THE EFFECT OF EXPANDING THE BLOOD VOLUME OF A DOG ON THE SHORT-CIRCUIT CURRENT ACROSS AN ISOLATED FROG SKIN INCORPORATED IN THE DOG'S CIRCULATION

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SUMMARY

1. A frog skin was incorporated into the blood circulation of a dog. Each side of the skin was supplied with blood at a constant temperature, flow and pressure.

2. In the experiments in which the blood volume of the dog was expanded with equilibrated blood, there was a fall in the short-circuit current across all eight frog skins. The fall in current began 10 min after the start of the transfusion and reached its lowest value 15-30 min after the end of the transfusion. The dog showed a simultaneous rise in urinary sodium excretion.

3. In the experiments in which the blood volume was not expanded, there was no change in the trend in the short-circuit current in five of the eight skins. There was a fall in current across the other three skins; the pattern of this fall differed from that which occurred when the blood volume was expanded. There was no rise in the rate of urinary sodium excretion in any of the dogs.

4. It is concluded that when a dog's blood volume is expanded the dog alters the concentration of some circulating substance, and that this change causes a fall in the rate of active sodium transport across the frog skin.

There is much work to suggest that the increased rate of urinary sodium excretion which results from expanding the blood volume or from the administration of saline is due in part to a change in the concentration of a circulating substance which influences tubular sodium reabsorption (de Wardener et al., 1961; Lockett, 1966; Johnston & Davis, 1966; Lichardus & Pearce, 1966; Tobian, Coffee & McCrea, 1967; Johnston et al., 1967; Bahlmann et al., 1967; Knox et al., 1968; Buckalew, Martinez & Green, 1969; Lichardus et al., 1969; Sealey, Kim, pdfs. 1969; Clarkson, Talner & de Wardener, 1970). In experiments performed on the whole animal and on the isolated kidney there has usually been a simultaneous rise in renal blood flow. In such experiments, therefore, it has not been possible to determine the mode of action of the circulating substance. The aim of the following experiments was to try to dis-
tlinguish whether it produces its effect on sodium reabsorption by causing a rise in renal blood flow or by acting directly on the cellular mechanisms for sodium transport. In order to determine whether the circulating substance has an effect on active sodium transport, an isolated living frog skin was incorporated into the circulation of a dog. The short-circuit current across the frog skin was observed before and after the blood volume of the dog was expanded with blood. When the blood volume of the dog was expanded, there was a fall in the short-circuit current across the frog skin at the same time that there was a rise in the urinary excretion of sodium by the dog.

METHODS

In these experiments an isolated living frog skin was incorporated in the blood circulation of a dog. Each side of the frog skin was supplied with cooled dog's blood at pressures, flows and temperatures which were controlled and which were independent of those of the dog. The blood volume of the dog was expanded by transfusing blood from a reservoir which was also incorporated in the dog's circulation. In this way the blood volume was expanded with blood in equilibrium with that in the dog.

Apparatus

The external circuits

The apparatus is shown in Fig. 1. Blood was pumped from a femoral artery of the dog into $R_1$, the transfusion reservoir; from there it was pumped directly into the circuits supplying the frog skin, and then back into a femoral vein. The other femoral artery and vein were used to measure arterial and venous pressures. The pumps were Watson-Marlow M.H.R.E. Flow-Inducer pumps. All tubing throughout the circuits was either silicone rubber or polyvinyl chloride (PVC).

The transfusion reservoir, $R_1$, consisted of a graduated cylinder of 1000 ml capacity, water-jacketed at 39°. Blood was pumped into the top of the reservoir and out of the bottom. It was slowly stirred by an electrically rotated multi-angled glass rod. The level of blood in the reservoir was controlled by adjusting the speed of the pump bringing blood to the reservoir. The flow of blood out of the reservoir was kept constant throughout any one experiment but differed between experiments over a range of 150–200 ml/min.

The circuits supplying the frog skin were similar to those described by Nutbourne (1968) and Hutchings, Nutbourne & Howse (1969). Identical circuits supplied each side of the frog skin with oxygenated solution at temperatures, flows and pressures which were constant and equal on both sides of the skin. It was found to be necessary to maintain a blood flow of more than 25 ml/min through each chamber of the cell. Lower flows caused anoxia of the frog skin. The hydrostatic pressure inside the cell was maintained at a higher level than that in the water-bath. This prevented leakage of water into the circuits. The flows and pressure of blood in the cell were dependent on the vertical distances between the cell and the reservoirs, $R_2$ and $R_3$, and also on the lengths and bores of the tubing used.

Blood was pumped from the bottom of the transfusion reservoir $R_1$ up to the reservoir $R_2$, which was approximately 210 cm above ground-level. The blood entered at the top of $R_2$ and flowed down the side. It left $R_2$ at O and fell under the influence of gravity through the glass cooling coils into the Perspex cell. The structure of the cell has been previously described.
Na\textsuperscript{+} transport after blood volume expansion (Hutchings et al., 1969). In most experiments two frog skins were used and the cells containing them were joined in series. The coils and the cells were approximately 90 cm below R\textsubscript{2}. They were immersed in a water-bath maintained at 20°. On entering each chamber of the cell, the blood impinged on the bottom edge of the frog skin and flowed upwards over its surface. After passing through the cells, the blood continued to fall under gravity through capillary tubing to the funnel-reservoir R\textsubscript{3}. This was approximately 30 cm below the cells. As in R\textsubscript{2}, the blood flowed down the sides of the reservoir to prevent frothing. The blood was pumped from the funnel-reservoir R\textsubscript{3} back to the dog. Before entering the dog, the blood was reheated as it passed through glass coils immersed in a water-bath maintained at 41°.

A constant head of pressure was maintained in the top reservoir R\textsubscript{2} by ensuring that there was a continuous overflow of blood through the chimney Q. The blood from Q passed directly through a wide-bore tube to the funnel-reservoir R\textsubscript{3}. In order to prevent hydrostatic pressure gradients arising across the skin, the chambers on the two sides of the skin were connected through holes in the Perspex membrane-fixing unit around the periphery of the frog skin.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{The circuits used in the determination of the effect of expanding the blood volume of a dog on the short-circuit current across a frog skin incorporated in the circulation of the dog. AP and VP, arterial and venous pressure-manometers, respectively; U, urine collection from bladder catheter; P, Watson-Marlow MHRE Flow-Inducer pump; R\textsubscript{1}, transfusion reservoir; R\textsubscript{2}, top reservoir for the frog skin; R\textsubscript{3}, funnel-reservoir; G, damp gauze; M, Perspex cell for frog skin; C\textsubscript{1} and C\textsubscript{2}, coils for cooling the blood; C\textsubscript{3}, coils for reheating the blood; Q, overflow chimney; mV, Vibron Electrometer; H\textsubscript{1} and H\textsubscript{11}, KCl-agar leads; I, calomel half-cell; J, saturated KCl solution; V, voltmeter; \textmu A, micro-ammeter; K, Ag-AgCl wires; L, saturated AgCl in saturated KCl.}
\end{figure}
The lengths of glass coil used to cool and reheat the blood were sufficient for the temperature of the blood to equilibrate with that of the water-baths at blood flows higher than could occur during an experiment, i.e. at 40 ml/min through the cooling coils and 220 ml/min through the heating coils. To lessen evaporation and loss of CO₂ from the blood, damp gauze was placed over all the reservoirs and a humidified mixture of 5% CO₂ in O₂ was passed over the surface of the blood in the three reservoirs and in the overflow tube Q. The rate of gas flow was adjusted so that the pH and Pa,CO₂ of the dog's arterial blood were maintained at the desired levels, i.e. at a pH of 7.3-7.4 and a Pa,CO₂ of 35-40 mmHg. When this blood reached the frog skins, it had been cooled to 20° and therefore had a measured pH of 7.5-7.6 and a Pa,CO₂ of 20-25 mmHg. Under these conditions, it was found that the plasma usually remained free of haemoglobin to naked-eye examination.

**Measurement of short-circuit current across the frog skin**

Short-circuit current across the frog skin was measured by the method of Ussing & Zerahn (1951). The KCl-agar leads used in the measurement of membrane potential were placed close to the surfaces of the centre of the skin; those applying the backing-off voltage were placed as far from the centre of the skin as possible (Fig. 1).

Dog's blood has a different ionic composition from that of a frog. In these experiments the frog skin was bathed in dog's blood for 8-10 hr. It was found that skins died before the end of the experiment if at any stage they were short-circuited continuously. Therefore, the short-circuit current was measured every 10 min. During each measurement the skin was kept short-circuited for as short a time as possible. Electrical interference was eliminated by the following measures: strapping polyvinyl tubing to rigid structures; placing some equipment on dry expanded polystyrene; changing the relative positions of the various plugs and sockets; earthing the blood in the funnel-reservoir R₃. In every experiment, tests were carried out to ensure that the potential difference measured across the frog skin was due entirely to membrane potential and not, in part, to electrical leakage from the equipment.

**Composition of solutions**

**Dog Ringer solution** Na⁺=130 mEq/l, K⁺=5 mEq/l, Cl⁻=102 mEq/l, HCO₃⁻=30 mEq/l, H₂PO₄⁻=0.32 mEq/l, HPO₄²⁻=2.6 mEq/l, Dextrose=1 g/l. The solution was made up with pyrogen-free water and adjusted to pH 7.4 with NaOH. It was sterilized in an atmosphere of CO₂. Just before use, 2 mEq/l Ca⁺⁺ (CaCl₂), 200000 i.u./l crystalline penicillin and 200 mg/l streptomycin sulphate were added.

**Albumin-Ringer solution.** The excess of CO₂ dissolved in the dog Ringer solution during the sterilization process was removed by bubbling air through the solution. Para-aminohippurate (PAH), 20 mg/l, and creatinine, 100 mg/l, were added. Bovine albumin was dissolved in the solution to give a 2.8% albumin concentration. The pH was again adjusted to 7.4 with NaOH.

**Experimental procedure**

**Preparation of the circuits**

On the day before the experiment the circuits were assembled, siliconized, washed through and filled with sterile dog Ringer solution. On the day of the experiment the circuits were
washed through and filled with albumin–Ringer solution. The transfusion reservoir contained 50 ml/(kg dog) of the albumin–Ringer solution. The rest of the external circuits contained approximately 300 ml of solution. The solution was pumped through the circuits. The levels of the solution in the transfusion reservoir $R_1$ and the funnel-reservoir $R_3$ were kept constant by adjusting the rates of the pumps. Moist 5% CO$_2$ in O$_2$ (325–410 ml/min) was distributed equally between the three reservoirs and the overflow chimney.

Preparation of the frog skin

The frogs (Rana temporaria) were kept at room temperature on growing grass. They had access to live mealworms and to a bath of deionized water. Most of the experiments were performed in the autumn and winter. Whenever possible, frightened frogs were not used. The frogs were killed by decapitation and then pithed. The abdominal skin was removed without stretching it, under dog Ringer solution. It was mounted loosely in the Perspex cell as previously described (Nutbourne, 1968; Hutchings et al., 1969). The cell was filled with dog Ringer solution and then joined into the circuits. The second frog skin was then prepared in a similar way and the second cell was joined into the circuits, distal to the first cell.

In the preliminary experiments, the skins were first joined into a separate circuit for 4–11 hr. This was filled either with albumin–Ringer solution or with blood from a different donor dog. Only when the skins were stable in this solution, were they transferred into the circuits joined to the experimental dog. This procedure was adopted in an effort to shorten the time taken by a frog skin to stabilize after being connected to the experimental dog. (It was desirable to shorten this time because in the anaesthetized dog the magnitude of the rise in urinary Na$^+$ excretion which takes place when the blood volume is expanded, tends to diminish with the duration of the anaesthesia.) However, it was found that these skins still took at least a further 5 hr to settle after they had been joined into the dog’s circulation. Therefore this method was abandoned. In the final series of experiments the frog skins were put directly into the experimental circuits 2–3 hr before the dog was joined into the circuits.

Preparation of the dog

Late on the day preceding the experiment, a 10–20 kg bitch was fed. Desoxycorticosterone acetate, 5 mg, and Pitressin tannate in oil (Parke-Davis & Co.), 5 pressor units, were given by intramuscular injection. The dog was given free access to water overnight. On the morning of the experiment, about 1 hr after the frog skins had been put into the experimental circuits, the dog was premedicated with intramuscular acetyl promazine (0.2 mg/kg). Anaesthesia was induced with intravenous sodium thiopentone (maximum dosage 0.5 ml/kg of a 2.5% solution). After a priming dose of 2 mg/kg Gallamine Triethiodide (Flaxedil), the trachea was intubated. A constant infusion of Flaxedil (3 mg kg$^{-1}$ hr$^{-1}$) was started and anaesthesia was maintained with N$_2$O and O$_2$ in the ratio of 3:1. Respiration was controlled by a Palmer Ideal Pump Assembly at 14 breaths/min and a tidal volume of 200–260 cc. The tidal volume was adjusted whenever necessary to maintain the dog’s arterial pH, $P_a$CO$_2$ and $P_a$CO$_2$ at constant physiological levels.

It was found that during an experiment a dog might lose about 500 ml of saliva. To prevent this loss, one end of a wide-bored rubber tube was passed into the stomach. The other end opened at the back of the mouth and was fixed in such a position that saliva flowed down the
tube into the stomach. The dog's temperature was measured in the oesophagus by a thermistor strapped to the saliva tube.

Both femoral arteries and veins were exposed and catheterized. One artery was connected to a mercury manometer and one vein was connected to a water manometer for the measurement of arterial and venous pressures respectively. The other two vessels were subsequently joined to the external circuits. A Foley catheter was inserted into the bladder. The preliminary experiments showed that it was easier to control the blood gases and blood pH when a dog was prone than when it was supine. The dog was therefore fixed in a prone position, lying in a V-shaped cradle, with its head raised to prevent loss of saliva.

Soon after the induction of anaesthesia the dog was given an infusion of 10 ml/kg of physiological saline solution over approximately 15 min. Before the dog was joined to the external circuits, it was given intravenous heparin (250 i.u./kg), creatinine (50 mg/kg), PAH (5 mg/kg), and also 10 mg chlorpheniramine (Piriton) to suppress possible adverse reactions of the dog to the bovine albumin. Heparin (5000 i.u. + 250 i.u./kg dog) was added to the solution within the circuits. The dog was then connected to the circuits (Fig. 1). This took place 1–2 hr after the induction of anaesthesia, i.e. 2–3 hr after the frog skins had been put into the circuits. The volume of blood in the external circuits was controlled by manipulating the pumps so as to keep the blood levels constant in both the transfusion reservoir, $R_1$, and the funnel-reservoir, $R_3$. As blood passed into the circuits and the albumin–Ringer solution flowed into the dog, there was usually a marked fall in arterial pressure lasting 10–60 min. The mean haematocrit fell from 40 to 26%.

**Maintenance of the dog.** During the 5–9 hr taken for the short-circuit current across a frog skin to reach a steady state, the dog's temperature was kept between 39 and 40°; arterial and venous pressures were measured. Blood gases and pH were checked at least every 30 min and corrected, as necessary, by adjusting the tidal volume and by occasional injections of NaHCO$_3$ (0·3 mEq/ml). It was found that NaHCO$_3$ was only needed during the first 4 hr of anaesthesia. The dog's lungs were hyper-inflated approximately six times an hour by blocking the outflow of the respirator for two breaths. Blood lost from the dog by bleeding was collected and weighed. This amount and that lost by taking blood samples was replaced from the transfusion reservoir. Steady intravenous infusions were given of Flaxedil (3 mg kg$^{-1}$ hr$^{-1}$), vasopressin (0·12 pressor units/hr), PAH (0·012 g kg$^{-1}$ hr$^{-1}$) and creatinine (0·03 g kg$^{-1}$ hr$^{-1}$). These substances were made up in physiological saline. A total of 48 ml saline/hr were given to the dog in this way. Heparin, 5000 i.u., was given 2-hourly or more often if the blood began to clot in the reservoirs.

**Control and expansion experiments**

The preliminary experiments demonstrated that a frog skin maintained in a dog's blood gradually deteriorates. The short-circuit current across the frog skin may show sudden falls even when the blood volume of the dog is not expanded (Fig. 2). A final series of experiments was therefore designed to differentiate between the fall in current produced by expanding the blood volume, and that which might occur irrespective of such expansion. In this series there were equal numbers of expansion experiments in which the blood volume was expanded, and control experiments in which the blood volume was not expanded.

The preliminary experiments also showed that when the short-circuit current was falling
Na⁺ transport after blood volume expansion

steeply, it was not possible to interpret the changes in current which took place when the blood volume was expanded. In addition, these experiments showed that when the blood volume of a dog was expanded after the dog had been anaesthetized for more than 8 hr, the rise in urinary sodium excretion was negligible. In the final series of experiments, therefore, skins were discarded 8 hr after the induction of anaesthesia, if the short-circuit current was rising or falling steeply or was still showing unexplained fluctuations. There were two frog skins in each experiment. If the current across both skins had failed to become sufficiently stable within 8 hr, the experiment was abandoned. If the current across only one skin had become stable, then this skin was used and the other was discarded.

After the decision had been taken that a skin was sufficiently stable for the next phase of the experiment to be performed, the experiment was either continued by expanding the blood volume of the dog (expansion experiment) or it was continued without expanding the blood volume of the dog (control experiment). These experiments were performed in a pre-arranged order which became known to the individuals involved only after the decision to continue the experiment had been made. The person responsible for measuring the short-circuit current was not told whether the blood volume was to be expanded, but only the time at which an expansion might take place.

In the expansion experiments, blood was transfused into the dog from R₁ at a steady rate over 15 min. A total of 28 ml/kg dog was given. This was done by slowing the speed of the pump taking blood to R₁. The rate at which blood flowed from R₁ to the frog skin circuits was not altered, nor was that of the blood returning to the dog. In the control experiments all the conditions were similar to those in the expansion experiments except that there was no transfusion from the transfusion reservoir into the dog. In both types of experiment the urine was collected over hourly periods until the skin began to stabilize. It was then collected over 10 to 20 min periods. A blood sample was taken in the middle of each period of urine collection.

Chemical analysis of samples

The dog's arterial pH, Pa₄CO₂ and Pa₂O₂ were measured at 37° immediately after the sample had been taken. The pH, Pa₄CO₂ and Pa₂O₂ of the blood in the frog skin circuits were measured at 20°. Glass electrodes and an E.I.L. Blood Analysing Trolley were used. Plasma and urine Na⁺ and K⁺ were measured with an Eppendorf flame-photometer. Plasma and urine creatinine, PAH and plasma total protein were measured on a Technicon AutoAnalyser. Urine osmolality was measured from the depression of freezing point using an Advance Osmometer.

RESULTS

Short-circuit current across the frog skin after its incorporation into a dog's circulation (Fig. 2)

Immediately after the dog had been joined to the circuits containing the frog skin, there was always a steep fall in the short-circuit current across the skin, which lasted 1–2 hr. After this initial fall, there were four common types of behaviour:

1. The current reached a steady level and remained stable for the rest of the experiment. In some skins the current gradually levelled out after the initial fall (Fig. 2, F). In others the initial fall was followed by a rise in current before it finally levelled (Fig. 2, A).

2. The current reached a steady level and then the skin died (Fig. 2, C and G).
(3) The current reached a steady level but then began to fluctuate and continued to do so for the rest of the experiment (Fig. 2, B and E).

(4) The current never reached a steady level. It either continued to fall (Fig. 2, H) or it fluctuated for the rest of the experiment (Fig. 2, D).

Fig. 2. Behaviour patterns of the short-circuit current (μA) across the isolated living frog skin incorporated in the circulation of a dog. Each skin was joined into the blood circulation of a dog at zero time.

Changes in the short-circuit current across the frog skin in preliminary experiments in which the blood volume of the dog was expanded

Forty-two preliminary experiments were performed. In all of them the blood volume of the dog was expanded. In twenty-six experiments the short-circuit current across the frog skin was either fluctuating or falling steeply throughout the experiment and it was not possible to interpret the changes in current which occurred when the blood volume was expanded. In fourteen of the remaining sixteen experiments the short-circuit current fell after the blood volume had been expanded. This fall had a consistent time relationship with the expansion and with the rise in the dog's urinary sodium excretion. In the other two experiments the short-circuit current did not change.
Changes in the short-circuit current across the frog skin in the final series of experiments

Twenty-nine skins were studied. One skin died. The short-circuit current across twelve skins was not stable; these were discarded before either the blood volume was expanded or a control experiment was performed. The short-circuit current across the remaining sixteen skins was considered to be sufficiently stable for the experiment to continue. Eight skins were used to determine the effect of expanding the blood volume of the dog. The other eight skins were used in control experiments in which the blood volume of the dog was not expanded.

Expansion experiments (Fig. 3). Eight frog skins were studied in five experiments. There was

Fig. 3. The effect of expanding the blood volume of the dog on the short-circuit current across the frog skin. The fifteen min period during which the blood volume was expanded is shown by the vertical stippled area, marked BVE. The short-circuit current is shown in $\mu$A. The curves are arranged in the above order to obtain a compact diagram. The numbers of the experiments are shown on the right (2, 6, 7, 8 and 10). The numbers in subscript (1 and 2) indicate which skin was used; skin$_1$ was proximal to skin$_2$ in the dog's circulation. The numbers on the left are the absolute values of $\mu$A at the beginning of each curve.
a fall in short-circuit current across all eight skins when the blood volume of the dog was expanded. In some, the fall was pronounced (Fig. 3, skin 2); in others it was much smaller (Fig. 3, skins 8 and 6). In seven of the skins there was no change in the current during the first 10 min of the transfusion. In the other skin (Fig. 3, skin 7), it is uncertain whether the current started to fall before or after the 10th min. In all eight skins there was a fall in current between the 10th and 20th min after the start of the transfusion. This fall accelerated and continued until the 15th–30th min after the end of the transfusion.

In six of the eight skins (2, 2, 10, 10, 7 and 6) the current then stopped falling and began to rise again. In none of them had the current reached its pre-transfusion level by the time that the experiment was terminated, 50–100 min after the start of the transfusion. In the sixth skin, 6, the current started to fall again after rising for only 10 min. In the remaining two of the eight skins, 6 and 8, there was no rise in the current after the initial fall. In one, 6, the current remained at the reduced level for the rest of the experiment. In the other, 8, the current started to fall again after having stayed at the reduced level for 20 min.

Two skins incorporated in the circulation of the same dog did not necessarily respond to the same degree when the blood volume was expanded. The two skins in Experiment 10 (Fig. 3) and those in Experiment 5 (Fig. 5) had similar responses, whereas in Experiment 2 (Fig. 3), there was a bigger response in skin than in skin.

Control experiments (Fig. 4). In these experiments the 'starting time' is defined as the time at which the transfusion would have started if the experiment had been one in which the blood volume was expanded. Eight frog skins were studied in six experiments. Observations were continued on the skins for 40–70 min after the 'starting time'.

In five skins, the trend of the short-circuit current did not change after the 'starting time' (Fig. 4, skins 4, 11, 9, 1 and 11). In the other three skins, a fall in current was evident 10 min after the 'starting time' (Fig. 4, skins 3, 5 and 5). This fall started earlier than that observed in the experiments in which blood volume was expanded. In one of these three skins, 3, the rate of fall rapidly increased until 60 min later it was almost vertical. Retrospectively, therefore, it appears that this fall was due to death of the skin and that the onset of this had coincided with the 'starting time'. In the other two of these three skins, 5 and 5, though the fall in current began earlier than in the expansion experiments, the subsequent changes in current did show some features which were similar to those observed when the blood volume was expanded. Because of these similarities, it was decided to continue the experiment on these two skins and to expand the blood volume of the dog after the control experiment had been completed. The dog was transfused 60 min after the 'starting time'. In this way it was possible to compare the changes in short-circuit current that occurred when the blood volume was not expanded, with those that occurred in these same skins when the blood volume was expanded. On expanding the blood volume of the dog, both skins behaved in the same way (Fig. 5). There was no fall in current during the first 10 min of the transfusion. The current fell throughout the next 20–30 min and then began to rise. This pattern was different from that observed when the blood volume was not expanded, whereas it was in keeping with that observed in most of the expansion experiments.

The relative changes in membrane potential and short-circuit current across the frog skin in the final series of experiments

Expansion experiments (Table 1). When the blood volume of the dog was expanded, the fall
in short-circuit current in all eight frog skins was accompanied by a simultaneous fall in the potential difference across the skins. Table 1 shows the values of the short-circuit current (μA) and the membrane potential (mV) in the 10 min period before the transfusion, and the lowest values in the 30 min period after the transfusion.

The ratio of the membrane potential to the short-circuit current, mV x 1000/μA, was calculated. After transfusion, the ratio was unchanged in one skin, 2₂; it rose in two skins, 6₂ and 7₁, and it fell in the remaining five skins, 2₁, 6₁, 8₁, 10₁ and 10₂. In the series of eight skins there was no significant difference (Student t-test) in the mean ratio before and after transfusion.
FIG. 5. The short-circuit current across two frog skins during a control experiment and subsequently when the blood volume of the dog was expanded (Experiment 5). The vertical line marked C is the ‘starting time’ of the control experiment when the blood volume of the dog was not expanded. The blood volume of the dog was subsequently expanded during the 15 min period marked BVE. $U_{\text{NaV}}$, urinary $\text{Na}^+$ excretion rate of the dog. $\mu\text{A}$, short-circuit current.

<table>
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Table 1. Comparison of the ratio of the membrane potential (mV) to the short-circuit current ($\mu\text{A}$) across frog skins before and after the blood volume of a dog was expanded. The Student $t$-test shows that the mean values of this ratio are not statistically different before and after expansion.
Control experiments. In the five skins in which the short-circuit current did not change, there was also no change in the membrane potential. In the other three skins (Fig. 4, skins 31, 51 and 52), there was a fall in membrane potential similar to that observed in the current. There was no significant change in the ratio mV × 1000/µA in any of the eight skins.

Comparison of the changes in the short-circuit current across the frog skin with the changes in the rate of urinary Na⁺ excretion by the dog in the final series of experiments

Expansion experiments (Figs. 6 and 8). Urinary Na⁺ excretion started to rise during the transfusion in four experiments and immediately after the transfusion in Experiment 6. It was
maximal within 35 min after the end of the transfusion. Thus the rise in Na\(^+\) excretion began just before the short-circuit current started to fall and was maximal at about the same time that the current was at its lowest value.

*Control experiments* (Figs. 5 and 7). In four of the five experiments the urinary sodium excretion was falling before the 'starting time' and continued to fall at about the same rate thereafter. In the other experiment (4) the urinary sodium excretion was rising before the 'starting time', after which it fell.

**Fig. 7.** The short-circuit current across the frog skin and the urinary excretion of sodium by the dog in four experiments in which the blood volume of the dog was not expanded. The vertical line marked C is the 'starting time'. \(\mu A\), short-circuit current. \(U_{Na}V\), the dog's urinary Na\(^+\) excretion in \(\mu Eq/min\).

*Other changes in the dog during the final series of experiments*

*Expansion experiments.* The temperature of the dog remained constant during and after the transfusion. In all experiments the rise in urinary Na\(^+\) excretion was accompanied by
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increased urine flow, and increased K⁺ excretion. In four experiments urine osmolality fell and in one experiment it rose (Table 2). Free water reabsorption rose in four experiments and fell in one experiment. In all experiments, expanding the blood volume caused a rise in arterial and venous pressures in the dog. There was no consistent change in any of the experiments in the dog's haematocrit or arterial pH, PaCO₂, PaO₂ or in the plasma Na⁺ or K⁺ (Fig. 8). PAH clearance, creatinine clearance and filtered sodium showed no consistent changes.

Table 2. Urinary sodium excretion, urine flow, urinary osmolality, osmolal clearance and free water reabsorption in the expansion and control experiments

<table>
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<tr>
<th>Type of Experiment</th>
<th>Expt.</th>
<th>Urinary sodium excretion (U Na⁺ × Uv) (µEq/min)</th>
<th>Urine flow (Uv) (ml/min)</th>
<th>Urine osmolality (U osmol) (mosmol/kg)</th>
<th>Osmolar clearance (C osmol = U osmol × Uv) (ml/min)</th>
<th>Free water reabsorption (C osmol - Uv) (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Expansion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>49</td>
<td>0:18</td>
<td>0:40</td>
<td>1260</td>
<td>900</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>35</td>
<td>0:20</td>
<td>0:26</td>
<td>1560</td>
<td>1710</td>
</tr>
<tr>
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<td>180</td>
<td>0:92</td>
<td>2:40</td>
<td>600</td>
<td>305</td>
</tr>
<tr>
<td>8</td>
<td>155</td>
<td>200</td>
<td>1:00</td>
<td>1:80</td>
<td>535</td>
<td>400</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>116</td>
<td>0:14</td>
<td>1:00</td>
<td>1360</td>
<td>500</td>
</tr>
</tbody>
</table>

'Before' = 30 min period, immediately before the start of the transfusion.
'After' = 30 min period, from 30-60 min after the start of the transfusion.

Control | | | | | | | | | | | | |
| Before | After | Before | After | Before | After | Before | After | Before | After | Before | After |
| 1 | 90 | 60 | 0:54 | 0:34 | 980 | 990 | 1:76 | 1:12 | 1:22 | 0:78 |
| 3 | No urine samples | | | | | | | | | | |
| 4 | 18 | 6 | 0:17 | 0:16 | 2465 | 2270 | 1:40 | 1:21 | 1:23 | 1:05 |
| 5 | 62 | 22 | 0:70 | 0:65 | 1010 | 1045 | 2:36 | 2:26 | 1:66 | 1:61 |
| 9 | 5 | 2 | 0:18 | 0:15 | 700 | 860 | 0:42 | 0:43 | 0:24 | 0:28 |
| 11 | 94 | 53 | 0:67 | 0:42 | 730 | 920 | 1:63 | 1:29 | 0:96 | 0:87 |

'Before' = 30 min period, immediately before the 'starting time'.
'After' = 30 min period, from 30-60 min after the 'starting time'.

There was a small rise in plasma protein concentration when the blood volume was expanded. This had no consistent relationship with the change in short-circuit current. In Experiment 7, there was no change in the plasma protein concentration when the blood volume of the dog was expanded. In the other four experiments there was a rise in protein concentration of 0:3-0:6 g% after the expansion. In two of these experiments (2 and 8), the rise in plasma protein took place between the 20th and 30th min after the start of expansion, i.e. it took place after the short-circuit current had begun to fall. In only two experiments did the rise in plasma protein concentration coincide with the fall in current.

Control experiments. There was a small fall in urine flow in all experiments in which observations were made. This was accompanied by a rise in urine osmolality in four experiments and a fall in one. Free water reabsorption fell in four experiments and rose in one (Table 2). There were no consistent changes in haematocrit, arterial pH, PaCO₂, PaO₂, plasma Na⁺, plasma K⁺, plasma protein, PAH clearance, creatinine clearance or filtered Na⁺.
DISCUSSION

The results show that when the blood volume of a dog is expanded, there is a fall in the short-circuit current across an isolated living frog skin incorporated in the blood circulation of the dog. The blood volume was expanded over 15 min by a transfusion of blood which was in equilibrium with that in the dog. The fall in short-circuit current across the frog skin began 10–20 min after the start of the transfusion into the dog. The current reached its lowest value 15–30 min after the end of the transfusion. It then either stabilized at the low level or began to rise again. The current had not regained the pre-transfusion level by the time the experiment was terminated, 50–100 min after the start of the transfusion. During these changes, the dog had a simultaneous rise in the rate of urinary sodium excretion.

In the control experiments, in which the blood volume of the dog was not expanded, the trend in the short-circuit current across five of the eight frog skins did not change. In the other three skins there was a fall in the short-circuit current. The pattern of this fall, however, differed
Na\textsuperscript{+} transport after blood volume expansion

from that usually observed when the blood volume was expanded. There was no rise in the rate of urinary sodium excretion in any of the dogs in these control experiments.

Ussing & Zerahn (1951) have shown that the short-circuit current across a frog skin maintained in a frog Ringer solution is due to active transport of sodium. We have assumed that this is also true when the frog skin is in dog's blood. In the experiments of Ussing & Zerahn, there was no communication between the solutions on either side of the frog skin. In the experiments described above, the blood on the two sides of the skin was connected round the edge of the skin through holes in the membrane-fixing unit. Hutchings et al. (1969), have shown that when such a cell is filled with Ringer solution, the presence of these holes leads to a falsely low absolute value for membrane potential and to a falsely high absolute value for the short-circuit current. Nevertheless, by using a variety of known membrane stimulants and depressants, they have shown that changes in the short-circuit current measured under these conditions parallel the changes observed when there is no communication round the membrane. It has therefore been assumed that the changes in the short-circuit current which occurred when the blood volume of the dog was expanded, reflect the changes in the rate of active sodium transport across the frog skin.

Knox et al. (1968) have shown that when the blood volume of a dog is expanded, the rise in urinary sodium excretion which takes place is due, in part, to a fall in the rate of sodium reabsorption in the proximal tubule. The frog skin experiments described here demonstrate that when the blood volume of a dog is expanded, the rise in the urinary sodium excretion in the dog is associated with a simultaneous fall in the short-circuit current across a frog skin incorporated in the dog's circulation. The fall in short-circuit current in the frog skin is probably due to a fall in the active transport of sodium. It is possible, therefore, that part of the decrease in sodium reabsorption in the tubule is also due to a fall in active sodium transport.

When the blood volume of the dog was expanded, the changes in active sodium transport across the frog skin could only have been brought about by a change in the blood bathing the skin. As the pressures and flows of the blood to each side of the skin were kept constant, the changes in cardiac output, arterial pressure and venous pressure which were induced in the dog when its blood volume was expanded could not have influenced sodium transport across the frog skin. In addition, the temperature of the blood supplying the frog skin was kept constant. Thus, it seems probable that the change in active sodium transport across the skin was due to some change in the chemical composition of the blood.

The arterial pH, \( P_a \text{CO}_2 \) and \( P_a \text{O}_2 \) were kept constant. The results show that there were no consistent changes in the haematocrit or the plasma Na\textsuperscript{+} and K\textsuperscript{+}. There was a small variable rise in plasma protein which had no consistent relationship with the change in short-circuit current. The techniques by which the blood volume of the dog was expanded were devised so as to minimize the chances of a coincidental change in the composition of the blood. Blood was continuously circulated through the transfusion reservoir, the frog skin circuits and through the dog. The blood volume was therefore expanded with blood which was in equilibrium with the blood in the dog (Bahlmann et al., 1967). It might be postulated that some substance which could either stimulate or depress active sodium transport was formed in the blood while it was outside the dog in the transfusion reservoir. Evidence against this possibility has been provided by Knox et al. (1968). In their experiments, a reservoir containing artificial plasma was connected to a dog's circulation until complete equilibration with the blood had taken place. The reservoir was then disconnected from the dog and the blood was kept at 37\degree for 30–60 min.
After this, the reservoir was reconnected to the dog's circulation. This did not produce any change in the fractional reabsorption of sodium from the proximal tubule of the dog. Further evidence against the possibility of a natriuretic substance being formed in the reservoir has been given by Clarkson et al. (1970).

It would seem likely, therefore, that the dog changes the concentration of some circulating substance which influences active sodium transport. This substance is unlikely to be a salt-retaining adrenal steroid or vasopressin, since both of these were present in gross excess throughout the experiment. Our experiments do not distinguish whether the concentration of this circulating substance increases or decreases. These conclusions are in line with the findings of Lichardus et al. (1966), Bahlmann et al. (1967) and Knox et al. (1968) who all used blood to expand the blood volume of dogs. They measured the changes in the urinary sodium excretion of the dog. Our conclusions also agree with the results obtained by Clarkson et al. (1970), who studied sodium transport in fragmented renal tubules incubated in plasma obtained from dogs before and after the blood volume was expanded with blood.

The experiments reported here provide a little evidence concerning the way in which the substance may influence active sodium transport. When the blood volume was expanded, there was no significant change in the ratio of the membrane potential to the short-circuit current across the frog skin. As the KCl-agar leads used in the measurement of membrane potential were not moved and the ionic composition of the blood surrounding the skin did not change when the blood volume was expanded, it therefore seems likely that the electrical resistance of the frog skin did not change as the short-circuit current fell. If this conclusion is valid, then it follows that the fall in short-circuit current was not due to a change in the permeability of the skin to anions. (If the permeability of the skin to anions had risen, the ratio mV/μA would have fallen, and vice versa.) The circulating substance which influences active sodium transport must therefore act on the Na+ pump itself. It could either act on this directly or it could act by decreasing the permeability of the outside of the skin to Na+ and hence diminishing the supply of Na+ to the pump. Both of these possibilities could leave the electrical resistance of the skin unchanged.

Many workers have also investigated the effect of intravenous infusions of saline on urinary sodium excretion. A review of this work has been given by Earley & Daugharty (1969). Saline dilutes the blood and hence alters its composition; it also causes haemodynamic changes. Both these factors may change tubular sodium reabsorption in the intact dog or isolated kidney. Therefore, it has been difficult to assess whether, in addition, there is a change in concentration of some circulating substance which, directly or indirectly, influences sodium transport. Recently there have been further experiments in which extracts were made from plasma and urine, taken before and after saline infusion. These extracts were then tested by various bioassay techniques. Sealey et al. (1969) and Lichardus et al. (1969) injected such extracts into hydrated rats. They obtained an increase in the rate of urinary sodium excretion with the extracts made from samples taken after the saline infusion. Lichardus et al. (1969) and Buckalew et al. (1969), using conventional Ussing-types of apparatus, studied the effect of the extracts on the short-circuit current across the frog skin and toad bladder respectively. They found that extracts obtained from plasma taken after saline infusion caused a fall in the short-circuit current.

In conclusion, therefore, it seems likely that in experiments in which saline is infused and also in experiments in which the blood volume is expanded with blood, there is a change in the
Na⁺ transport after blood volume expansion

concentration of some circulating substance which influences sodium transport in the renal tubule. It is not yet established whether it is the same substance which is involved in the two types of experiment. The frog skin experiments described here, in which the blood volume of the dog is expanded with blood, show that the substance acts directly on the mechanism for active sodium transport. Lichardus et al. (1969) and Buckalew et al. (1969) have reached a similar conclusion in experiments in which saline is infused.

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