THE ACTION OF SODIUM AND POTASSIUM ON BLOOD VESSELS AND ITS RELATION TO HYPERTENSION

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Received September 16, 1955

It is well established that electrolyte imbalance results in hypertension. There is increasing evidence for an association between the metabolism of sodium and experimental hypertension. Recent evidence indicates that the cause of essential hypertension is peripheral and ultimately resides in the smooth muscle of the blood vessels. The action of sodium and potassium on unstriated muscle is of importance in view of its possible relation to hypertension. Dogs and rats with renal hypertension showed increased concentrations of sodium in various tissues (Larmore and Grollman, 1950).

The increase in the sodium content of unstriated muscle appears to increase its tone. Thus isolated unstriated muscle contains more sodium than striated muscle and it also shows greater tone and viscosity. If unstriated muscle is soaked in Ringer's solution for a few hours, its tone, viscosity (Winton, 1930) and sodium content increases (Singh, 1938 *a*). Sodium content as well as tone increases if the muscle is cooled; in these muscles, excessive tone and viscosity is reduced by treating them with sodium-deficient solutions. Frequent stimulation increases tone as well as sodium content (Singh, 1939 *a*). Tone as well as sodium content increases in the absence of calcium (Singh, 1938 *a*, *b*). Dog's stomach muscle contains more sodium than frog's stomach muscle and the former shows greater tone than the latter (Gokhale and Singh, 1945); a greater sodium content implies a lesser potassium content and *vice versa*. Some unstriated muscles rapidly gain potassium from an isotonic solution of potassium chloride (Singh, 1938 *a*, 1944), and show marked reduction in tone and viscosity (Singh, 1938 *c*, 1943). Increase in the osmotic pressure of the medium increases the concentration of potassium inside the muscle fibres (Gokhale and Singh, 1945), and causes reduction of tone (Singh, 1939 *b*).

These experiments suggest that intracellular sodium increases tone, and intracellular potassium has the opposite action. Extracellular sodium and potassium may increase or decrease tone. Intracellular ions will act on the proteins of the muscle and thus produce permanent effects. A tonic

contraction by an intracellular ion will not require expenditure of energy, and thus will be sustained. To study the intracellular action of ions on the contractile mechanism, the excitatory system has to be excluded.

Two methods have been employed to study the action of ions on the contractile mechanism of unstriated muscle. In the first method, the muscle is allowed to die in a solution (Singh and Singh, 1949 *a*, 1950 *a*). As the excitatory system and the permeability of the muscle is destroyed, the substances in the solution then act on the contractile mechanism. By such method, it has been found that sodium has a contractile effect, and potassium has the opposite action. The effect of sodium chloride is counteracted by small concentrations of calcium, potassium and magnesium. The effect of potassium is antagonised by small concentrations of calcium and increased by magnesium.

In the second method, the muscle is killed by heating to 50° C. for a few minutes (Singh and Singh, 1954 *a*, *b*, *c*). Most substances that cause denaturation of proteins cause the heat killed muscle to relax; heating beyond 50° C. to 60° C. has similar action. According to modern theory, denaturation consists of an alteration of the specific internal structure of the protein wherein the closely folded peptide chains unfold. Similarly the contraction of muscle is supposed to be due to folding of the contractile protein; so relaxation would be due to unfolding of the muscle proteins. Thus according to these views the process of relaxation of muscle would be similar to denaturation of proteins. These experiments therefore give an indirect evidence of chain-folding during contraction.

The effect of various substances on the heat-killed muscle is similar to that on the dying muscle, though there is an important difference between the reactions of these two preparations. The heat-killed or denatured muscle does not contract, except when treated with some protein coagulants (Singh, 1955 *a*), or by heating to 70° C. (Singh, 1955 *b*). Substances that cause active relaxation of dying muscle have similar effect on the heat killed muscle. By active relaxation of dead muscle is implied, that it relaxes without the application of any external force; criteria for active relaxation of living muscle are many more (Singh and Singh, 1952 *a*). Thus potassium chloride causes active relaxation of dying as well as heat-killed muscle. Substances that cause contraction of dying muscle do not cause contraction of heat-killed muscle. They either have no effect, or cause slight active relaxation of the latter. These substances may, therefore, be considered to have a contractile effect (Singh and Singh, 1955 *c*). Sodium chloride has such an action, and if part of the potassium chloride is replaced with

sodium chloride, then the effect of the former in causing active relaxation of the heat-killed muscle is correspondingly diminished. Sodium chloride may therefore be considered to have a contractile effect on the contractile mechanism of unstriated muscle.

There is every likelihood that the unstriated muscle of the arterioles reacts like other smooth muscle. In the present research, an attempt has been made to test this possibility.

EXPERIMENTAL

Small sized dogs were killed by an overdose of chloroform. The hind legs were perfused with isotonic solutions (0·154 *M*) of sodium or potassium chloride. A cannula was tied into the femoral artery; the leg was then severed from the body and the cannula secured to the femur by a ligature round the neck of the bone. Saline (sodium chloride solution) was run into the cannula from a 50 c.c. burette. The skin of the leg was then removed. In some experiments the leg was heated to 50° C. for 10 minutes to destroy the excitatory system of the arterioles. The experiments were performed at room temperature (30° C.).

The limbs were perfused from the burette throughout the experiment. The advantage of perfusion from the burette was, that as the perfusion pressure head decreased, the flow might stop and the perfusion time might be considered as infinity. If now change of the solution caused the flow to restart, then the second solution might definitely be considered to have a vasodilator effect, if other factors remained constant.

Perfusion was started with unoxygenated sodium chloride solution for about an hour or two. Time was noted for the flow of 50 c.c. of the solution. When the readings had become constant, then one leg was perfused with potassium chloride for about 2 hours and the other with sodium chloride serving as a control, the legs being immersed in their respective perfusion fluids. The legs were then left immersed for about 18 to 24 hours; the idea was to obtain the reactions of dying or heat-killed arterioles, as with isolated pieces of muscle.

After immersion, the perfusion was restarted till the reading had become constant. When the perfusion is stopped for some hours and then restarted, the flow at first might be slow, and then reaches a constant rate after 3 or 4 perfusions.

Rings of dog's aorta were also used. They were similarly immersed for 24 hours in solutions of sodium or potassium chloride. Some were heated to 50° C. before immersion.

Direct microscopic observations using a micrometer eyepiece, were also made on the small arterioles and venules in the mesentery of the dog and guinea pig. The mesentery was pinned over a small hole in a piece of cork. After initial observations, the mesentery with the piece of cork was immersed in sodium or potassium chloride for 24 hours. In some observations it was heated to 50° C. before immersion.

Results

Perfusion of the legs at room temperature (30° C.) with sodium or potassium chloride caused them to swell, so that the blood vessels might have been compressed. Potassium chloride causes more swelling than sodium chloride. It will be seen from the experiments described below that swelling plays a minor role in affecting the rate of perfusion. Rigor also plays a minor role as judged by its effect in the control limb.

Isotonic solution of potassium chloride is a strong stimulant for all kinds of unstriated muscle. In some, the contraction lasts for 10 to 20 minutes, and in others, for some hours. The contraction is due to extracellular potassium, and the relaxation occurs when the potassium passes inside the cells. Thus the action of intracellular potassium is opposite to that of extracellular potassium. Extracellular sodium has variable effect; some smooth muscles contract, and others relax. Immersion for 24 hours results in contraction of the dying muscle; some, like *Mytilus* muscle, contract strongly, and others, moderately. Intracellular sodium has thus a contractile effect.

Smooth muscle of the arterioles reacts like other smooth muscle. The small arterioles and venules in the mesentery contract strongly in isotonic potassium chloride. After immersion for 18 to 24 hours they dilate. Prolonged immersion in sodium chloride causes them to contract.

Rings of dog's aorta also relax in potassium chloride after prolonged immersion. Owing to the presence of elastic tissue they do not show much active relaxation. The contraction, either in sodium or potassium chloride, is not significant, presumably for the same reason. The initial handling also causes them to contract, so that sodium or potassium chloride do not cause further contraction.

Twelve pairs of legs were perfused with sodium or potassium chloride. In 3 experiments, when the legs were first perfused with sodium chloride, the rate of perfusion decreased till it stopped altogether. One leg of each pair was then immersed in potassium chloride for 18 to 24 hours, and the other continued in sodium chloride serving as a control. Perfusion restarted in potassium chloride but not in sodium chloride. The initial perfusion

time for 50 c.c. of sodium chloride solution varied from 4 to 6 minutes. When perfusion restarted in potassium chloride, it varied from 24 to 31 minutes; in sodium chloride, it remained infinite. As the swelling in potassium chloride was greater than in sodium chloride, these results definitely show that sodium chloride has a constricting and potassium chloride a dilating effect on the dying arterioles. These results resemble those on the dying unstriated muscle. The initial stoppage of the perfusion flow was due to spasm of the blood vessels.

In 8 experiments, the perfusion time for the flow of 50 c.c. of sodium chloride was 2 to 10 minutes. In potassium chloride, the rate diminished immediately, and continued to decrease so that it took over 60 minutes. After 2 to 3 hours the perfusion time in sodium chloride was doubled and remained constant throughout. After prolonged immersion in potassium chloride, the perfusion time had diminished to 5 to 8 minutes, and in some experiments, it was less than the initial time in sodium chloride (Figs. 1, 2 *a*, *b*).

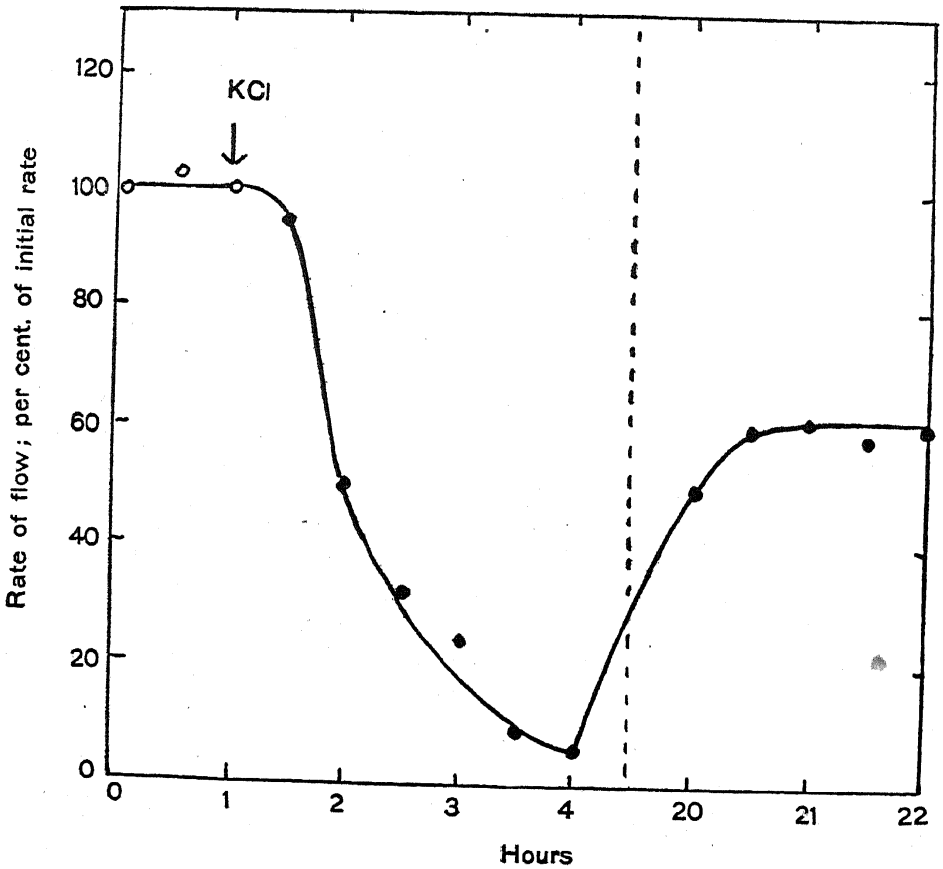

FIG. 1. Dog's hind legs. Effect of perfusion with potassium chloride. At first, constant rate of perfusion was obtained with sodium chloride. Then perfusion with potassium chloride was begun. Note immediate diminution of flow indicating spasm of blood vessels, which partly disappeared after prolonged immersion in potassium chloride.

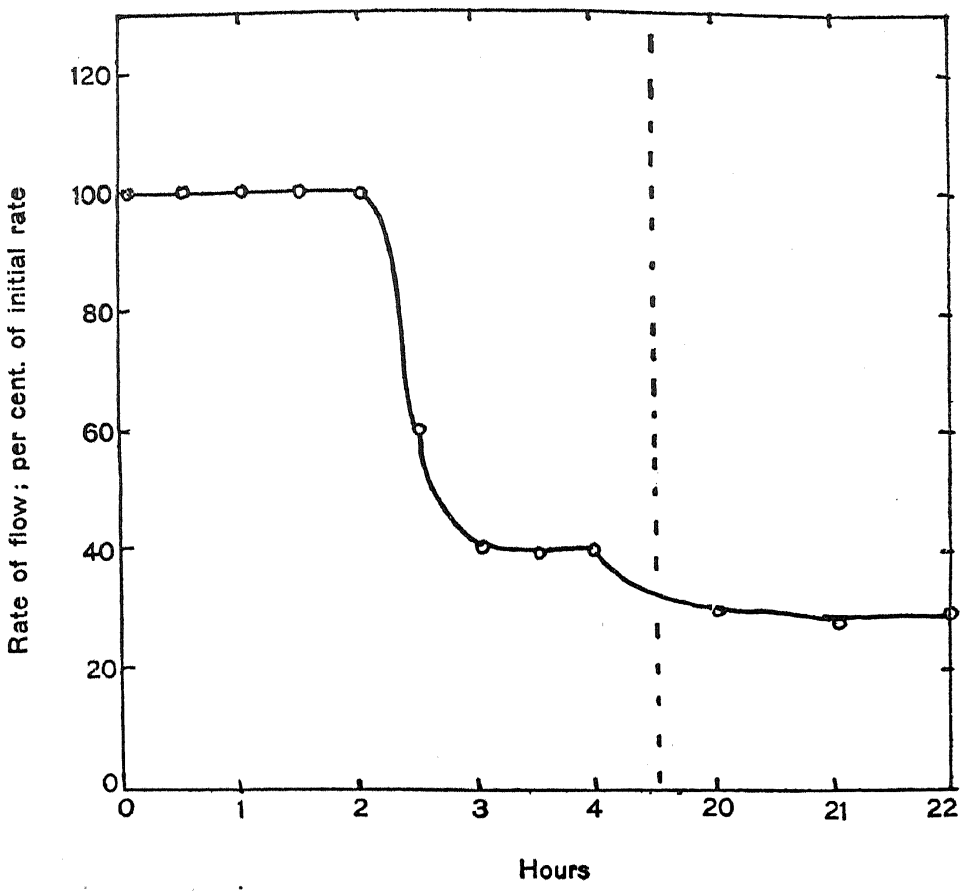

FIG. 2 *a*. Dog's hind legs. Effect of perfusion with sodium chloride. Note after 2 hours the rate diminished, which further diminished after prolonged immersion.

In one experiment, the perfusion time reached 26 minutes after one hour's immersion in potassium chloride, and began to diminish so that in 2 hours it had come down to 5 minutes and remained so throughout, the perfusion time in sodium chloride being 7 minutes. Thus if the flow did not stop, then also the effects of sodium and potassium chloride were similar to those described above, and resembled the effects on isolated muscle. The main factor which affects the perfusion rate is therefore the calibre of the blood vessels. A piece of dog's stomach muscle contracts strongly if immersed in isotonic potassium chloride solution. The contraction is maintained for some hours, but after prolonged immersion, the muscle relaxes. In some muscles the relaxation may occur in a few minutes. In sodium chloride, the muscle remains contracted.

As with isolated muscle, sodium and potassium chlorides lose their constricting effect, if the leg is heated to 50° C. for 20 minutes. In 3 such

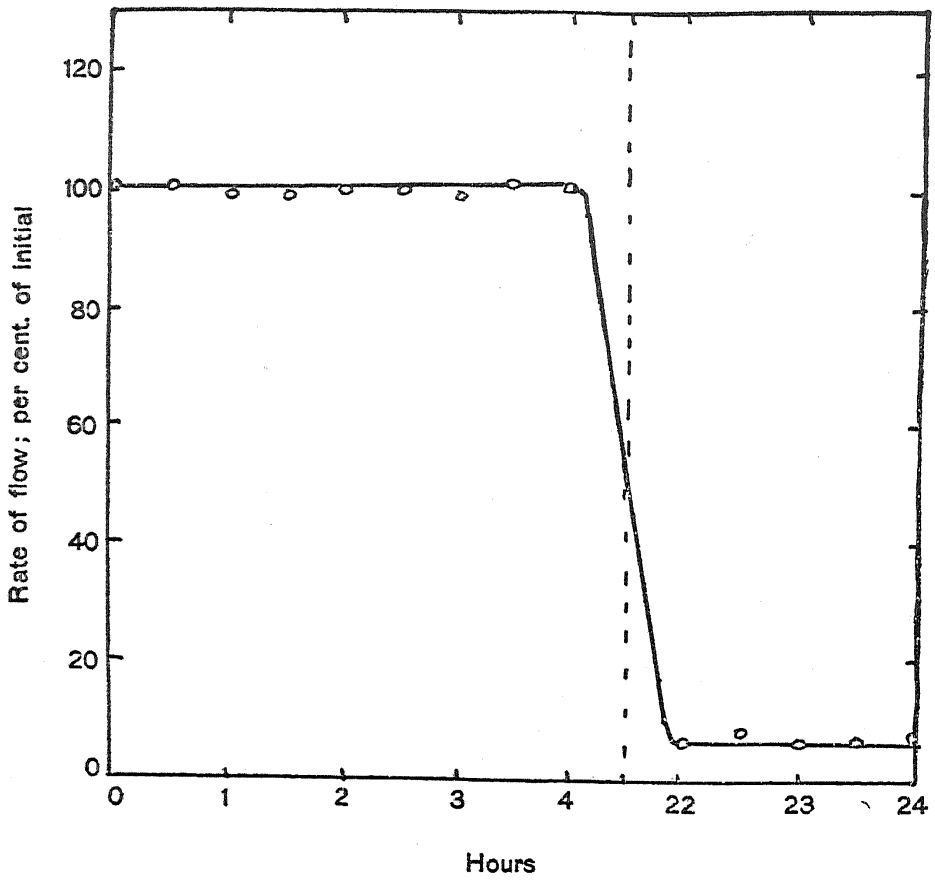

FIG. 2 *b*. Dog's hind legs. Effect of perfusion with sodium chloride. Note that the fusion rate remained constant for 4 hours, but greatly declined after prolonged immersion.

experiments, the legs were perfused with sodium chloride. When the rate of flow had become constant, they were heated to 50° C. This caused great retardation of flow, possibly due to contraction of the blood vessels; this could be observed with the naked eye in the femoral arteries. They were again perfused with sodium chloride till the rate of flow had become constant. One leg of each pair was then perfused with potassium chloride, the other being perfused with sodium chloride, thus serving as a control. When the perfusion rates had become constant, they were respectively immersed in potassium and sodium chlorides for 18 to 24 hours. They were again perfused respectively with potassium and sodium chlorides, and the perfusion rate had increased for both (Figs. 3, 4). This exactly resembles the response of the heat-killed muscles, which relax by about 6 per cent. in sodium chloride and 40 per cent. in potassium chloride (Singh and Singh, 1954 *c*).

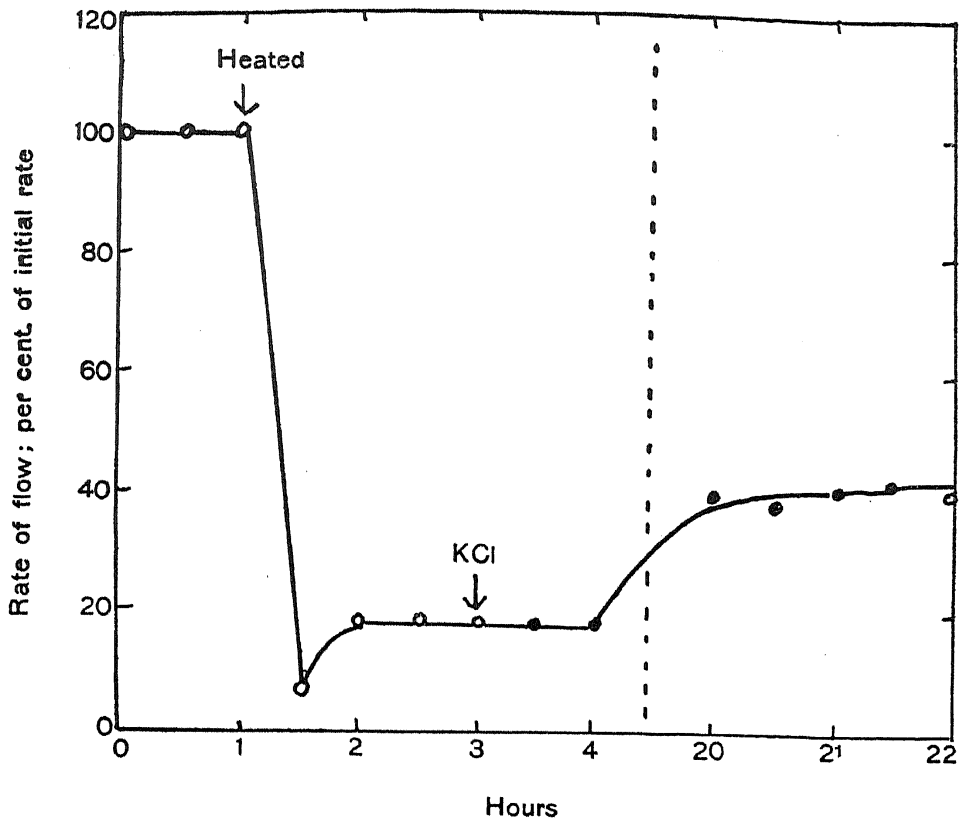

FIG. 3. Dog's hind legs. Effect of perfusion with potassium chloride in a heated leg. At first constant rate of perfusion was obtained with sodium chloride. The leg was then heated to 50° C. for 20 minutes. When perfusion rate was constant, sodium chloride was replaced with potassium chloride. In dead arterioles, there is no immediate effect, but after prolonged immersion there is dilatation.

DISCUSSION

These experiments explain the role of sodium in essential hypertension. It thus appears that sodium enters the smooth muscle of the arterioles, and acts on the contractile proteins, producing a persistent contraction. The action is a direct one on the contractile mechanism, with the result that the arterioles do not relax in response to substances that cause relaxation through the excitatory mechanism.

Another contributory cause would be the failure of active relaxation. It is known that if smooth muscle is continuously stimulated, then the contraction may become permanent and does not subside on the withdrawal of the stimulating agency (Singh and Singh, 1949 *b*, 1952 *c*). If dog's stomach muscle is continuously stimulated with potassium chloride or other stimulants, the contraction may not subside on withdrawal of the stimulants, and thus becomes permanent. Thus contraction started by nerves or pressor substances such as noradrenaline or renin would become permanent,

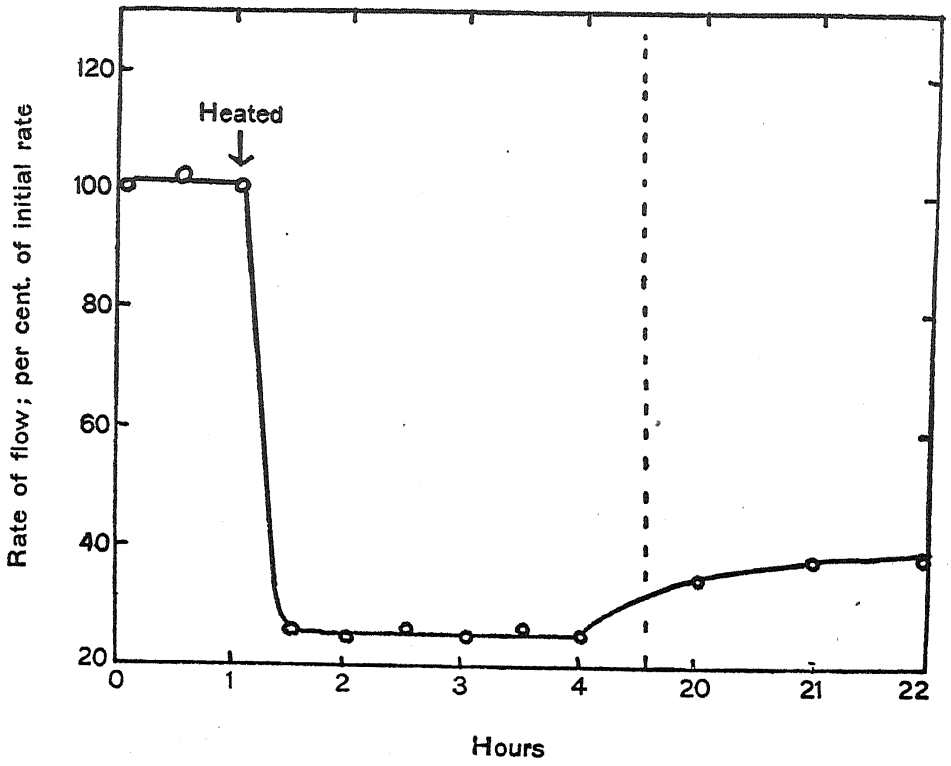

FIG. 4. Dog's hind legs. Effect of perfusion with sodium chloride in a heated leg. At first constant rate of perfusion was obtained with sodium chloride. The leg was then heated to 50° C. for 20 minutes. Note immediate spasm of blood vessels, which partially recover after prolonged immersion in sodium chloride.

even after the original causative factor had disappeared, and the pressor substances are no more demonstrable in the blood. The nature of tone changes and the muscle does not relax (Singh and Singh, 1950 *b*). Drugs which previously lowered blood pressure, would gradually lose their efficacy till their effect is more or less completely lost, as these tonic changes become established.

The next question arises, as to which is the primary event, the high blood pressure or the entrance of sodium into the smooth muscle of the blood vessels. Both factors are likely to precede other events. Thus if the smooth muscle of the blood vessels was stimulated, its permeability would be increased, and sodium would then enter the cells and produce persistent contraction (Singh and Singh, 1952 *b*, *c*). If sodium entered the smooth muscle cells, due to its retention in the body, then hypertension would be a secondary event.

It is also likely that sodium chloride might enhance the sensitivity of the smooth muscle of the blood vessels to chemical stimulants. Thus some smooth muscle react more strongly to chemical stimulation if there is a slight excess of sodium chloride in the surrounding medium (Singh, 1938 *b*). Intracellular potassium decreases the sensitivity to chemical stimulation, so that its partial replacement with sodium would make the muscle more sensitive to such stimulation (Singh, 1938 *b*, 1939 *b*). Sodium retention in the body might also make the nervous system hyperirritable for similar reasons, and thus increase the tone of vasomotor centres. Potassium retention, such as in Addison's disease, would lower blood pressure. In nephritis, sodium retention would increase the tone of the musculature of the alimentary canal and thus cause vomiting and diarrhœa; the increased irritability of the nervous system would cause convulsions.

Intracellular potassium has an inhibitory effect on tonic stimulation, so that its partial replacement with sodium will result in increased irritability. This appears to be true of muscle and the nervous system. In unstriated muscle (Singh, 1938 *b*; 1939 *c*), extracellular ions have both an inhibitory and an excitatory action in the order

$$K > NH_4 > Na > Li$$

so that increased irritability would result, if (*a*) the concentration of the inhibitory ion is decreased; (*b*) the inhibitory ion is removed; (*c*) the ion in question is replaced with another ion, which has a lesser inhibitory effect.

Concentration of the intracellular inhibitory ion can be lowered by decreasing the osmotic pressure of the extracellular medium, as this would result in the passage of water into the cell. This results in increase of irritability in unstriated muscle (Dale, 1913; Singh, 1938 *b* and 1939 *b*), as well as the nervous system, as indicated by convulsions in water intoxication. If extracellular potassium has an inhibitory action, then its replacement with sodium increases the irritability of unstriated muscle. It is therefore reasonable to conclude that partial replacement of intracellular potassium with sodium would result in increased irritability.

The above conclusion is supported by two sets of observations: (*a*) unstriated muscle contains more sodium and less potassium than striated muscle and it is more sensitive to chemical stimulation than the latter; (*b*) with continued stimulation, striated muscle fatigues and its sodium content increases; it also becomes more sensitive to potassium (Gelhorn, 1932). Thus sodium retention would increase the irritability of vascular smooth muscle, as well as that of the nervous system. It is interesting to note that a certain increase in hydrogen-ion concentration diminishes the permeability

of unstriated muscle to sodium, and decreases tone. The effect of acidity in diminishing the irritability of the nervous system and epileptic convulsions is well known. This action of sodium will be on the excitatory mechanism. Sodium retension would thus produce hypertension in three ways: (*a*) by increasing the irritability of the nervous centres; (*b*) by acting on the excitatory mechanism of vascular smooth muscle; (*c*) by acting on the contractile mechanism of vascular smooth muscle. The last action constitutes the irreversible phase of high blood pressure.

SUMMARY

1. The reaction of blood vessels to sodium and potassium ions have been studied: (*a*) by perfusing the hind limbs of the dog with isotonic solutions of sodium and potassium chlorides; (*b*) by direct microscopic observation of the blood vessels in the mesentery of the guinea pig and dog; (*c*) by studying the reaction of rings from dog's aorta.

2. The effect of the above ions on the contractile mechanism of the smooth muscle of the arterioles was studied by the dying muscle technique and by prior heating to 50° C.

3. Sodium has a contractile and potassium, a relaxing effect on the contractile mechanism of the smooth muscle of the arterioles. This explains the role of sodium in essential hypertension.

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