

PROPERTIES OF THE SUBSTANCE LIBERATED BY
ADRENERGIC NERVES IN THE RABBIT'S EAR

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THE identity of the substance liberated by adrenergic nerves is not yet known with certainty. The evidence of experiments with isolated organs has all supported the view that this substance is adrenaline.

(1) Loewi [1921] showed that fluid removed from a frog's heart after stimulation of the adrenergic nerves stimulated a second heart. Brinkman & van Dam [1922] showed that it inhibited the frog's stomach. Tschannen [1933] showed that it constricted frog's arteries. The active substance is destroyed by irradiation [Loewi & Navratil, 1926], or even by prolonged standing at room temperature [Lanz, 1928]. It is dialysable, and when the solution is made alkaline it gives the bright green fluorescence in ultra-violet light described by Gaddum & Schild [1934] as a specific test for adrenaline [Loewi, 1936]. The roughly quantitative estimate of the concentration of adrenaline that can be obtained by this test agreed with the results of biological tests on the frog's heart. Loewi was convinced by this evidence that the active substance actually is adrenaline.

(2) The substance liberated by the adrenergic nerves in the eye of rabbits and dogs has been demonstrated by its effect on the toad's heart, and on pilomotor muscles when the solution was injected intradermally. The solution collected after stimulation of the nerves also gave Viale's test for adrenaline more strongly than the fluid from the other eye [Bacq, 1933].

(3) Finkleman [1930] found that stimulation of the adrenergic nerves to a piece of rabbit's intestine liberated a substance which inhibited a second piece of intestine.

(4) Lehmann [1932] perfused the leg vessels of frogs and found that stimulation of the lower abdominal sympathetic chains liberated a substance which had an effect like adrenaline on the frog's heart.

(5) Bain [1933] perfused a dog's tongue, and found that stimulation of the sympathetic nerves liberated a substance which inhibited the tonus and contractions of a piece of rabbit's intestine.

Experiments in which the active substance was carried by blood to produce its effect on distant organs have, on the other hand, given results which are not compatible with the view that adrenaline is the only substance liberated [Cannon & Rosenblueth, 1933, 1937].

It has been found [Gaddum & Kwiatkowski, 1938] that stimulation of the adrenergic nerves in a rabbit's ear causes the liberation of a substance giving Shaw's [1938] colour test for adrenaline. By this test it has been shown that the substance is not noradrenaline or any other of a series of allied substances which have been found to give different results in this test. The present paper is an account of a more detailed study of the properties of this substance, and represents an attempt to apply to adrenergic nerves the methods which have been successful in identifying the substance liberated by cholinergic nerves.

METHODS

The method used for perfusing the rabbit's ear was identical with that used previously [Gaddum & Kwiatkowski, 1938]. In most of the experiments the rate of flow was kept approximately constant by raising the reservoir of perfusion fluid to a height of 2-3 m. and interposing a capillary tube between the reservoir and the ear.

The active substance liberated by the nerves was detected by its actions on the ear itself, the frog's heart and the fowl's rectal caecum and by the colorimetric method described by Shaw. When the ear itself was used as a detector the perfusion pressure was kept constant at between 18 and 50 cm. Active solutions were injected into the special cannula previously described, and effects on the outflow recorded as before. Adrenaline caused vasoconstriction in concentrations of 10^{-8} and over.

Frogs' hearts (*R. esculenta*), isolated by Straub's method, were filled with Locke's solution diluted to 1.4 times its volume with water. The perfusion fluid was diluted in the same way before testing. In order to minimize possible distortion of the record by acetylcholine from the ear, the fluid with which the heart was washed, but not the perfusion fluid, contained atropine sulphate (10^{-6} to 10^{-7}). This preparation usually reacted with an increase in the size of the beat to a concentration of 10^{-9} of adrenaline, and sometimes to a concentration of 10^{-10} .

The fowl's rectal caecum was used to test whether the substance liberated in the ear caused inhibitor effects as well as excitor effects, since

this preparation is the most sensitive of the tissues known to be inhibited by adrenaline, which is effective in a concentration of 10^{-9} [Barsoum & Gaddum, 1935]. In order to economize material the tissue was suspended in a small bath (2 c.c.). It was kept at 30° C., since according to Blaschko & Schlossmann [1938] this increases its sensitivity, and since it also diminishes the frequency of large spontaneous movements which are apt to spoil the record. The solutions were warmed to this temperature before being added to the bath.

Gaddum & Kwiatkowski [1938] found that the adrenaline equivalent of the effluent was considerably increased by the addition of ephedrine to the perfusion fluid. It was hoped that this observation would make it easy to study the pharmacological effects of the active substance, but unfortunately it has been found that the concentration of ephedrine which produces this effect (10^{-5}) is sufficient to interfere with the pharmacological tests. In most of the experiments recorded below no ephedrine was used, but in some experiments the perfusion fluid contained a concentration of 10^{-7} . Such concentrations did not inhibit the effect; their value was doubtful.

The perfusion fluid was Locke's solution equilibrated with oxygen containing 5% CO_2 .

The postganglionic sympathetic nerves were stimulated near the ganglion, either with supramaximal shocks by condenser discharges (36 per sec.), or with an induction coil with 4 V. in the primary circuit and a coil distance of not less than 10 cm.

In most cases the nerves were stimulated for 15–20 min., during 1 min. out of every 3 min., until the collection of 20 c.c. of perfusion fluid was finished. In some experiments the nerves were stimulated for 3 min. with an interval of 1 min. A volume of 10 c.c. of the fluid was used for the colour test, and the rest was used for immediate testing on the ear, heart or gut.

In order to prevent oxidation, the perfusion fluid was collected in a vessel containing sufficient ascorbic acid to produce a final concentration of 10^{-5} .

The adrenaline solutions were prepared from the pure base with HCl.

RESULTS

Stimulation of the adrenergic nerves causes the liberation of a substance which stimulates the frog's heart (Fig. 1). The control sample *C* had a small effect like that of adrenaline. The sample collected after stimulation of the nerves had a larger effect, and was equivalent to a

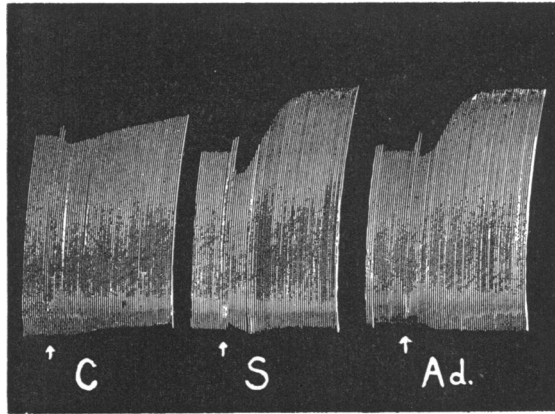


Fig. 1. Frog's heart. Fluid from perfused ear of rabbit diluted to be isotonic. *C*, no stimulation. *S*, fluid collected during stimulation of sympathetic. *Ad.* adrenaline 10^{-9} .

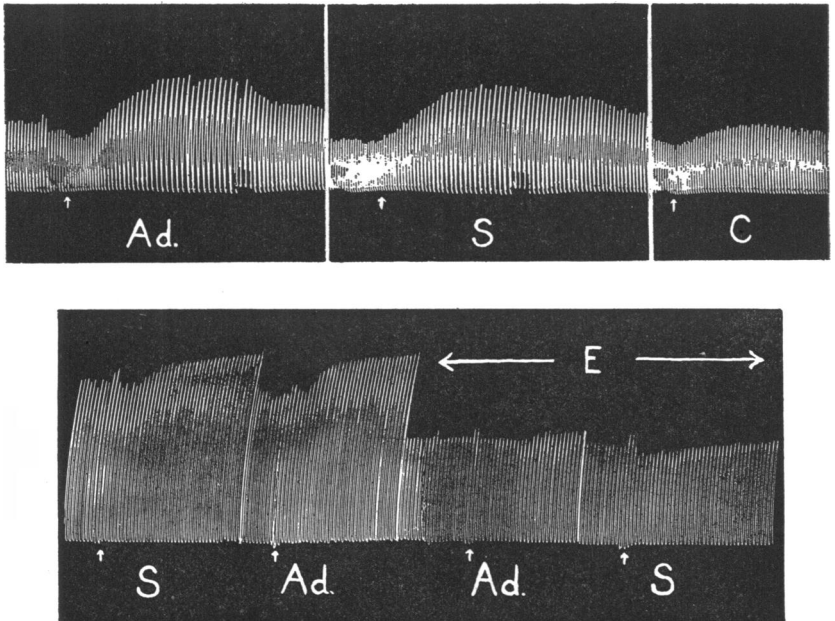


Fig. 2. Above: outflow from perfused rabbit's ear. Height of record represents drop interval. Reinjection of fluid collected during stimulation of sympathetic. Below: same fluids tested on frog's heart. *Ad.* 1 c.c. adrenaline 10^{-8} . *S*, 1 c.c. fluid collected during stimulation. *C*, 1 c.c. fluid collected without stimulation. *E*, ergotamine 10^{-5} .

concentration of 10^{-9} of adrenaline. It will be seen that the stimulant effect was preceded by transient inhibition. The cause of this inhibition has not been studied in detail, but it is unlikely to be acetylcholine, since the heart was treated with atropine to make it insensitive to acetylcholine. The stimulation was due to a substance which resembled adrenaline in the fact that its effect was inhibited by high concentrations of ergotoxine (Fig. 2), and that it disappeared from the solution when it was kept at room temperature for 2-3 hr.

Fig. 2 shows the result of an experiment in which the effluent was tested both on the frog's heart and on the vessels of the ear. At *S*, 1 c.c. of the fluid collected during stimulation of the nerves was injected into the inflow cannula and caused vasoconstriction which was shown as an increase in the drop interval of the outflow. The effect was equivalent to that of a concentration of 10^{-8} of adrenaline. Fluid collected before stimulation of the nerves had a comparatively small effect. The same active fluid was also tested on a frog's heart, and again found equivalent to a concentration of 10^{-8} of adrenaline. Ergotoxine (10^{-5}) abolished both the effects of this fluid and of the equivalent concentration of adrenaline.

The fluid collected in this experiment was also tested by Shaw's [1938] colorimetric method, and again found to be equivalent to the same concentration of adrenaline.

The fluid collected during stimulation of the nerves also usually inhibits the fowl's rectal caecum. In some experiments this effect was large, but in others there was no effect or even a slight stimulation of the caecum. In several experiments the adrenaline equivalent in this test has appeared lower than that on the frog's heart, but it has not been possible to obtain satisfactory quantitative evidence on this point.

On the other hand there can be no doubt that a substance inhibiting the fowl's rectal caecum is actually liberated when the adrenergic nerves to a perfused rabbit's ear are stimulated [Gaddum, Jang & Kwiatkowski, 1939].

Fig. 3 shows a phenomenon which has been observed in several experiments, but which has no connexion with the substance liberated by adrenergic nerves. In this experiment the ear was perfused with Locke's solution containing eserine (4×10^{-6}), and the effluent was tested on leech muscle, which had previously been sensitized with eserine. Stimulation was applied to both the great auricular nerve and the posterior auricular nerve simultaneously. This caused the liberation of a substance acting like acetylcholine on the leech muscle. The substance

was not derived from sympathetic nerves because in the particular experiment from which Fig. 3 was made the superior cervical ganglion had been removed, and 16 days had been allowed for the degeneration of the postganglionic fibres. It was thought, at first, that such experiments might provide evidence of the presence of cholinergic vasodilator nerves in the rabbit's ear, but it was observed that stimulation of the nerves caused contraction of small voluntary muscles at the base of the ear.

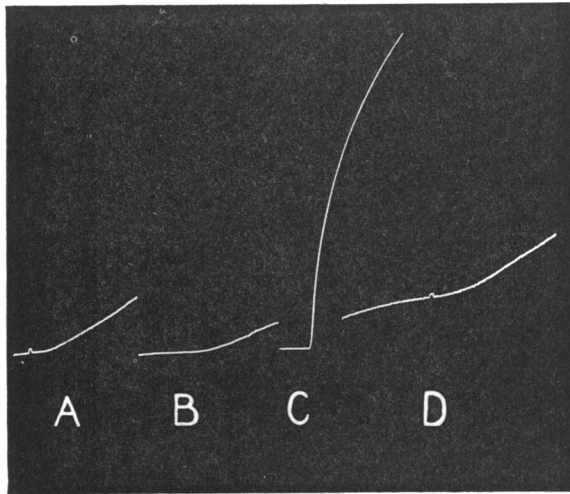


Fig. 3. Eserinizated leech muscle. Samples of fluid from rabbit's ear, perfused with Locke's solution containing eserine (4×10^{-6}). Sympathetic removed 16 days earlier. Stimulation of great and posterior auricular nerves during the collection of sample B (1 c.c. in 5 min.). Rate of flow constant.

These muscles could not easily be excluded from the perfusion system, and it is possible that the effect shown in Fig. 3 was due to the stimulation of motor nerves to these muscles, which would be expected to cause the liberation of acetylcholine [Dale, Feldberg & Vogt, 1936].

DISCUSSION

The experiments described above show that the substance liberated by the adrenergic nerves in a rabbit's ear resembles adrenaline in its action on the frog's heart, the rabbit's ear and Shaw's colorimetric test. These tests agreed quantitatively with one another in their estimate of the adrenaline equivalent. The value of such quantitative agreement between different tests in the identification of pharmacologically active

substances was emphasized by Chang & Gaddum's [1933] experiments with choline esters. Feldberg & Gaddum [1934] applied the principle to the substance liberated in the cat's superior cervical ganglion, and so were able to show this substance was acetylcholine and not any other of a series of choline esters. The same principle has since been used to show that the substance liberated by other cholinergic nerves is also acetylcholine. Taken in conjunction with the observations that the colour in Shaw's test is increased by suitable treatment of the solutions with alkali [Gaddum & Kwiatkowski, 1938], and that the substance liberated by the nerves inhibits the fowl's rectal caecum [Gaddum *et al.* 1939], these experiments leave little doubt that the active substance is adrenaline.

SUMMARY

The evidence that the substance liberated by the adrenergic nerves in a perfused rabbit's ear is adrenaline has been strengthened by the observation that it stimulates the frog's heart, and constricts the vessels of the rabbit's ear, and that estimates of the adrenaline equivalent obtained by these two tests agreed with colorimetric estimates.

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