A VASO-DILATOR ACTION OF ACETATES.

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The action here described was discovered accidentally. We had under investigation a depressor substance occurring in extracts from blood, obtained under certain experimental conditions. The original alcoholic extract, after concentration, was treated with basic lead acetate, to effect a further purification. Excess of lead having been removed by H_2S from the filtrate, the latter was neutralised with NaOH. When the action of this neutral extract was tested on the blood-pressure of a cat, it was found that its depressor effect was unusual in form: instead of the rapid recovery of blood-pressure following the initial fall—such as occurs after injection of small doses of histamine or choline—there occurred, after a sharp initial fall, a slow recovery.

Further experiments were made in which known amounts of histamine were added to normal blood, and the blood then extracted, in one of two ways:

(*a*) by the use of Schenk's reagent only;

(*b*) by Schenk's reagent, followed by a further purification with basic lead acetate.

In extracts (*a*) histamine was found to have been recovered almost quantitatively. Extracts (*b*), on the other hand, had acquired a more powerful and more prolonged depressor action than was produced by pure histamine, in the doses which had been originally added to the blood.

There were two possibilities: the treatment with lead acetate might have caused the liberation of a depressor substance from a previously inactive constituent of the extract, or it might have introduced a new depressor substance from without. The latter possibility was easily tested. The only substance newly added by the treatment was acetic acid, liberated in removing the excess of lead as sulphide, and converted into sodium acetate by the neutralisation; and it was found, in fact, that the addition of sodium acetate, in quantities roughly equivalent to those inevitably produced in the lead treatment, to dilute solutions of

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pure histamine, produced an increase and prolongation of depressor effect precisely similar to that under discussion. Thus, a combined injection of 0·0025 mg. histamine (·0075 mg. histamine acid phosphate) plus 27 mg. neutral sodium acetate, produced a depressor effect nearly equal to that of 0·005 mg. histamine (Fig. 1, *A*).

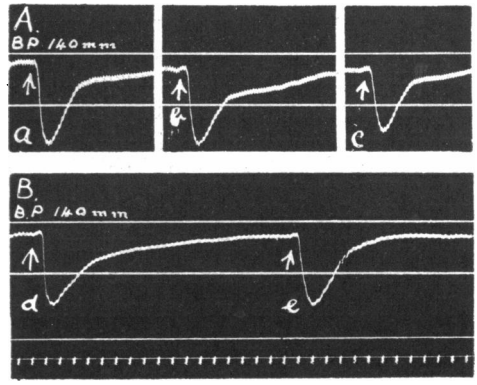

Fig. 1. Cat's blood-pressure. *A*. At (*a*), intravenous injection of 0·005 mg. histamine; (*b*), 0·0025 mg. histamine and 27 mg. sodium acetate; (*c*), 0·0025 mg. histamine. *B*. At (*d*), intravenous injection of 0·005 mg. histamine; (*e*), 0·00125 mg. histamine and 27 mg. sodium acetate and 17 mg. KCl. Time marker in all tracings shows intervals of 10″.

The observations above described had more than an immediate significance. In the course of investigations, for some time in progress in this laboratory, on the depressor constituents of certain tissue extracts, it has been the usual practice to assay these extracts quantitatively, by a physiological test on the cat's blood-pressure, during different stages in their purification. A knowledge of the depressor effects of acetates might throw light on anomalies which have appeared, from time to time, after chemical procedures involving their introduction.

General character of the depressor action. In its usual form, the depressor effect of a small intravenous injection, such as 0·25 c.c. to 0·5 c.c. of a normal solution of neutral sodium acetate into a cat, is characteristic, and consists of an initial sharp fall of 3–5 mm., followed by a momentary check, and by a more gradual fall and a still slower recovery (Fig. 2). Sodium acetate in these doses does not always produce the initial slight and rapid fall; this, on the other hand, often occurs after the injection of other hypertonic salt solutions in similar amounts, and is apparently a non-specific effect. The secondary gradual fall and recovery has not

been observed with any salts that have been tested, other than acetates. The extent of the fall of blood-pressure after a given dose of sodium acetate varies from one cat to another, and in the same cat at different stages in the experiment; in this respect its action resembles that of more potent depressors, such as histamine and choline.

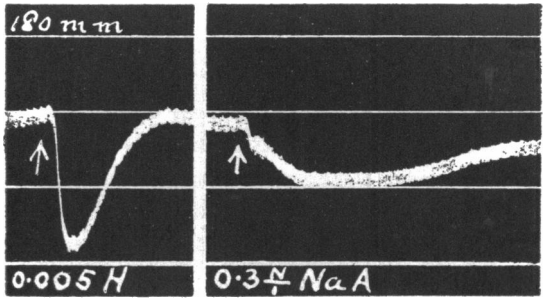

Fig. 2. Cat's blood-pressure. Intravenous injection of 0·005 mg. histamine, compared with that of 0·3 c.c. of *N*/1 neutral sodium acetate.

Mechanism and location of the depressor action. The following observations contributed to the analysis of the effect.

1. A pronounced and definite depressor effect, such as that obtained by injecting 0·5 c.c. of normal sodium acetate into a cat, was not accompanied by any perceptible change in the rate of the pulse. Accordingly it seemed probable that the effect was mainly, if not entirely, due to a dilator action on the peripheral vessels.

2. This was confirmed by direct experiments.

(*a*) In a cat under ether, with arterial blood-pressure recorded from the carotid artery, the volume of one hind limb, freshly denervated by section of the sciatic and crural nerves, was registered by a plethysmograph and bellows-recorder. Heparin was given and a cannula for intra-arterial injection introduced, through the iliac artery of the other side, into the aorta just central to its bifurcation, as described by Dale and A. N. Richards(1). A small injection of histamine into the aorta by this route produced the usual expansion of the limb, followed by a relatively small fall of the general arterial pressure. Sodium acetate solution produced similar evidence of vasodilator action when injected in the same way, the effects, as with ordinary intravenous injection, being more prolonged in proportion to their intensity than those of histamine. The action of sodium acetate was accordingly, like that of histamine, a peripheral effect on the vessels, independent of the nervous system

(Fig. 3). It will be seen that the expansion of the limb in response to the acetate is preceded by a sharp, evanescent constriction. This is a non-

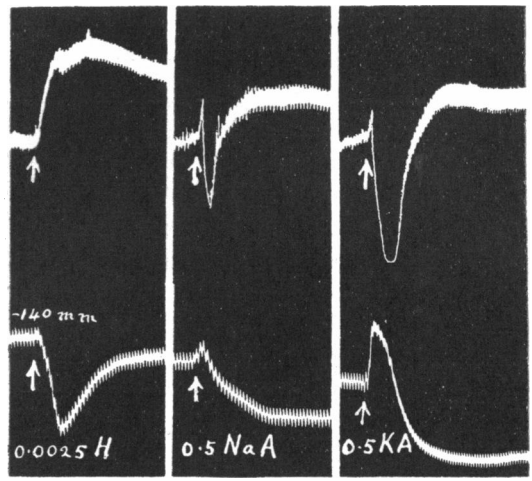

Fig. 3. Cat's blood-pressure (below), and plethysmographic record of limb volume (above). Intra-arterial injection of 0·0025 mg. histamine, compared with those of 0·5 c.c. *N*/1 sodium acetate, and of 0·5 c.c. *N*/1 potassium acetate.

specific effect of the hypertonic solution, and is seen with similar injections of normal solutions of salts other than acetates.

(*b*) A dog's isolated hind limb was perfused with the Dale-Schuster pump (2), the dog's own blood being used, and the venous blood oxygenated by perfusion through the lungs with the second pump of the system. An attempt to obtain significant plethysmograph records failed, any fall of the arterial pressure, however produced, being accompanied by passive shrinkage of the part of the limb in the plethysmograph. The record of changes in the pressure in the arterial cannula showed, however, quite adequately the changes in peripheral resistance, which alone could affect the pressure produced by the uniformly acting pump. Fig. 4 shows the

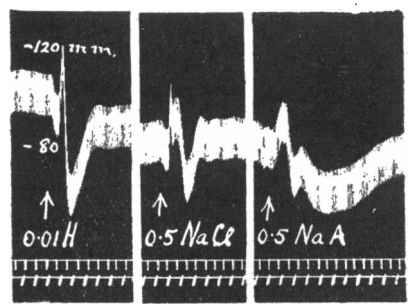

Fig. 4. Artificial perfusion of isolated dog's hind limb. Record of arterial pressure (above), rate of outflow, and time. Comparative effects of intra-arterial injections of 0·01 mg. histamine, 0·5 c.c. of *N*/1 NaCl, and 0·5 c.c. of *N*/1 Na-acetate. Outflow recorder in this and Figs. 5 and 6 marks volumes of 10 c.c.

effect of injecting, into the blood going to the arterial cannula, 0·5 c.c. of normal solutions of NaCl and NaĀ respectively. The former produces, like all hypertonic salt solutions which we have tried, a sharp, evanescent fall of pressure. With sodium acetate solution this effect of mere tonicity is followed by a specific, and quite definite, slower fall and recovery, indicating a vaso-dilator effect similar to that seen on the peripheral vessels of the whole cat.

3. The effect of sodium acetate on the blood-pressure was unaffected by the previous administration of atropine, in doses sufficient to abolish the action of choline or its esters in ordinary doses. The activity of acetates on the blood-pressure could, accordingly, not be related to the peculiar potency of acetyl-choline, as suggested by Le Heux(3) in explanation of the stimulating effect of acetates on intestinal activity. We shall see that the depressor effect of acetates resembles that of histamine rather than that of choline.

4. The depressor action being due to peripheral vaso-dilatation, it was desirable to identify more closely the size of the vessels participating in the response. It was stated by Dale and A. N. Richards(1) that a preparation of the isolated mesenteric arterial branches of the cat, perfused with blood, shows a good vaso-dilator response to acetyl-choline, but only constriction with histamine. According to Burn and Dale(4) the similar arterial preparation from the dog shows some vaso-dilator response to histamine as well as to acetyl-choline. The effect of acetate has been tried on both.

(*a*) The preparation of the cat's mesenteric arterial branches, perfused with whipped cat's blood by means of the Dale-Schuster pump, gave a full vaso-dilator response to acetyl-choline, and a just perceptible trace of vaso-dilatation with very small doses of histamine, larger doses of the latter substance producing vaso-constriction. With sodium acetate, in any dose, it exhibited only a small vaso-constrictor effect.

(*b*) In a similar preparation of the dog's mesentery the finding of Burn and Dale was confirmed, both histamine and acetyl-choline producing vaso-dilatation. Sodium acetate in this case also gave only a small vaso-constrictor response (Fig. 5).

(*c*) An isolated strip was prepared by spiral section of iliac artery of a dog, and suspended in 100 c.c. of warm oxygenated Ringer's solution according to the familiar technique. Contraction to maximal tone was caused by addition of 0·02 gm. of adrenaline, a smaller contraction by 1 mg. of histamine, and relaxation by sodium nitrite. Sodium acetate, added in amounts up to 1 c.c. of normal solution, had no perceptible

effect in either direction. According to the indications of these experiments, therefore, the vaso-dilator effect of the acetates corresponds, in

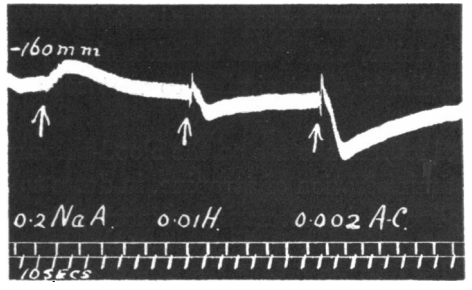

Fig. 5. Perfusion of isolated dog's mesentery. Record of arterial pressure, volume of outflow, and time. Injections of 0·2 c.c. *N*/1 Na-acetate, 0·01 mg. histamine, and 0·002 mg. acetyl-choline.

its distribution, much more nearly to that of histamine than to that of choline and its esters; where the action differs from that of histamine in location, it appears to be even more exclusively on the minute peripheral vessels.

5. The correspondence of the action to that of histamine in its localisation could be tested in another way. In the rabbit anæsthetised with ether or urethane, in contrast to the cat under similar conditions, it is unusual to observe any depressor vaso-dilator effect with histamine. In one experiment on a rabbit under urethane we observed a full depressor effect with acetyl-choline (0·001 mg.), a very small but definite depression even with small doses of histamine (0·0025–0·005 mg.), but no trace of depressor action with sodium acetate in any dose, a small rise of arterial pressure being the only effect perceptible. Again the indication is in favour of the depressor effect of acetates being even more selectively peripheral than that of histamine.

6. Another rather striking correspondence between acetates and histamine may be mentioned, in an action not vaso-dilator. We have been engaged with Dr Dale in experiments on the response, to various conditions, of the vessels of a dog's liver, artificially perfused through hepatic artery and portal vein simultaneously. In the later stages of such experiments a small dose of histamine produces only two pronounced effects in this preparation—a rise of pressure in the portal vein, and a restriction of the outflow through the hepatic veins and vena cava. For reasons which will be fully discussed in a later publication, we have been

led to attribute these effects to that action of histamine which Mautner and Pick(5), and more recently Baer and Rössler(6), have detected, namely a stimulation to constriction of the branches of the efferent, hepatic veins. Under such conditions sodium acetate likewise causes rise of portal pressure and restriction of hepatic outflow—both due, as we believe, to constriction of hepatic venules—while salts of other acids, such as sodium chloride, in equal doses of equimolecular solutions, have no trace of this action (Fig. 6).

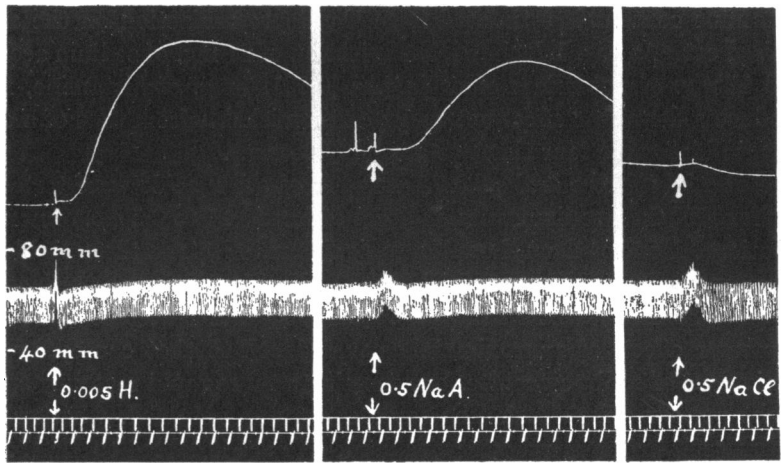

Fig. 6. Perfusion of isolated dog's liver. Records of portal vein pressure, hepatic artery pressure, and volume of hepatic outflow. Effects of intra-arterial injections of 0·005 mg. histamine, 0·5 c.c. of *N*/1 Na-acetate, and 0·5 c.c. of *N*/1 NaCl.

Effects of other acetates. The effects of the Ca^{++} and Mg^{++} ion were not sufficient, in the intravenous doses in which calcium and magnesium acetates were tested, to interfere with or modify those due to the $\bar{A}'$ ion; their depressor effects were not to be distinguished from that of sodium acetate in equimolecular dosage. Slight deviations from neutrality in either direction did not alter the action. The effects, that is to say, were due to the acetate ion, and not to the hydrogen or hydroxyl ion. Only in the case of potassium acetate, of the salts tested, did the kation contribute independently to the effect. When potassium acetate was injected intravenously, the first visible effect was the rapid fall of the arterial pressure, due to the depressor effect of potassium ions on the heart-muscle. This persisted during the vaso-dilator effect of the acetate ions, and the resulting total depression was deeper than that produced

by the equivalent of sodium acetate. When potassium acetate was injected directly into the aorta, the plethysmographic record for the limb showed an immediate vaso-constriction, which was obviously the vaso-constrictor effect of potassium salts described by Mathison (7), followed by the vaso-dilator effect of the acetate ion (Fig. 3).

From the point of view with which we began these observations it should be noted that a tissue extract, purified by the lead acetate treatment, is likely to contain excess of both potassium and acetate ions, and that the combined effect of these may produce material errors, if the histamine content is estimated from the depressor action on the atropinised cat. As Fig. 1 *B* shows, the injection of 27 mg. of sodium acetate and 17 mg. of potassium chloride with 0·00125 mg. of histamine, may produce a depressor effect not easily distinguishable from that of a dose of pure histamine four times as great (0·005 mg.).

Effect of other neutral salts. A number of other acids were tested in the form of their sodium salts, in doses of from 0·25 to 0·5 c.c. of normal solutions. None of them showed any significant effects on the cat's blood-pressure. Those tested were NaCl, $NaNO_3$, Na_2SO_4, NaI, and the lactate, butyrate, benzoate and salicylate. Only the aceto-acetate showed a depressor effect, of approximately equal depth to that caused by the equivalent of sodium acetate, but showing a more rapid recovery and a slight pressor after-effect. The same effect was obtained, whether the sodium salt or the ethyl ester was used. We are indebted to Mr W. W. Starling for the preparations of these substances.

SUMMARY.

Acetate solutions, in doses of the order of 25 mg. or more, exert a vaso-dilator effect in the dog and cat; the location of the action appears to be upon the smaller blood vessels, and is similar in this respect to the dilatation produced by histamine.

The dilator action of acetates takes place independently of the central nervous system, and is not affected by atropine.

We wish especially to express our gratitude to Dr Dale for guidance both in planning and in carrying out these experiments.

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