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THE ACTIONS OF ADRENALINE AND OF ACETYLCHOLINE ON THE ISOLATED PULMONARY VESSELS AND AZYGOS VEIN OF THE DOG.

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THE work described in this paper was done at the request of Prof. I. de Burgh Daly, and was designed to accompany the investigations which have been, and are being, carried out in his laboratory on the pulmonary and bronchial vascular systems of the dog *in situ* [Berry, Brailsford and Daly, 1931; Berry and Daly, 1931; Daly and Euler, 1932].

PREVIOUS WORK.

The literature on the reaction of isolated pulmonary vessels to adrenaline begins with the work of Meyer [1906], who found that the drug contracted the extrapulmonary artery of the ox. Langendorff [1907] found contraction in most cases in experiments with extrapulmonary arteries from the sheep, pig, calf and cat. Neither of these two observers gave very full details of his work. Dixon and Halliburton [1910] found that the dog's pulmonary artery contracted with adrenaline for a distance of 1·5 cm. from its cardiac origin. The contraction became progressively less the farther away from the heart the sections were taken. Cow [1911] used the arteries of the sheep and ox for most of his experiments, but occasionally human, goat, or rabbit vessels. He does not state which species he used for the pulmonary vessels, but gives tracings from the rabbit and the sheep. He found that the extrapulmonary arteries showed progressively less reaction to adrenaline as they approached the lung, and that the intrapulmonary arteries were unresponsive. Campbell [1911] recorded contraction of the pulmonary artery of the sheep and of the rabbit, and, in seven out of ten cases, of the sheep's pulmonary vein. Barbour [1912] found an extremely strong contraction in two experiments with the main pulmonary artery of the rabbit. The extrapulmonary artery of the calf gave in four cases contraction, in one no reaction, and in two, where the sections were taken from the neighbourhood of the lungs, doubtful dilatation. In four cases

(three from the calf, one from the pig) intrapulmonary arteries showed no response. Macht [1914] used strips of the pulmonary arteries of pigs, oxen, and human beings, these strips being of various sizes and tested at various periods of time after death (49 days after death in one case). His concentrations of adrenaline varied between 1 : 1,000,000 and 1 : 1000. In every case the effect was contraction. Adrenaline produced no active dilatation after ergotoxine. Rothlin [1920] found that the extrapulmonary arteries of the ox and of the horse responded regularly by contraction. The rise to the maximum was rapid and the contraction was not long sustained. The intrapulmonary arteries also showed a contraction with 1 : 2,000,000 and 1 : 1,000,000, but it was not great. No qualitative change in reaction was produced by varying the concentration of adrenaline, and lengthening never occurred. Waterman [1930] worked with isolated veins of the dog and of the cat. In eight experiments he obtained contraction, and in the ninth a negative result. All the intrapulmonary branches he used gave a contraction. The greatest dilution of adrenaline was 1 : 70,000,000. Wissler finally [1931] recorded the results of sixty experiments with arteries from twenty-three oxen of varying age. He found that the regular response in the central vessels was a contraction. In some peripheral vessels definite dilatation resulted. Between these two areas lay an indifferent zone. The circumference of the vessels giving contraction was from 9 mm. upwards, that of the vessels giving dilatation from 11 mm. downwards. Contractions showed a rapid rise to their maximum, and usually disappeared within 5 min. The percentage shortening under optimum conditions was up to 5 per cent., whereas barium chloride caused a shortening of as much as 20 per cent. Some vessels responded to adrenaline 1 : 40,000,000, but the threshold was usually between 1 : 20,000,000 and 1 : 10,000,000. The reaction as a rule increased in degree with increase in concentration, but in some few cases, in which the threshold was high, there was no increase in degree. There was never any change in sign in the response with increase in concentration. The largest dilatation recorded was 7 per cent. Dilatations, in contra-distinction to contractions, were long-lasting, and also required in general higher concentrations of the drug (1 : 1,000,000) for their production.

The only experiments with acetylcholine on isolated pulmonary vessels are those of Waterman [1930]. He found no effect with acetylcholine in three experiments on pulmonary veins. On the other hand, pilocarpine in three cases gave vaso-constriction, and no effect in two others.

Method.

Nine dogs altogether were used, approximately a hundred experiments were performed, and about three times this number of applications of drugs were made. The dogs were killed with the humane killer of the slaughterhouse, so that there was no contamination of the vessels by anæsthetics. Ring preparations were used in all experiments (except one), and they were suspended in oxygenated Ringer's solution. The apparatus employed was an optical recording one, which is described elsewhere [Franklin, 1930], and the movements were magnified × 166 or × 160. The acetylcholine employed was the bromide (B.D.H.), and the adrenaline the ordinary 1 : 1000 solution (Parke, Davis and Co.), except in a few experiments in which, as a control, crystalline adrenaline (kindly supplied by the same firm) was used. The routine dilutions of acetylcholine used were 1 : 100,000,000, 1 : 10,000,000, and 1 : 1,000,000; some experiments were made with 1 : 10,000,000,000 and 1 : 1,000,000,000, but the effects were negative. The routine dilutions of adrenaline used were 1 : 10,000,000 and 1 : 1,000,000, but 1 : 100,000 was used in some cases when the tissue was old, and also in some of the reversal experiments. It was not possible to work with vessels much less than 4 mm. in circumference, hence the azygos vein was the only representative of the bronchial vascular system to be tested. The measurements of the vessels were made at room temperature and in general were somewhat less than in Ringer's solution at 37° C.; the relaxation on warming was greater in the case of the veins than of the arteries.

Results.

Adrenaline. The pulmonary aorta gave no effect once with 1 : 10,000,000, the extrapulmonary artery no effect twice with this concentration. Both vessels, in one instance each, gave a relaxation with adrenaline. In all other cases, *i.e.* in ten experiments with the aorta and twenty with the artery, the effect was a contraction. The highest degree of shortening was over 5 p.c. in the case of the aorta (Fig. 1), and over 12 p.c. in the case of the artery. The intrapulmonary arteries gave very small and variable responses with contraction predominating. The intrapulmonary veins also gave very small responses or none at all, and the relaxations balanced in number the contractions. The smaller extrapulmonary veins always contracted with adrenaline, the large extrapulmonary veins gave contraction or no response, never relaxation. The azygos vein responded by

contraction in eleven cases, by relaxation in two or three, and showed no effect in one or two.

The pulmonary aorta (Fig. 1), the extrapulmonary artery, large extrapulmonary vein (Fig. 2), and azygos vein (Fig. 3), all showed a

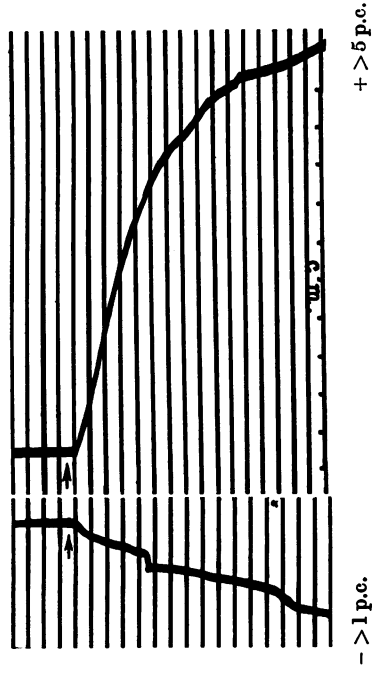

Fig. 1.

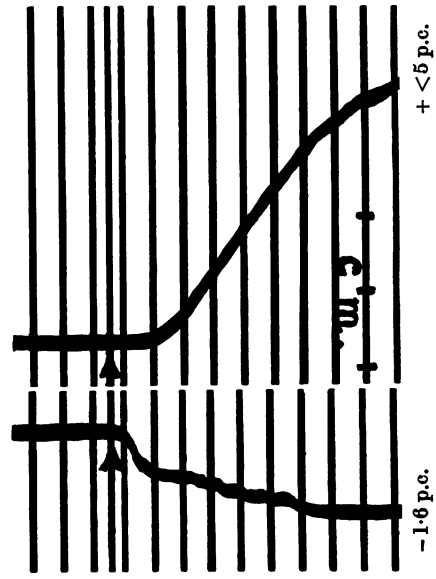

Fig. 2.

Fig. 1. Pulmonary aorta ring. Circumference 26 mm. Upper curve normal, lower after immersion of ring in ergotoxine ethanesulphonate solution. At arrows adrenaline hydrochloride 1 : 100,000. Time-grids minutes. Magnification × 160[1].

Fig. 2. Extrapulmonary vein ring. Circumference 9 mm. Upper curve normal, lower after immersion of ring in ergotoxine ethanesulphonate solution. At arrows adrenaline hydrochloride 1 : 100,000. Time-grids minutes. Magnification × 160.

reversal of adrenaline contraction after immersion in ergotoxine ethanesulphonate 1 : 50,000 or 1 : 20,000.

Acetylcholine. The pulmonary aorta once showed no effect with 1 : 100,000,000. In all other cases it gave relaxation. The extrapulmonary artery invariably relaxed (Fig. 4). The intrapulmonary arteries gave no

[1] Magnification in all figures refers to that shown in the *unreduced* records. The centimetre scales indicate the original size of the records before reduction.

response to the drug, while that of the intrapulmonary veins was variable, with contraction predominating. The smaller and larger extrapulmonary veins showed contraction (Fig. 5), which can be reversed by atropine sulphate in a concentration between one and ten times that of the acetylcholine (Fig. 6). The azygos vein never relaxed, and in most cases showed contraction, though in some the effect of the drug was negative.

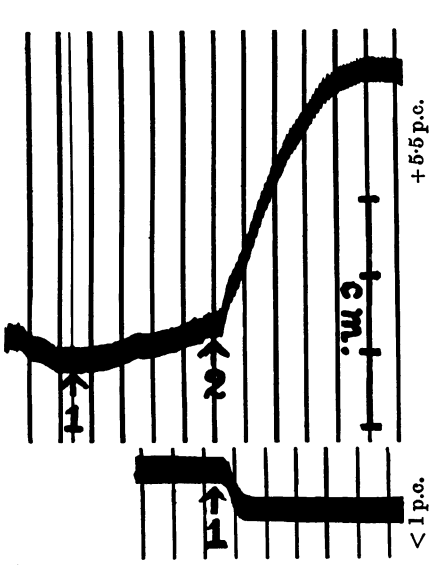

Fig. 3.

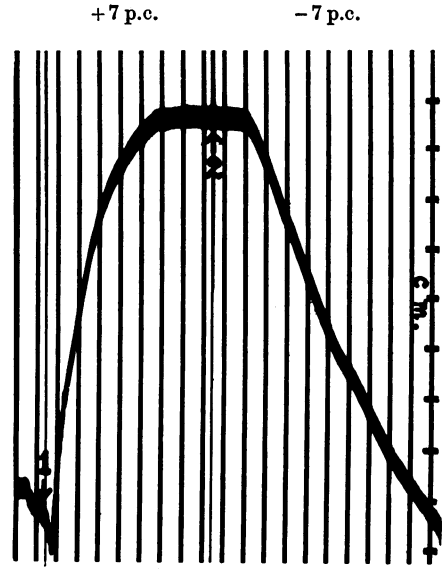

Fig. 4.

Fig. 3. Azygos vein ring. Circumference 8 mm. Upper curve normal, lower after immersion in ergotoxine ethanesulphonate solution. Upper curve (1) adrenaline hydrochloride 1 : 10,000,000, (2) 1 : 1,000,000; lower curve adrenaline hydrochloride 1 : 1,000,000. Time-grids minutes. Magnification × 166.

Fig. 4. Extrapulmonary artery ring. Circumference 16 mm. (1) Adrenaline hydrochloride 1 : 1,000,000, (2) Acetylcholine bromide 1 : 1,000,000. Time-grids minutes. Magnification × 160.

Discussion.

Taken on the whole, then, the extrapulmonary vessels of the pulmonary vascular system of the dog showed the following remarkable features. Both arteries and veins usually contracted with adrenaline, while the arteries relaxed and the veins contracted with acetylcholine.

Increase in concentration of either drug never changed the sign of an effect from contraction to relaxation or *vice versa*.

All parts of the extrapulmonary system, and also the azygos vein,

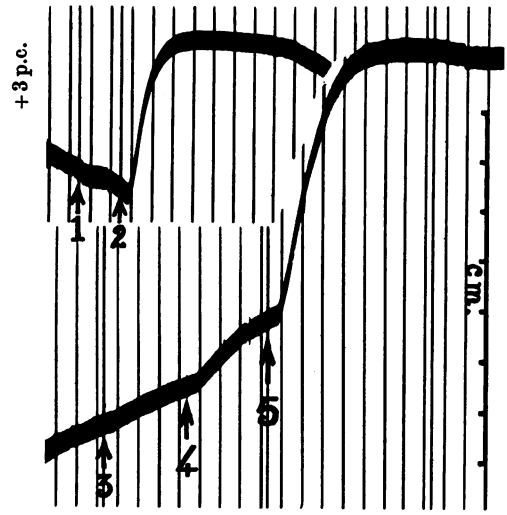

Fig. 5. Extrapulmonary vein ring. Circumference 8 mm. (3) Acetylcholine bromide 1 : 100,000,000, (4) 1 : 10,000,000, (5) 1 : 1,000,000. After replacement of Ringer's solution (1) adrenaline hydrochloride 1 : 10,000,000, (2) 1 : 1,000,000. Time-grids minutes. Magnification × 166.

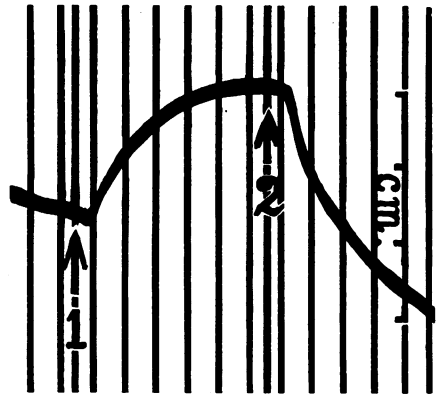

Fig. 6. Extrapulmonary vein. Circumference 10 mm. (1) Acetylcholine bromide 1 : 1,000,000, (2) Atropine sulphate 1 : 100,000. Time-grids minutes. Magnification × 160.

gave presumptive evidence, in the ergotoxine reversals of adrenaline contractions, of the presence of sympathetic vaso-dilator mechanisms in addition to the sympathetic vaso-constrictor ones. Control experiments with crystalline adrenaline proved that the reversals were not due to any other component of the stock 1 : 1000 solution (Parke, Davis and Co.).

In the single case in which a ring of pulmonary aorta relaxed with an initial dose of adrenaline, the piece was used 25 hours after the death of the animal. The isolated case in which relaxation occurred in a ring from the artery was similar, though it was from another dog. It was conceivable that in these instances the sympathetic vaso-dilators were naturally predominant over the constrictors, or that the lapse of time since death had affected the dilator endings less than it had the constrictor endings. The effect of lapse of time was therefore tested on the pulmonary aorta and artery from another dog, but after 50 hours the reaction to adrenaline was only changed in degree, and not in sign. This result, and Macht's instance of a contraction 49 days after death, incline one to the first alternative, and one may, in view of all the evidence, expect occasional dilator effects with adrenaline on isolated pulmonary vessels, as the literature tends to prove.

The azygos vein showed effects which were in general similar to those exhibited by the pulmonary veins, but there was a somewhat greater tendency to dilatation with adrenaline.

As adrenaline and, very probably, acetylcholine [Dale, 1929] are chemical agents produced in the normal body for the execution of definite functions in connection with the autonomic nervous system, it is important to consider if the concentrations of the two substances used in these experiments bear any adequate relation to their physiological concentrations. In this connection it may be stated that all the effects on the vessels described above have been obtained with 1 : 100,000,000 acetylcholine, and with 1 : 10,000,000 adrenaline, even if their degree has been increased with increase in concentration. Further, smooth muscle usually requires, in its isolated *in vitro* state, a higher concentration of a given drug than it does *in vivo*. It should also be noted [Hülse, 1922] that the pulmonary vascular system is more likely than the systemic to have a higher concentration of adrenaline in the blood passing through it. The final decision as to the validity of transferring the results of these experiments to one's conception of the vessels acting *in situ* must, however, be left until more precise knowledge exists of the concentrations and behaviour of adrenaline and of acetylcholine in the normal body.

At all events the amounts used in the present series compare favourably with those recorded in the literature.

One possibility, which is suggested by the action of acetylcholine on the extrapulmonary arteries and veins, is that congestion of the lungs, like asthma, may be due to excessive action of the parasympathetic system. If this is so, the neutralization of the venous effect (Fig. 6) by atropine points to a rational method of treatment. But more knowledge must first be obtained of the reactions of the intrapulmonary vessels, and one must also find out if human pulmonary vessels respond in a similar way to those of the dog. This latter point is to be investigated as soon as suitable material is obtained.

With regard to the intrapulmonary vessels, the writer's opinion is that they are much less reactive than the extrapulmonary ones, but that this point should be settled by the use of another technique (this is to be done by the aid of the microscope in Prof. Daly's laboratory). By comparison, however, with other small structures (*e.g.* trachea of fœtal kitten), the reactions of which have been recorded with the same apparatus as used in the present experiments, the small degree of the response of these vessels must be ascribed to weakness of innervation or scantiness of smooth muscle rather than to any fault of the apparatus.

In one case adrenaline was used on a longitudinal piece of pulmonary artery, instead of a ring, and it was found to cause contraction.

Summary.

1. In general isolated ring preparations of the extrapulmonary part of the pulmonary vascular system of the dog gave the following reactions with the concentrations used:

(*a*) Adrenaline constricted the arteries and the veins.

(*b*) Acetylcholine relaxed the arteries and constricted the veins.

(*c*) Adrenaline constrictor effects were reversed by ergotoxine.

2. Similar preparations of the azygos vein of the dog gave reactions similar, in large measure, to those given by the extrapulmonary veins.

3. The intrapulmonary vessels gave weak and variable reactions.

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