pilocarpine have always been very small in the few experiments in which they were present, no matter how large the amount injected.

3. We have confirmed the evidence of Bacq and Fredericq that there is a small proportion of cholinergic fibres in the sympathetic supply of the nictitating membrane.

4. We conclude that the effects of KCl, $CaCl_2$, histamine, and pilocarpine in the whole animal after exclusion of the adrenals are indirect effects due to the liberation of an adrenaline-like substance from some other organ. These effects are in any case sometimes absent and usually small.

5. Since denervation of the nictitating membrane has been shown to cause a fall in amine oxidase and in true cholinesterase, the hypersensitivity of the membrane to adrenaline and ACh can be explained by the decline in the amount of these enzymes.

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