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# Actions of adrenaline on the potassium balance of the isolated heart

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1. The action of adrenaline on the $K^+$ balance of the isolated heart was found to depend on the ionic composition and the temperature of the perfusion fluids used.

2. When the perfusion fluid contained 145 mM $Na^+$, 1·7 mM $Ca^{++}$ and 3–9 mM $K^+$, adrenaline caused the hearts to gain $K^+$ ; when the $K^+$ concentration was reduced to 1·2 mM, adrenaline caused a loss of $K^+$. Both were actions on $\beta$ receptors.

3. When the $Ca^{++}$ or the $Na^+$ concentration in the perfusion fluid was reduced, together with a reduction in $K^+$, adrenaline no longer produced $K^+$ loss from the hearts, but produced a gain of $K^+$.

4. When the temperature of the perfusion fluid was reduced to 25° C, adrenaline still produced a gain of $K^+$ by hearts perfused with fluid containing 3·2 mM $K^+$, but did not produce a loss of $K^+$ from hearts perfused with fluid containing 1·2 mM $K^+$.

Measurements of the action of adrenaline on $K^+$ fluxes in isolated atria were made by Waddell (1961), and by Stafford (1962). Both authors were in agreement that the main action of adrenaline was to cause an increased uptake of $K^+$ into the atria, thus producing a net increase in their $K^+$ content. The action of adrenaline was studied mostly in concentrations ranging from $2 \times 10^{-6}$M to $7{\cdot}5 \times 10^{-5}$M, but higher concentrations produced the same effect. Melville, Mazurkiewicz & Korol (1955), however, found that adrenaline increased the rate of loss of $K^+$ from isolated hearts perfused with $K^+$-free fluid. Later, Melville & Korol (1958) showed that, in hearts perfused with several fluids containing different $K^+$ concentrations, adrenaline, noradrenaline or isoprenaline produced a net loss of $K^+$ from the heart.

Waddell (1961) and Stafford (1962), who found that adrenaline caused an increase in $K^+$ content, were using atria and Melville and his co-workers, who found a decreased $K^+$ content, were using isolated hearts, so it seemed possible that the conflicting results could have been due to a difference between the actions of adrenaline on $K^+$ fluxes in atrial and ventricular muscle ; differences in the ionic composition of the fluid might also have been an important factor.

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The experiments described in this paper were done on isolated hearts perfused with fluids of various ionic compositions, and show that adrenaline may cause either a gain or a loss of $K^+$ from the heart, depending on the experimental conditions.

**Methods**

Small rabbits (0·7–1·3 kg) were killed by a blow on the head. The heart was quickly removed and dissected free from the pericardium and fat. The stump of the aorta was cannulated and the heart was perfused through the coronary vessels as in Langendorff's method. Perfusion was begun 1–2 min after the rabbit was killed. A water-jacketed chamber (supplied by Aimer) was placed around the heart. The temperature of the circulating water was $30° \pm 0·2°$ C, unless otherwise stated.

The perfusion fluid was a modification of Krebs solution, of the following composition: NaCl 119 mM, KCl 5·8 mM, $CaCl_2$ 1·7 mM, $NaHCO_3$ 25 mM, $NaH_2PO_4$ 1·2 mM, $MgSO_4$ 1·2 mM, dextrose 11 mM; gas phase 5% carbon dioxide in oxygen. In some experiments the $K^+$ concentration of this solution was reduced to 3·2, 2·5, 2·0 or 1·2 mM; the $Ca^{++}$ was changed to 0·68 or 5·0 mM, or the $Na^+$ was reduced to 70 mM. When less KCl or $CaCl_2$ was added, isotonicity was maintained by adding more NaCl; when less NaCl was added, isotonicity was maintained with sucrose. Each heart was perfused with a modified Krebs solution (details in **Results**) for 25 min, and then adrenaline was infused from a motor driven syringe through polythene tubing which terminated in the aortic cannula. The rate of the adrenaline infusion was 0·052 ml./min, and the adrenaline ($7·4 \times 10^{-4}$M) was dissolved in an acid solution (pH 4·5–5·5), the $Na^+$ and $K^+$ concentrations of which were the same as those of the perfusion fluid in each experiment. The concentration of adrenaline in the fluid reaching the heart depended upon the coronary flow, but was usually about 3·8 mM, and was always between 2·7 and 4·9 mM.

The effluent fluid from the heart was collected for 30 sec periods into graduated 10 ml. tubes and the volume, to the nearest 0·1 ml., noted. Collections were made during the fifteenth, twentieth and twenty-fourth min of perfusion of the heart, and every minute after the adrenaline infusion was started. The heart rate was measured during the collection periods. After 15 min of adrenaline infusion, the heart was removed from the cannula; the fluid was squeezed out of the ventricles, and the heart was weighed to the nearest 0·05 g. Results are expressed in terms of wet weight of tissue.

Samples of the perfusion fluid and of the effluent were diluted until their $K^+$ concentrations were 100–150 $\mu$M, and their $K^+$ contents were measured with a flame photometer (EEL). Two or three readings were taken for each effluent. The smallest difference in the $K^+$ concentrations of perfusion and effluent solutions that could be measured corresponded to a mean difference of 0·5–1 division on the flame photometer scale. This represented changes of approximately 20, 30 and 55 $\mu$M when the $K^+$ concentrations of the undiluted solutions were 1·2, 2·0 and 3·2 mM respectively. Thus, the higher was the $K^+$ concentration of the perfusion fluid, the less precise were the results. The difference between the $K^+$ concentrations of the effluent and perfusion fluids was multiplied by the coronary flow to obtain the amount of $K^+$ lost or gained by the heart during that period. This amount was then divided by the weight of the heart. Observations were made on three to five hearts in each perfusion medium.

### *Uptake of $^{42}K^+$ by the isolated heart*

Sixteen isolated hearts were perfused with Krebs solution containing either 5·8 or 1·2 mM $K^+$ for 25 min. Then part of the $K^+$ in each of the media was replaced with $^{42}K^+$. The hearts were perfused with fluid containing $^{42}K^+$ for 5, 10, 15 or 20 min, and then the $^{42}K^+$ and $K^+$ contents of the atria and apices of the ventricles were determined as described by Rayner & Weatherall (1959).

## Results

Modified Krebs solution was used in many of the experiments on isolated hearts. The extent to which the Krebs solution was modified was limited by the following considerations. Only solutions were used in which the hearts (*a*) maintained an apparently normal sinus rhythm for 25 min, (*b*) were not gaining or losing $K^+$ at a rate greater than 0·05 $\mu$-mole/g per min before the adrenaline infusion was started and (*c*) responded to adrenaline with a clear increase in rate of beating. In practice, the following limitations arose. The $K^+$ content of the perfusion fluid could not be decreased beyond 1·2 mM because sinus rhythm was not maintained and $K^+$ loss occurred. In perfusion fluid containing 2·0 mM $K^+$, the $Ca^{++}$ could not be reduced below 0·68 mM, or the hearts became grossly oedematous. In perfusion fluids with the $Na^+$ reduced to 70 mM, hearts beat regularly and responded to adrenaline provided that the $K^+$ was below 2·5 mM ; if the $K^+$ was increased to 3·2 mM, the hearts stopped beating.

### *Effect on the action of adrenaline of changes in the $K^+$ concentration of the perfusion fluid*

#### *$K^+$ balance*

Figure 1 shows the effect of adrenaline on the $K^+$ balance of isolated hearts perfused with fluids containing 1·2, 2·0, 2·5 or 3·2 mM $K^+$. When the perfusion fluid contained a low concentration of $K^+$ (1·2 mM), adrenaline caused a loss of $K^+$ from the hearts (Fig. 1a). The maximum rate of loss of $K^+$ occurred 9–10 min after the adrenaline infusion was started, and was as high as 1·1 $\mu$-mole/g per min. When the $K^+$ concentration in the perfusion fluid was 2·0 mM, adrenaline caused no appreciable change in the $K^+$ balance of the hearts (Fig. 1b). With a slightly higher $K^+$ concentration in the perfusion fluid (2·5 mM), adrenaline produced a small gain of $K^+$ during the first 5 min of infusion (Fig. 1c). When the $K^+$ concentration in the perfusion medium was 3·2 mM, adrenaline caused the hearts to gain $K^+$ (Fig. 1d). The maximum rate of gain of $K^+$ occurred 4–5 min after the adrenaline infusion was started, and was as high as 1·2 $\mu$-mole/g per min. Thus, the maximum rate of gain of $K^+$ produced by adrenaline in hearts perfused with fluid containing 3·2 mM $K^+$ occurred earlier than the maximum rate of loss of $K^+$ in hearts perfused with fluid containing 1·2 mM $K^+$. This difference in the rate of onset of the two effects probably accounts for the biphasic action of adrenaline on the $K^+$ balance of hearts perfused with fluid containing 2·5 mM $K^+$ (Fig. 1c).

Some measurements were made of the effect of adrenaline on the $K^+$ balance of isolated hearts perfused with fluid containing higher concentrations of $K^+$: 5·8 or 9·0 mM. Adrenaline caused the hearts to gain $K^+$ in each case, but the gain appeared to be no greater than that occurring with fluid containing 3·2 mM $K^+$. However,

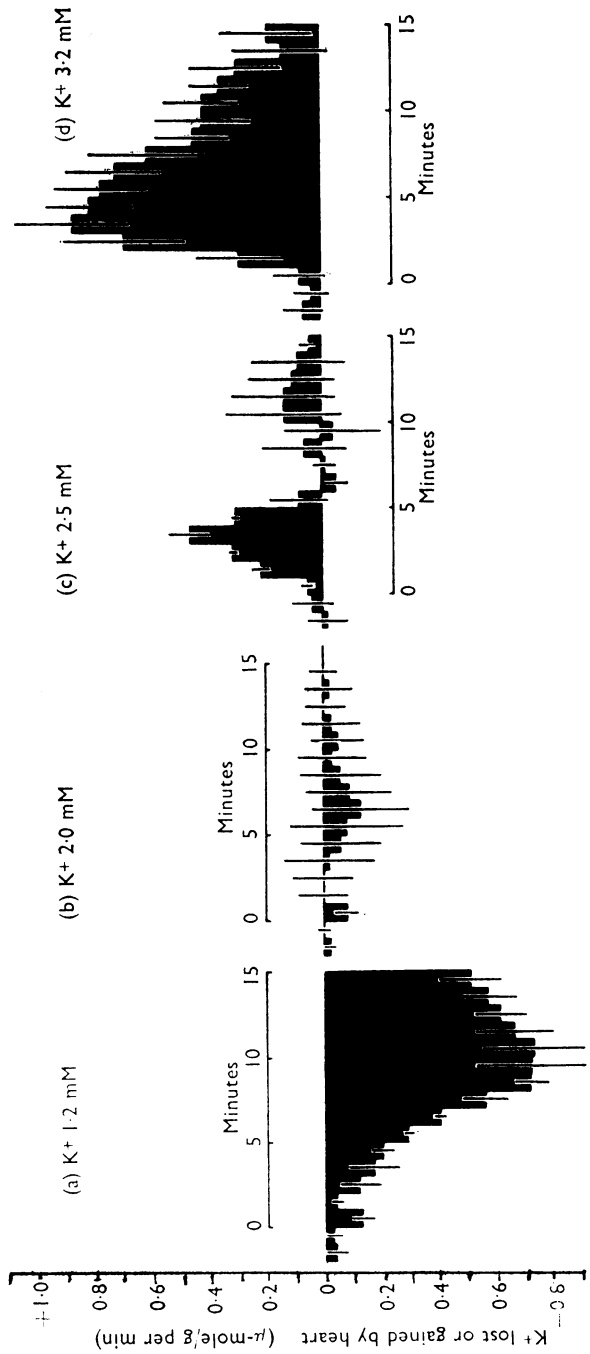


FIG. 1. Action of adrenaline on the $K^+$ balance of isolated hearts perfused with modified Krebs solution containing 1·2, 2·0, 2·5 or 3·2 mM $K^+$. The infusion of adrenaline (0·038 μ-mole/min) was started at time 0, and continued for 15 min. $K^+$ gained or lost from the hearts was calculated from the $K^+$ concentration in the effluent fluid, sampled at 1 min intervals throughout the experiments. Mean results from three to five hearts are illustrated ; standard errors of the means are indicated by the vertical lines. When the concentration of $K^+$ in the perfusion fluid was 1·2 mM, adrenaline produced a loss of $K^+$ from the hearts (a) ; with 3·2 mM $K^+$ in the perfusion fluid, adrenaline caused the hearts to gain $K^+$ (d).

this indirect method of measuring changes in cardiac $K^+$ becomes very inaccurate when the $K^+$ content of the perfusion fluid is high.

*Heart rate*

The mean initial rates of beating of hearts perfused with fluids containing 1·2, 2·0 and 3·2 mM $K^+$ were $80 \pm 2$, $108 \pm 6$ and $114 \pm 1$. In the presence of adrenaline these heart rates increased to $193 \pm 10$, $183 \pm 16$ and $210 \pm 10$ respectively. Thus, the increase in heart rate occurred whether the hearts were gaining or losing $K^+$, so apparently the change in rate of beating was not the only factor that determined changes in $K^+$ content of the hearts. However, it seemed important to find out whether adrenaline still caused a loss of $K^+$ from isolated hearts perfused with low $K^+$ fluid, when their rate of beating was maintained constant by electrical stimulation. It was found that only two out of five hearts perfused with fluid containing 1·2 mM $K^+$ would respond to each stimulus when stimulated electrically at 200/min, although all of them would beat at this rate under the influence of adrenaline. In the two hearts that did follow electrical stimulation, the increase in rate of beating from the normal rate of approximately 80/min up to 200/min caused a measurable loss of $K^+$ from the hearts of about 0·2 $\mu$-mole/g per min. Then, when adrenaline was infused, the rate of loss of $K^+$ from the hearts increased 4–5 fold, up to 0·8–1·0 $\mu$-mole/g per min. Thus, while the increased rate of beating itself produced a small loss of $K^+$ from the hearts, adrenaline produced a much greater loss, despite there being no further change in rate of beating.

*Coronary flow*

The mean coronary flow was $14{\cdot}4 \pm 0{\cdot}4$ ml./min, and was not altered by the $K^+$ concentration in the perfusion fluid. Adrenaline always caused an initial decrease in coronary flow, probably due to the increased force of the contractions. During the infusion of adrenaline, the coronary flow gradually increased again, but did not return to the control rate. The mean coronary flow during the adrenaline infusions was $10{\cdot}2 \pm 0{\cdot}6$ ml./min.

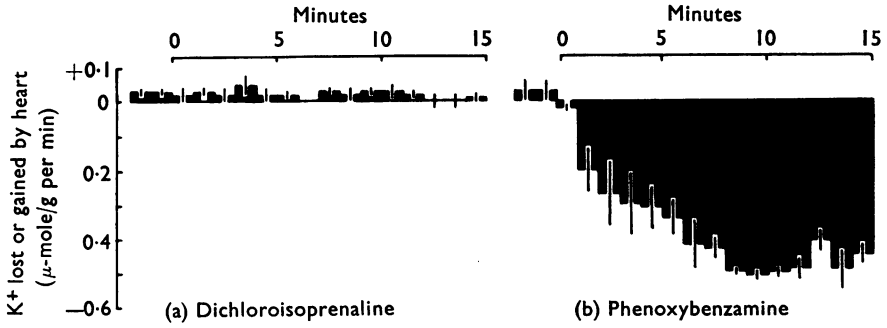


FIG. 2. Effects of anti-adrenaline drugs on the action of adrenaline on $K^+$ balance of isolated hearts perfused with modified Krebs solution containing 1·2 mM $K^+$. Dichloroisoprenaline ($3{\cdot}5 \times 10^{-6}$M) or phenoxybenzamine ($1{\cdot}5 \times 10^{-6}$M) was added to the perfusion fluid at the beginning of the experiment, so the hearts had been exposed to the blocking drug for 25 min before the infusion of adrenaline was started. In the presence of dichloroisoprenaline, adrenaline did not produce a loss of $K^+$ from the hearts (a). Phenoxybenzamine did not block the action of adrenaline (b).

*Effects of isoprenaline and anti-adrenaline drugs*

Isoprenaline produced effects like those of adrenaline: it caused a gain of $K^+$ in a heart perfused with fluid containing 3·2 mM $K^+$, no change in $K^+$ balance in two hearts perfused with fluid containing 2·0 mM $K^+$ and a loss of $K^+$ from two hearts perfused with fluid in which the $K^+$ was reduced to 1·2 mM. However, isoprenaline was more potent than adrenaline; when infused at a rate of 0·0007 $\mu$-mole/min, it produced effects comparable with those produced by adrenaline infused at 0·038 $\mu$-mole/min. Reducing the rate of infusion of adrenaline to 0·0038 $\mu$-mole/min greatly reduced its effect in causing $K^+$ loss from two hearts perfused with low $K^+$ fluid.

Dichloroisoprenaline ($3{\cdot}5 \times 10^{-6}$ M), added to perfusion fluid containing 1·2 mM $K^+$, blocked the ability of adrenaline to cause a loss of $K^+$ from the heart, but

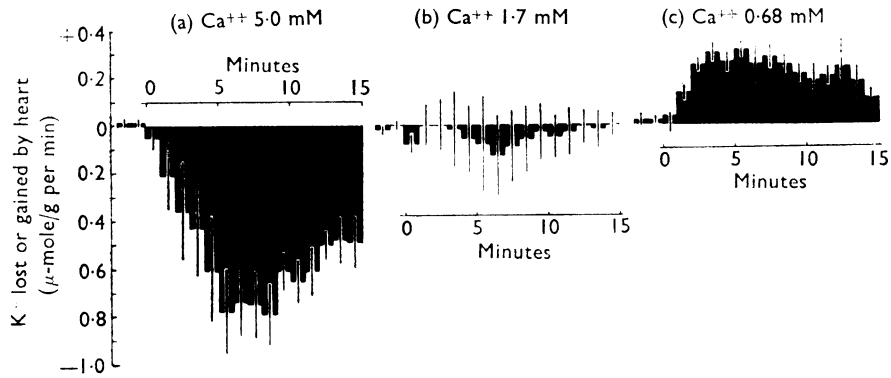


FIG. 3. Effect of changes in the $Ca^{++}$ concentration on the action of adrenaline on $K^+$ balance. Hearts were perfused with modified Krebs solution containing 2·0 mM $K^+$, and 5·0, 1·7 or 0·68 mM $Ca^{++}$. When the $Ca^{++}$ was increased to 5·0 mM, infusion of adrenaline (0·038 $\mu$-mole/min for 15 min) caused the hearts to lose $K^+$ (a); when the $Ca^{++}$ was decreased to 0·68 mM, adrenaline caused a gain of $K^+$ by the hearts (c).

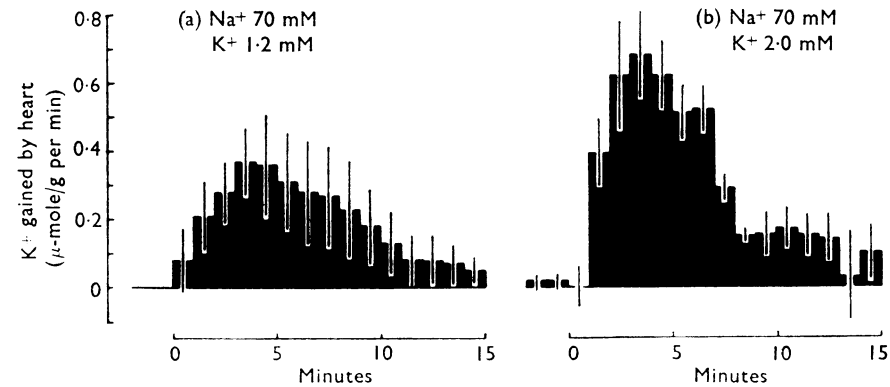


FIG. 4. Effect of reducing the $Na^+$ concentration on the action of adrenaline on $K^+$ balance. Infusion of adrenaline (0·038 $\mu$-mole/min) for 15 min caused a gain of $K^+$ in hearts perfused with modified Krebs solution in which both the $Na^+$ and $K^+$ concentrations were reduced.

phenoxybenzamine ($1{\cdot}5 \times 10^{-6}$ M) did not (Fig. 2). The loss of $K^+$ from the isolated heart produced by isoprenaline was also blocked by dichloroisoprenaline, but not by phenoxybenzamine. It has been shown before (Stafford, 1962) that the increased uptake of $K^+$ produced by adrenaline is the result of an action on $\beta$ receptors.

*Effect of changing the $Ca^{++}$ concentration on the action of adrenaline*

Isolated hearts beat regularly and responded to adrenaline by an increase in rate when perfused with fluids containing 2·0 mM $K^+$ in which the $Ca^{++}$ was 0·68 mM, 1·7 mM or 5 mM. As described above (Fig. 1b and 3b), in perfusion fluid containing reduced $K^+$ (2·0 mM) and usual $Ca^{++}$ (1·7 mM), adrenaline had very little effect on the $K^+$ balance of the heart. But when the $Ca^{++}$ in the perfusion fluid was increased to 5 mM, adrenaline caused a pronounced loss of $K^+$ from the hearts (Fig. 3a). When the $Ca^{++}$ concentration in the perfusion fluid was reduced to 0·68 mM, adrenaline caused the hearts to gain $K^+$ (Fig. 3c). Hearts perfused with the high and low $Ca^{++}$ fluids showed mean increases in rate of $100 \pm 8$ and $72 \pm 4$ beats/min respectively when adrenaline was infused.

*Effect of reducing the $Na^+$ and $Cl^-$ concentration on the action of adrenaline*

When the NaCl concentration in the perfusion fluid was less than normal, isotonicity was maintained with sucrose, but this meant that both the $Na^+$ and $Cl^-$ concentrations were reduced simultaneously. The effect of replacing part of the NaCl with sucrose was to decrease the action of adrenaline in releasing $K^+$ from the hearts. Mean results are shown in Fig. 4. When the $Na^+$ was reduced to 70 mM in perfusion fluid containing 1·2 mM $K^+$, adrenaline produced a slight increase in the $K^+$ contents of the hearts (Fig. 4a), whereas it caused a pronounced loss of $K^+$ from hearts perfused with fluid in which the $Na^+$ was 145 mM and the $K^+$ 1·2 mM (Fig. 1a). In perfusion fluids containing 2·0 mM $K^+$, adrenaline caused a marked gain of $K^+$ by the hearts when the $Na^+$ concentration was 70 mM (Fig. 4b), but practically no change in $K^+$ balance when the $Na^+$ concentration was 145 mM (Fig. 1b).

*Effect of temperature*

Experiments were done on hearts perfused with fluid at a temperature of $25° \pm 0{\cdot}2°$ C and containing 1·2, 2·0 and 3·2 mM $K^+$. The results are shown in Fig. 5.

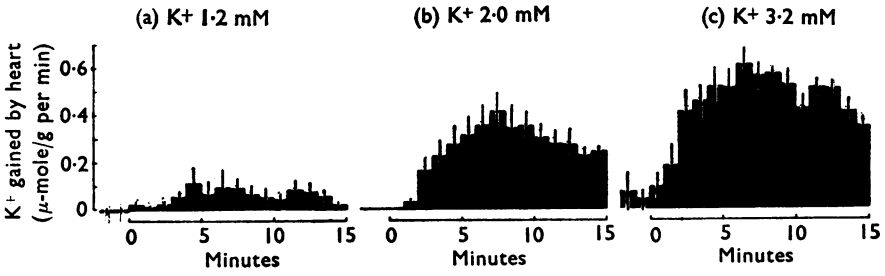


FIG. 5. Effect of a reduction in temperature on the action of adrenaline on $K^+$ balance. Experimental conditions were the same as those described in the legend to Fig. 1, except that the temperature of the perfusion fluid was 25° C. A reduction in temperature of 5° C abolished the $K^+$ loss produced by infusion of adrenaline.

The effect of adrenaline in releasing $K^+$ from the hearts perfused with low $K^+$ was abolished by reducing the temperature to 25° C (Fig. 5a); the $K^+$ gain produced by adrenaline in hearts perfused with fluid containing 3·2 mM $K^+$ was scarcely altered by lowering the temperature (Fig. 5c). In hearts perfused with fluid containing 2·0 mM $K^+$ at 25° C, adrenaline caused a gain of $K^+$ (Fig. 5b), whereas at 30° C, it had no effect on $K^+$ balance (Fig. 1b).

Three experiments were done at 35° ±0·2° C on hearts perfused with fluid containing 3·2 mM $K^+$; the gain of $K^+$ by the hearts produced by adrenaline was no greater than it was at 30° C.

### *Uptake of $^{42}K^+$ by the isolated heart*

The rate of uptake of $^{42}K^+$ by atria and ventricles of isolated, spontaneously beating hearts is shown in Fig. 6. Uptake of $^{42}K^+$ into atria and ventricles was much slower from the perfusion fluid containing 1·2 mM $K^+$ than it was from Krebs solution, which contains 5·8 mM $K^+$. It was shown before that the rate of exchange of $^{42}K^+$ in isolated atria depended on the rate at which they were beating (Stafford, 1962), and isolated hearts perfused with low $K^+$ fluid did beat at a lower rate than normal (see above). However, the marked reduction in the rate of $^{42}K^+$ entry in low $K^+$ fluid is too great to be due to a reduction in rate of beating alone.

With both Krebs and low $K^+$ perfusion fluids, less $^{42}K^+$ was taken up by the atria than by the ventricles. It was found before (Stafford, 1962) that the uptake of $^{42}K^+$ into isolated atria beating at 125/min was about 15 $\mu$-moles/g in 15 min; this agrees well with the values shown in Fig. 6a for uptake into the atria of isolated hearts under comparable experimental conditions. Since the rate of turnover of $K^+$ was no greater in perfused than in isolated atria, it seems unlikely that the lower rate of turnover in the atria than in the ventricles of perfused hearts can be ascribed to

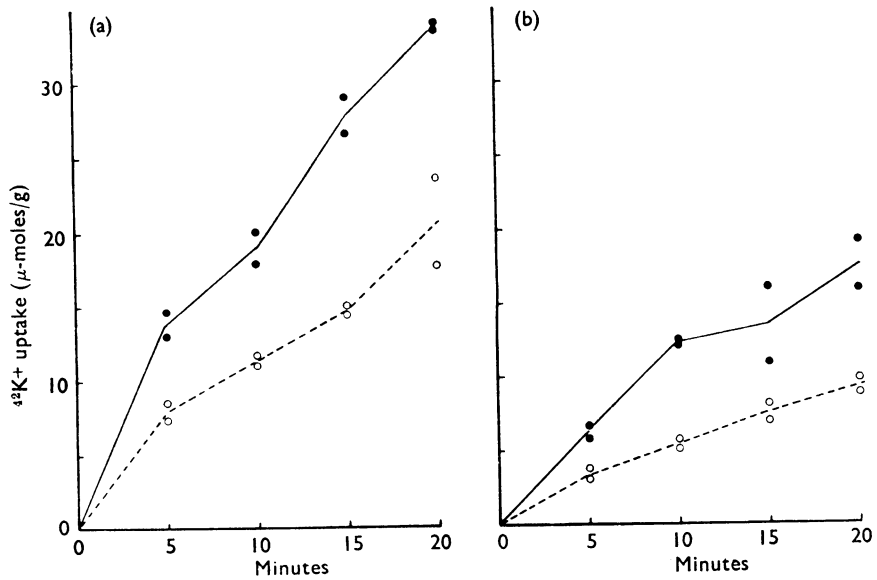


FIG. 6. $^{42}K^+$ uptake by the atria (○——○) and ventricles (●——●) of isolated hearts perfused with Krebs solution (a) or modified Krebs solution containing 1·2 mM $K^+$ (b).

inadequate perfusion of the atria. Thus the difference probably reflects a difference between the properties of atrial and ventricular muscle, and not simply a difference in vascularity.

The mean $K^+$ contents of the atria and ventricles which had been perfused with low $K^+$ fluid for 25–45 min were $53{\cdot}9 \pm 1{\cdot}5$ and $46{\cdot}5 \pm 1{\cdot}0$ $\mu$-moles/g respectively. These are lower, but not significantly ($P > 0{\cdot}1$) lower, than the corresponding values for atria and ventricles from hearts perfused with Krebs solution, $56{\cdot}0 \pm 2{\cdot}2$ and $50{\cdot}4 \pm 1{\cdot}8$ $\mu$-moles/g respectively. This shows that hearts perfused with fluid containing only 1·2 mM $K^+$ do not lose $K^+$ rapidly. These figures provide direct confirmation of the estimated $K^+$ balance of hearts perfused with low $K^+$ fluid (Fig. 1a) which were initially losing $K^+$ at a rate of about 0·04 $\mu$-mole/g per min. If this rate of loss of $K^+$ persisted for 2 hr, there would only be a 10% decrease in the $K^+$ content of the heart.

## Discussion

The results of these experiments show clearly that the action of adrenaline on the $K^+$ balance of the isolated heart is altered by the conditions under which it is investigated. The effects of adrenaline can be summarized as follows: in perfusion fluids containing 145 mM $Na^+$, 1·7 mM $Ca^{++}$ and 3–9 mM $K^+$, adrenaline caused the hearts to gain $K^+$; when the $K^+$ in the perfusion fluid was reduced to 2 mM, adrenaline had no appreciable effect on the $K^+$ balance of the hearts, and when the external $K^+$ was reduced still further, to 1·2 mM, adrenaline caused a loss of $K^+$ from the hearts. This loss of $K^+$ produced by adrenaline from hearts perfused with low $K^+$ fluids was blocked by dichloroisoprenaline, but not by phenoxybenzamine; isoprenaline also caused a loss of $K^+$ from hearts perfused with low $K^+$ fluid, and it was more potent than adrenaline. These results show that the $K^+$ releasing action of adrenaline is an action on $\beta$ receptors, as is also the action of adrenaline in producing increased $K^+$ uptake in isolated atria bathed in Krebs solution (Stafford, 1962). The action of adrenaline in promoting $K^+$ loss from hearts perfused with low $K^+$ fluids depended upon the $Na^+$ and $Ca^{++}$ concentrations; when the $Na^+$ or $Ca^{++}$ concentration was also reduced, adrenaline caused the hearts to gain $K^+$.

These findings are difficult to explain on the basis of previously published results on the effects of adrenaline on $Na^+$ or $K^+$ fluxes (Glitsch, Haas & Trautwein, 1965) or on the electrical properties of cardiac muscle (Dudel & Trautwein, 1955; Webb & Hollander, 1956; Vaughan Williams, 1958; Furchgott, Sleator & de Gubareff, 1960; Fozzard & Sleator, 1967). However, all these reports are concerned with atrial muscle, and it is clearly unwise to extrapolate from one kind of cardiac tissue to another. Because the rate of turnover of $K^+$ is faster in the ventricles than in the atria, and because of the relative amounts of tissue involved, it would seem reasonable to assume that most of the net changes of $K^+$ measured in the present series of experiments reflect the action of adrenaline on ventricular muscle. There is a lack of relevant information on the extent to which $Na^+$ and $K^+$ fluxes are linked in ventricular muscle, on the proportion of active to passive transport of $Na^+$ or $K^+$, and on the effects of changes in the ionic composition or the temperature of the perfusion fluid on these processes (see Langer, 1968). Thus, the difficulty in analysing the mechanism of action of adrenaline is due to lack of knowledge about the fluxes affected, and about whether the action of adrenaline on $K^+$ fluxes is direct, or the consequence of an alteration in $Na^+$ transport or in myocardial metabolism.

The only clue to a possible explanation of these results arises from consideration of the effects of changes in temperature on the actions of adrenaline. It is not unreasonable to suppose that the temperature-dependent effect of adrenaline—that is, the loss of $K^+$—is exerted on that component of $K^+$ fluxes which is linked with active transport. The gain of $K^+$, which was less temperature-dependent, might then be an effect of adrenaline on $K^+$ transport that is not energy-dependent. In a previous paper (Stafford, 1962) describing the actions of adrenaline on $K^+$ fluxes in isolated atria, no evidence linking the gain in $K^+$ with a loss in $Na^+$ emerged. Therefore, stimulation of $Na^+$ extrusion was not proposed as a likely explanation of the results. The present result, that the gain of $K^+$ by the heart produced by adrenaline was independent of temperature over the range 25°–30° C, again suggests that it is an effect on passive rather than active transport. However, a net gain in $K^+$ cannot easily be explained solely by an increase in $K^+$ permeability.

A decrease in the external $K^+$ concentration has been shown to decrease $K^+$ permeability in ventricular muscle (Langer & Brady, 1966). Other studies with rabbit isolated hearts have shown that the $Q_{15}$ for the initial rate of exchange with $^{42}K^+$ increased from 1·3 to 1·9 when the external $K^+$ concentration was decreased from 6 to 2·7 mM (Humphrey & Johnson, 1960). These observations suggest that decreasing the external $K^+$ concentration increases the proportion of active to passive transport of $K^+$ in ventricular muscle. Thus it seems that the action of adrenaline in causing a loss of $K^+$ in hearts perfused with a low $K^+$ fluid is more likely to be consequent upon an alteration of the active transport of $K^+$. This is supported by the fact that a decrease in temperature decreased the loss of $K^+$ produced by adrenaline under these conditions. If the activity of the $Na^+$ pump were reduced by adrenaline, with a consequent decrease in $Na^+$ extrusion this might explain the net loss of $K^+$, which would then be due to decreased rate of $K^+$ entry. However, as yet there is no evidence to support this suggestion, nor even to allow the assumption that the temperature dependence of this action of adrenaline points to an action on linked $Na^+/K^+$ exchange. Whether the $K^+$ loss produced by adrenaline in low $K^+$ fluids was due to a decreased influx of $K^+$ or to an increased efflux has not yet been investigated. This is because of the difficulty of maintaining a constant rate of beating in isolated hearts perfused with low $K^+$ solutions, in which conduction time is known to be depressed (de Carvalho & Langan, 1963); anomalous results are obtained if the effect of adrenaline on $K^+$ fluxes is measured without controlling the rate of beating (Waddell, 1961; Stafford, 1962).

Irrespective of how adrenaline is producing a net loss of $K^+$ from the hearts, its effect on $K^+$ fluxes must be quite substantial. The rate of turnover of $^{42}K^+$ in hearts perfused with low $K^+$ fluid was calculated from $^{42}K^+$ entry during the first 5 min (Fig. 6b), and was about 0·7 $\mu$-mole/g per min for the atria, and 1·6 $\mu$-mole/g per min for the ventricles. Since adrenaline produced a net loss of $K^+$ from the hearts of over 0·7 $\mu$-mole/g per min (Fig. 1d), its effect on fluxes must be of the order of a 50% change.

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