THE SENSITISING ACTION OF ALKALIES.

By A. HEMINGWAY.

(*From the Department of Physiology, King's College, London.*)

THAT the tone and activity of tissues containing plain muscle, such as blood vessels, intestine, uterus, etc., is largely dependent upon the acidity or the alkalinity of the fluids with which they are bathed has long been known. Amongst earlier papers those of Schwarz and Lemberger(1) and Hooker(2), working on blood vessels, showed the dilating power of acids. Lately, Evans and Underhill(3) have investigated in detail the changes in tone of plain muscle when subjected to fluids of various concentrations of hydrogen-ions. They showed that, within normal limits, increase of hydrogen-ion concentration caused relaxation of muscle, whilst a decrease had the opposite effect, increasing the tone and causing a quickening of the rhythmic movements.

In addition to these changes in tone and activity there is considerable evidence that certain drugs are more effective when used in an alkaline medium, and the present investigation has had its starting-point in this observation and more particularly in the work of McDowall(4) upon the relationship between the action of alkalies and pituitary extract upon pulmonary vessels. It is now shown that continuous exposure of the tissue to changes towards the alkaline side is not necessary for the exhibition of this action, but that sudden and repeated excursions towards slight alkalinity, with a return to normal following the change, are even more effective in bringing about the exaggerated response.

Method.

Three preparations have been studied, mammalian blood vessels, small intestine and uterus. In the first, the hind limbs of the cat have been perfused and changes in the peripheral resistance observed. This has been measured by using a scheme based upon that of Schafer(5). Fluid is fed from a Mariotte bottle (*A*) maintained at a suitable height. After passing through a heating coil (*B*), it is fed to a five-way tube (*C*) furnished with an air-trap, thermometer, connection to a side tube (*D*) and to the perfusion cannula. This cannula is inserted into the abdominal aorta, and the resistance is measured in the side tube, and recorded by connecting to a piston recorder or a water manometer. All branches of

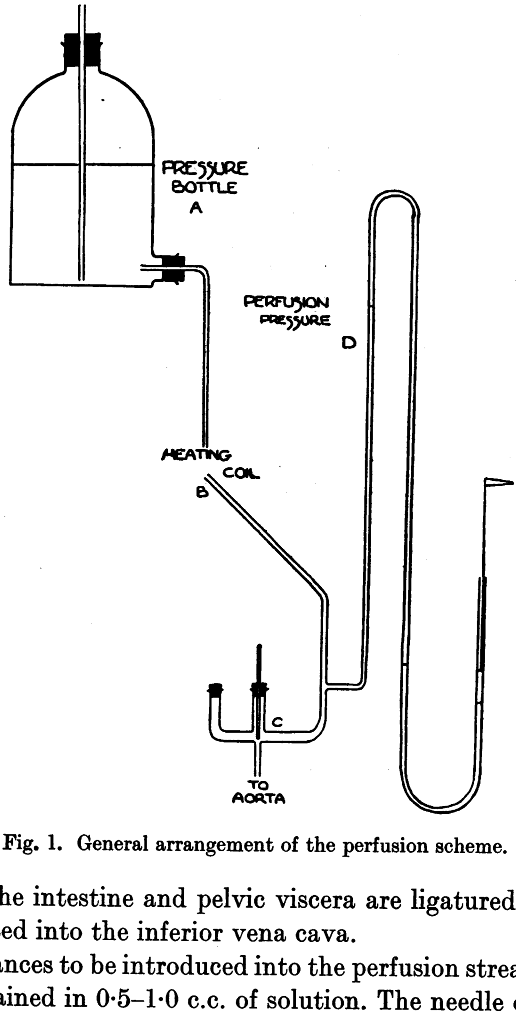

Fig. 1. General arrangement of the perfusion scheme.

the aorta to the intestine and pelvic viscera are ligatured and a return cannula inserted into the inferior vena cava.

Any substances to be introduced into the perfusion stream were made up to be contained in 0·5–1·0 c.c. of solution. The needle of the syringe containing the fluid was pushed through the rubber tube leading to the aortic cannula and the addition made as rapidly as possible. This modification, suggested by Prof. McDowall, ensures that an accurate record of the point of injection is taken since a small increase of pressure is caused momentarily in the manometer. In addition, there is no question as to the varying dilutions of successive injections which a slower rate of injection might introduce. Control injections of Ringer's fluid show that no other effects are induced. In all the tracings shown, therefore, there is a small curve indicating the exact point of injection and this is then followed by a curve due to the reaction of the vessels, if any.

The perfusing fluid consisted of Ringer's solution (NaCl 0·9 p.c.; KCl ·042 p.c.; $CaCl_2$ ·024 p.c.), no buffer substances were added, and the solution was adjusted to pH 7·4 by the addition of N/100 NaOH until it was slightly alkaline and finally brought back to the required concentration by blowing in CO_2.

The animals were killed by bleeding after preliminary anæsthetisation with chloroform and ether, and the procedure was then to commence the perfusion as speedily as possible (in some cases this being done whilst the circulation was still undisturbed and afterwards tying off the aorta and vena cava), but the rapidity with which the operation is performed does not appear to affect the results obtained. The perfusion pressure was then adjusted to the desired level and, a steady flow being established, injections of alkali were made into the stream of the perfusing fluid. Usually about 0·5–1·0 c.c. of fluid was added; the solutions used being N/100 NaOH, 5 p.c. $NaHCO_3$ (pH 8·5), and buffered phosphate solutions of pH 9.

Observations.

The invariable response of the vessels to a change in hydrogen-ion concentration thus induced was a slight constriction, showing itself as an increase in the peripheral resistance, although sometimes this was almost imperceptible at the commencement. If, now, the injections were continued at, say, intervals of 2 minutes, the reaction of the vessels became gradually more pronounced until finally, after perhaps some 12

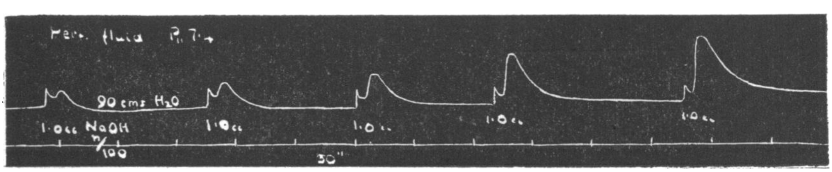

Fig. 2. Perfusion of hind-limbs of cat. Gradual increase of response to addition of alkali. The preliminary rise in each curve is due to the injection and this is followed by the rise due to vaso-constriction.

injections, it remained fairly constant. The course of such an experiment is shown in Fig. 2.

An attempt was made to determine what changes in alkalinity were occurring during the reaction and in this particular experiment the perfusing fluid was at pH 7·4. The addition of 1 c.c. $N/100$ NaOH raised the pH of the fluid issuing from the inferior vena cava to 7·5 within 30 seconds and 1 minute after the injection this had returned to normal again. So that the vessels were subjected to their original solution before the next addition of alkali was made.

Parallel to this increased sensitivity to alkali there runs an exaggerated response to adrenalin and also to pituitary extract. An early effect of the drug, before sensitisation has commenced, is taken as standard and

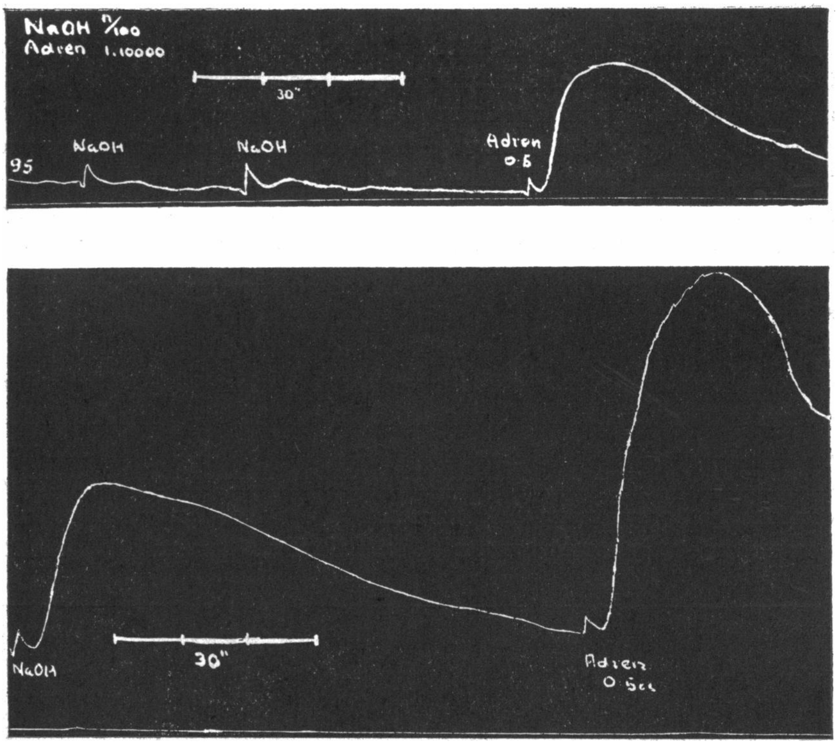

Fig. 3. Sensitisation to adrenalin. The upper tracing shows the commencement of the perfusion with slight increase in response to the second addition of alkali. The lower tracing gives the reaction to the fourteenth injection of NaOH and the exaggerated response to adrenalin. Perfusion pressure in cm. H_2O.

then, later, a second response is always much increased. A typical result is shown in Fig. 3.

In the preliminary experiments either sodium hydrate or sodium bicarbonate was used as the alkaline solution, and it was thought that the Na-ion might perhaps play some part in bringing about the results. To test this possibility buffered solutions of sodium phosphates and potassium phosphates were prepared of the same alkalinity, and, as expected, could be interchanged without altering the degree of response. The changes, therefore, are brought about by differences in hydrogen-ion concentration.

This confirms the result of Schmidt(6), who pointed out the importance of hydroxyl-ions in determining the response of vessels to adrenalin.

If the increased excitability is associated with the decrease in the hydrogen-ion concentration, then additions of acids to the perfusing fluid should bring about a desensitisation. To verify this idea a preparation was first rendered sensitive in the usual way and then this was shown to be reduced by repeated injections of HCl, for after three or four injections of acid and the reaction of the returning perfusing fluid having returned to the original state, the constriction due to the alkali was considerably reduced, but could be increased again by further alkali, the reaction being reversible (see Fig. 4).

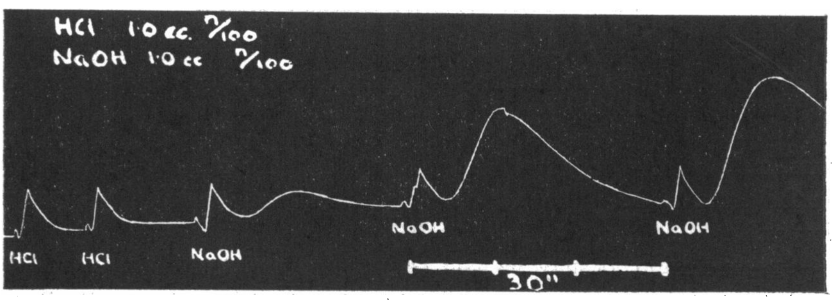

Fig. 4. Showing resensitisation after disappearance of the effect with injections of acid.

The uterus and the small intestine of the cat were found to give similar results, in addition to observations made upon the pulmonary vessels. The changes in alkalinity in the case of the former tissues were brought about by rapidly filling the vessel containing them with a solution of sodium bicarbonate *p*H 8·5 or adding 1 c.c. alkali to the solution, immediately emptying and washing two or three times with Ringer's solution. With the uterus the response to histamine and pituitary extract was determined before and after treatment with alkali, and for the intestine, pilocarpine was used as the stimulating agent. In both cases an increased response was elicited after washing with alkali, although the time of exposure to the changed solution was not more than 20 seconds (see Fig. 5). The response showed a slight increase with each

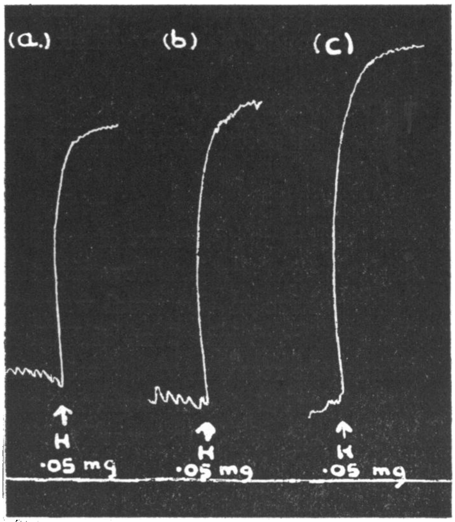

Fig. 5. Increasing contractions of the uterus to a constant amount of histamine added to 100 c.c. of Ringer's solution. After each addition, the tissue was bathed in $NaHCO_3$ solution *p*H 8·5 and then washed.

washing for three or four times and then became constant, or changes in the general tone of the preparation made further accurate comparison impossible.

In conclusion, it may be said that the response of plain muscle to changes in *p*H is not due to a difference in concentration of ions inside and outside the cell, but is more likely due to changes occurring in the cells themselves, whether the membrane or the interior. It is suggested by McSwiney (7) that the varying tone of plain muscle is due to a changing distribution of the concentration of water in the phases of its colloidal systems. If this be so, then there is no reason why this change should not be induced by rapid changes such as are described and, with each variation in equilibrium, a condition of greater susceptibility to stimulation is left behind, thus accounting for the sensitising action.

Summary.

1. There is an increasing reaction of mammalian blood vessels to successive changes in hydrogen-ion concentration towards the alkaline side.

2. An exaggerated response to adrenaline accompanies the reaction to alkalies.

3. The plain muscle of the uterus and the small intestine gives similar results.

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