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DIGOXIN, OUABAIN AND POTASSIUM MOVEMENTS IN RABBIT AURICLES

BY

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Movements of potassium have been observed in isolated right and left auricles of rabbits by means of radioactive tracer. The tissues have been immersed in a modified Krebs saline at 37°, and in these conditions showed only small changes in ionic content over periods up to 6 hr. The extracellular volume, determined with inulin and with ^{24}Na, was large (44.1 ml./100 g. tissue), and accounted for about 3.8% of the tissue potassium. Left auricles exchanged without gross inhomogeneity: the rate of exchange was about 1.5% of the total tissue potassium/min., though it was probably higher in the first half-hour or hour after preparation. In right auricles the exchange was less homogeneous and included a faster component than that observed in left auricles. Digoxin and ouabain reduced the influx and had no appreciable effect on the efflux of potassium, so that the auricles lost potassium. The threshold concentrations which produced effects within 20 min. were of the order of 10^{-6} for ouabain and somewhat higher for digoxin. Irregularities or failure of contraction occurred when the tissue potassium was reduced by about 15%. Loss of potassium was accompanied by gain of sodium, but the tissue appeared unsuitable for making estimations of the rate of sodium movement.

Hearts are generally less sensitive to cardiac glycosides when they are in a medium rich in potassium (Clark, 1912; Baker, 1947; Friedman and Bine, 1947; Lown, Salzberg, Enselberg and Weston, 1951; Hazard, Hazard, and Thouvenot, 1956), and these drugs, at least in toxic doses, cause loss of potassium from the heart muscle (Calhoun and Harrison, 1931; Wood and Moe, 1938; Wedd, 1939; Friedman and Bine, 1947; Hajdu, 1953; Regan, Talmers, and Hellems, 1956), and diminish the rate of exchange of potassium (Conn, 1956). It is therefore likely that the potassium influx is diminished, as in erythrocytes (Schatzmann, 1953) and other tissues, but it is less obvious whether the efflux is modified. These fluxes have therefore been estimated in rabbit auricles, and the effects of digoxin and ouabain on them have been observed.

METHODS

Saline.—Unless otherwise stated, the salt solution used contained Na^+ 146 mM, K^+ 5 mM, Ca^{++} 1.7 mM, Mg^{++} 1.2 mM, Cl^- 124 mM, HCO_3^- 25 mM, SO_4^{--} 1.2 mM, $H_2PO_4^-$ 1.2 mM, dextrose 0.2% (w/v) and was equilibrated during use with 95% O_2+5% CO_2. When appropriate, part of the Na or K was replaced by ^{24}Na or ^{42}K. The radioactive salts were prepared from spectroscopically pure Na_2CO_3 and K_2CO_3 which had been exposed to neutron bombardment at A.E.R.E., Harwell, and was converted to the chlorides by addition of N HCl and sufficient water to make neutral 154 mM solutions.

Auricles.—Young rabbits, mostly weighing 1.0 to 1.5 kg., were concussed and bled from the carotid arteries. The heart was removed from the chest and rinsed in saline, and the auricles were freed by dissection partly in air and partly in saline at room temperature. In some experiments they were used free and in others they were attached by nylon threads to a frame so that most of the auricle was stretched as a flat sheet: surplus tissue was then cut away. In most experiments left and right auricles were studied separately; the right generally beat spontaneously, while the left did not beat, or did so at infrequent intervals. A few experiments were performed with pairs of auricles suspended so as to beat isotonically, when the contractions were recorded on smoked paper.

Measurement of ^{42}K Influx and Changes in Total K and Na Content.—Free or stretched auricles were immersed in tubes containing 15 ml. radioactive saline medium in a water bath at 37°. After various periods, the auricles were removed, rinsed with 0.15 M choline chloride to remove saline adhering to the outside of the tissue, weighed, dried for 2 hr. or overnight at 105° and weighed again. The dry tissue was ashed with 0.5 ml. conc. HNO_3 for 1 to 2 hr., followed by a further hour after dilution with 5 ml. H_2O. The solution was made up to 25 or 50 ml. for estimation

of potassium and sodium by means of a flame photometer and ^{42}K by means of a liquid counting tube and conventional scaler and power supplies. Calculation of the rate of influx from these results is described in connexion with the results. Alternatively, an estimate of the rate of influx was obtained at the same time as one of the efflux as described below.

Measurement of ^{42}K Efflux.—Stretched auricles were immersed in radioactive saline medium for various periods and then transferred to an apparatus similar to that described by Creese (1954) in which the tissue was perfused with oxygenated inactive saline at 37° while it lay over a shielded Geiger tube separated by a window of Perspex (polymethylmethacrylate resin) approximately 0.15 mm. thick. The decline in radioactivity was followed by counting from time to time as required. At the end of the experiment the auricle was treated as described above and its specific activity was compared with that of the saline in which soaking-in of tracer had occurred. The sensitivity of the efflux counter was determined from the final count of the efflux bath, the ratio of specific activities of auricle and saline when counted in a liquid counter, and the potassium content of the auricle, so that the total potassium entry during the soak-in period could be determined.

As there was some uncertainty about the adequacy of counts made in this way on beating tissue, efflux was sometimes followed by placing the auricles in a bath containing 50 ml. of inactive medium and replacing the medium after 1, 2, 5, 10 min. and then every 5 or 10 min. for 1 to 4 hr. The radioactivity of each sample of medium was then determined with a liquid counter.

Estimations of Radioactivity.—All estimates were corrected for background, resolving time of the counting equipment used, and decay, the half-time for ^{42}K being taken as 12.4 hr. and for ^{24}Na as 15.1 hr. The activity of standard solutions was checked and found to be consistent with these values for at least six half-lives; all experiments were completed within this time.

Inulin Space.—The inulin spaces were estimated by immersing auricles in saline containing 1.0% inulin (w/v) for 60 min., blotting lightly, rinsing with an isotonic solution and blotting again before transfer to an inulin-free saline for a second hour. The inulin was estimated in both solutions by the method of Bacon and Bell (1948), and the sodium, potassium, and water content of the auricles was determined as usual. Estimations were made on free and stretched left and right auricles and on isotonically contracting pairs of auricles.

Cell Dimensions.—Auricles were cooled or were poisoned with ouabain until they ceased to beat and were then fixed in saline containing 10% (v/v) of neutralized formalin, and embedded in polyethyleneglycol-1000 monostearate (Miles and Linder, 1952). Sections were stained with haematoxylin and van Gieson, magnified ×1,000 and projected on a screen so that the diameter of the cells could be measured.

Drugs.—Pure crystalline ouabain and digoxin were used as supplied (Burroughs Wellcome) in concentrations (w/v) stated in the description of results. Digoxin was dissolved in 70% ethanol before addition to the saline, so that the final solutions contained up to 0.5% ethanol. Control observations were made when appropriate with saline containing the same concentrations of ethanol.

Results

Cell Dimensions.—Three divided pairs of auricles were used. One pair was cooled to stop beating and fixed as soon as it had been dissected. A second pair was treated similarly after 3 hr. immersion in the usual oxygenated saline; and the third pair was immersed for 50 min. in the same medium, to which ouabain (10^{-6}) had been added.

Sections of the six auricles were examined independently by two persons, each of whom measured the maximum width or diameter of 30 cells in each slide. The sections showed cells cut at various angles; in view of the arrangement of fibres in the myocardium, this was probably unavoidable. Where cells were cut predominantly longitudinally, so that opposite sides of the cell were approximately parallel, the distance between the walls at the widest point was taken as the cell diameter; with cells cut predominantly transversely, so that the cells appear oval or round on cross section, the minimum diameter was used. A number of areas were used on each slide, and the measurements were made on the best defined cells in each field. No means was devised for obtaining unbiased samples, and no correction was applied for possible shrinking during preparation. The mean diameter of 360 cells was 7.0 μ: the mean for left auricles was 6.2 μ and for right auricles 7.7 μ, each with a standard deviation of ± 2.7 μ. Analysis of variance showed the difference between left and right sides to be significant, but did not indicate appreciable significance in the effects of immersion or ouabain on the cell size. It was assumed that the cells were sufficiently nearly circular on cross section for the diameter so estimated to provide an adequate estimate both of circumference and of cross sectional area, and the ratio of these measurements, using 7.0 μ as diameter, was taken as the ratio of surface area to volume.

Composition of Auricles.—Observations on the sodium, potassium and water content of auricles immersed without stretching for various periods of time are shown in Table I. Right auricles were generally smaller and consistently contained less potassium, even with allowance for the difference in weight. There were no significant changes in

TABLE I
MEAN COMPOSITION OF UNSTRETCHED RABBIT AURICLES IN EXPERIMENTAL CONDITIONS

Condition	No. of Auricles L	R	Wet Wt. (mg.) L	R	Dry Wt. (mg.) L	R	H_2O (g./kg. Dry Wt.) L	R	K^+ (mEq./kg. Dry Wt.) L	R
Dissected and rinsed ..	3	4	135·5	130·0	24·7	23·0	4·48	4·65	374	342
Immersed for 5 to 30 min. ..	7	6	127·8	96·1	20·2	16·9	5·33	4·69	350	325
,, ,, 120 ,, ..	6	6	135·3	95·7	24·4	18·2	4·61	4·28	332	308
,, ,, 360 ,, ..	3	3	142·9	123·7	26·6	23·4	4·37	4·29	299	290

the mean sodium and water content of the tissues with time, but there was a small, significant ($P<0.02$ left, <0.1 right) diminution in the potassium content on either side, at a rate of about 2% of the total K/hr. No loss of potassium occurred in a few experiments in which the concentration of potassium in the medium was 7.5 instead of 5 mM. The inulin spaces were surprisingly large, 2.40 ± 0.085 (S.E.) ml./g. dry weight of tissue, or 44.1 ml./100 g. wet weight. This high value was supported by observations, described later, on the efflux of ^{24}Na after a period of immersion in saline containing this tracer. Estimations of the intracellular ionic concentration by deducting the amounts expected to be present in the extracellular fluid are therefore rather unreliable. In the case of sodium, the mean deduction is about 85% of the total in the tissue, and the estimate so obtained is practically worthless: with potassium the deduction is much smaller (about 3.8%) and such variation as may occur in the extracellular volume is comparatively unimportant.

Stretching the auricles had no detectable influence on the composition of tissues which had been immersed for an hour or more. Observations made less than an hour after immersion gave unusually low values for potassium, but these observations were not comparable with those on unstretched tissues because the dissection and setting up of the preparation, which was done at room temperature, took longer (10 to 20 min. instead of 3 to 5 min. from the time of killing the animal to immersion) and the initial loss of potassium may have been due to cooling and manipulating rather than to an effect of stretch.

^{24}Na Efflux.—The large volume of the inulin space suggested that observations on the movement of ^{24}Na would give information mainly about the extracellular sodium. Some experiments were therefore done in which beating auricles were immersed for varying periods in saline medium containing ^{24}Na and were then transferred to an inactive medium, which was changed frequently and collected for counting after contact with the auricles. By adding the amounts of tracer lost in each portion of fluid to the amount remaining in the auricle at the end of the experiment, graphs were constructed to show the count of the tissue at different times (Fig. 1). These counts, plotted on semi-logarithmic paper, lay on a line which was considerably curved for at least 25 min. The shape of the curve was similar whether influx had proceeded for 5, 30 or 60 min. The estimate of the total tracer in the tissue at the beginning of the efflux was higher than would have occurred if the auricle Na had reached the specific activity of the medium; there was no evidence that the tissue Na had varied during the experiment, and the excess was probably due to small amounts of the highly active influx medium being carried over on the surface of the auricle and not removed because rinsing and blotting were less thorough at this rapid transfer than at the end of the experiment. It is therefore not directly shown how completely the tissue Na had exchanged at the beginning of the experiment, but the difference in position of the efflux curves after 5 or 60 min.

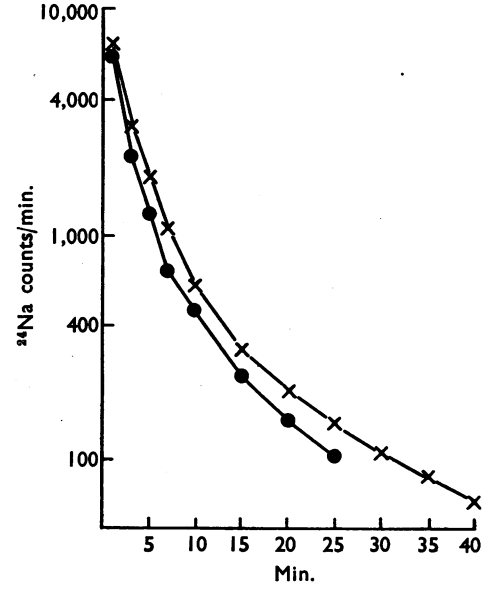

FIG. 1.—The loss of ^{24}Na from two pairs of rabbit auricles, plotted semilogarithmically. ●, 5 min. soak-in. X, 30 min. soak-in.

influx suggests that only a small additional amount of the total tissue Na exchanged after the first 5 min. The continued flattening of both curves makes it difficult to distinguish clearly between the exchange of intracellular and extracellular sodium, but retropolation of the later points suggests that the slowly exchanging, presumably intracellular fraction, accounts for less than 7 to 8% of the total tissue Na. This agrees with the results of inulin determinations and supports the large estimate of the extracellular space.

^{42}K Influx, Normal Auricles.—Measurements of influx were obtained by immersing auricles, usually unstretched in active saline, removing them at various times, and estimating their activity and potassium content after ashing. In some experiments, the auricles were immersed in active saline immediately after dissection. The results of six such experiments with 56 auricles are shown in Fig. 2. Left and right auricles behaved differently. In both, the uptake of ^{42}K did not follow the exponential course which would arise if the tissue potassium was uniformly exchangeable at a constant rate. The departure from an exponential course was greater in right auricles, which were generally beating spontaneously, than in left auricles: but in both the early part of the uptake was much more rapid than the late part. The rapid phase was too large to be due solely to the extracellular potassium, which accounts for less than 4% of the total in the tissue. Part of the apparent decline in rate is a consequence of averaging the uptake of a number of different auricles, which varied somewhat in size and probably in individual flux rates: the mean uptakes at different times from a population of single exponential uptake curves do not necessarily lie on a single exponential curve. However, there was also a real decline in the rate of uptake as the time of immersion increased, as was seen when auricles were immersed in inactive saline for 90 min. after dissection and then transferred to the active medium (Table II). Right auricles showed a much larger departure from a single exponential uptake, and a greater decrease in rate with time. This difference was not simply a consequence of beating, as the following experiment showed. Undivided pairs of auricles were immersed in active saline for various periods and were removed and then divided for drying, ashing and estimation. The pairs of auricles were observed to beat, but the uptake of radioactivity of the left auricles showed little difference from that of left auricles separated before immersion in the active medium (Table III). There was more difference between left and right auricles when they were separate than when they were undivided; division appeared both to accelerate the exchange in right auricles and to retard it in left auricles. None of the differences were large, and it is at least clear that the differences between left and right auricles did not

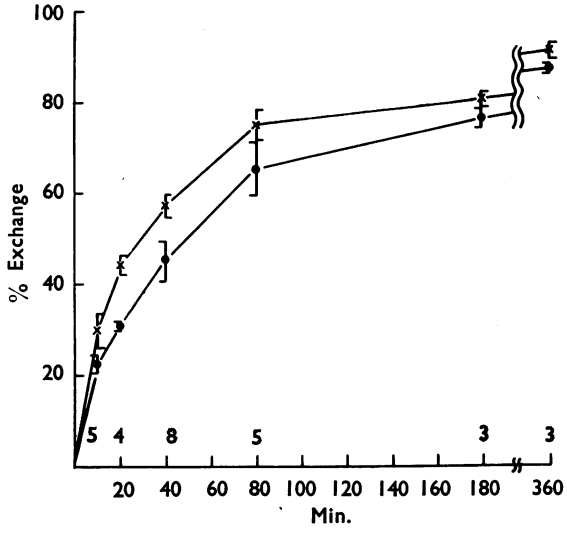

FIG. 2.—The uptake of ^{42}K by rabbit auricles. X, Right auricles. ●, Left auricles. The range marks show the standard errors of the mean. The figures above the abscissa show the number of observations on which each pair of points is based. The ordinate shows the specific activity of the auricles as % of that of the medium.

TABLE II

UPTAKE OF ^{42}K BY LEFT AND RIGHT AURICLES IMMERSED IN RADIOACTIVE SALINE IMMEDIATELY OR 90 MIN. AFTER DISSECTION

Left/right differences in exchange are highly significant ($t=4{\cdot}31$, $P<0{\cdot}01$).

Delay min.	Side	No. of Obs.	Dry Wt. (mg.) Mean±S.E.	K Content (mEq./kg. Dry Wt.) Mean±S.E.	K Exchanged After 20 min. (%) Mean±S.E.	Significance of Difference of Exchanges	
						t	P
0	Left	4	18·0±1·3	360±5	30·3±0·6	1·26	>0·3
90	,,	4	17·1±2·3	365±11	27·6±2·0		
0	Right	4	16·0±1·1	358±9	42·2±2·0	4·00	<0·01
90	,,	4	16·7±1·9	362±14	33·2±1·1		

TABLE III

UPTAKE OF ^{42}K BY LEFT AND RIGHT AURICLES IMMERSED IN RADIOACTIVE SALINE BEFORE OR AFTER DIVISION

Side	Condition During Immersion	No. of Obs.	Dry Weight (mg.) Mean±S.E.	K Content (mEq./kg. Dry Wt.) Mean±S.E.	% K Exchanged at End of Immersion Mean±S.E.	Significance of Difference	
						t	*P*
20 min. Immersion							
Left	Undivided	4	24·3±3·6	371±7	33·0±2·0	1·29	≃0·25
	Separate	4	18·0±1·3	360±5	30·3±0·6		
Right	Undivided	4	18·9±2·4	358±12	36·7±1·0	2·50	≃0·05
	Separate	4	16·0±1·1	358±9	42·2±2·0		
40 min. Immersion							
Left	Undivided	4	26·7±1·9	376±10	44·0±2·8	0·50	≃0·63
	Separate	4	21·9±2·1	342±7	42·0±2·9		
Right	Undivided	4	21·6±2·4	319±35	50·5±1·9	0·75	≃0·48
	Separate	3	15·8±0·7	281±22	54·2±4·6		

depend only on the inactivity of the separated left tissues. The longest period over which uptake was followed was 6 hr., by which time both left and right auricles had exchanged about 90% of their potassium (Fig. 2). In some experiments in a medium containing 7.5 mM K instead of 5.0 mM K, the exchange at this time was virtually complete (98.6%, mean of six observations: range 94.6 to 102.1%).

^{42}K Efflux, Normal Auricles.—The efflux of ^{42}K after a period of immersion in active saline was measured by repeated direct counting of the same auricle immersed in inactive saline, or by allowing efflux to occur in a bath of moderate volume, the contents of which was changed frequently and counted after removal from the tissue. With direct counting of the auricles, errors may arise from the change in geometry which occurs at each contraction. These errors were minimized by gently stretching the tissue on a frame and allowing contraction to occur isometrically; and such errors were minimal with the usually inactive, left, auricles. Anomalous results arise if pairs of auricles are used and cover an area substantially larger than the window of the counter, presumably because of the different rates of exchange of the two halves: and experiments of this sort have been disregarded. When efflux occurs in a bath whose contents is changed intermittently, some error results because the specific activity of the medium is not kept at zero; but with frequent changes, and a large bath as used here, this effect is trivial. Experiments have been done in both ways, and the reasonable agreement between the results suggests that neither source of error is important.

In some experiments with direct counting, the auricles have been transferred from the soak-in bath to the counting bath from time to time during the period of uptake, and their radioactivity and its initial rate of decline observed over three counts within 4 min. at each time. This procedure is inaccurate because it is difficult to ensure that the auricle is brought to exactly the same position in relation to the counting window each time, and small variations in position can have a large influence on the counting rate. However, the procedure gives a rough check on the rate of uptake of ^{42}K and the lability of what has been taken up. It is preferable to allowing the influx to occur in the same bath as the efflux, as it is difficult to be certain that the highly active soak-in medium is eliminated from the bath before counting during efflux.

The efflux curves obtained in these ways (Figs. 3 to 5) showed differences between right and left auricles like those which occurred in the influx. The left auricles exchanged fairly and uniformly, though with a moderate decline in the rate after the first hour. In the right auricles, a semi-logarithmic plot was more curved, and not readily divisible into separate fractions. The initial rate was always faster than that observed in left auricles, and the final rate after 3 to 4 hr. was slower (Fig. 3).

Calculation of Potassium Fluxes in Normal Auricles.—These results make possible approximate calculations of the potassium fluxes of the auricles. In left auricles it seems reasonable to assume that there is no serious inhomogeneity in the cells, and that the movements decline somewhat during immersion. Most of the decline in rate probably occurs in the first half-hour; and it has not much influence in interpreting efflux experiments in which the measurements of radioactivity are made after the first 90 min. The basis of the calculations has been given by Keynes and Lewis (1951) and Keynes (1954). The rate of

influx is given by the product of initial rate of entry of tracer to the tissue and the sensitivity of the apparatus. The initial rate of entry of tracer can be obtained from the count of the tissue, Y, at time T (for example at the end of a period of influx and beginning of efflux) by the expression

$$\left(\frac{dY}{dt}\right)_{t=0} = \frac{Y}{T}\left(\frac{kT}{1-e^{-kT}}\right) \qquad (1)$$

where k is the rate constant and is given by the observations on the efflux. The sensitivity of the counting apparatus was obtained by ashing the auricle at the end of the experiment and comparing its specific activity with that of the active saline used for influx when each was counted in a liquid counter. The final count in the efflux apparatus then corresponded to the quantity of potassium found to be exchanged at this time, and the uptake at other times could be obtained proportionally. Thus in the experiment shown in Fig. 5 at $T=80$ min. $Y=9140$ counts/min. and $k=-0.0115$ min.$^{-1}$, giving the factor 1.53 for the conversion of Y/T to $(dY/dt)_{t=0}$. This factor is rather large, but it seems preferable not to use counts earlier in influx because of the uncertainty whether the auricle was located in exactly the same position on each transfer to the counting bath. The counting sensitivity was 4.73×10^{-4} micromoles/count, so the average entry to the whole tissue was 0.0955 micromoles/min., corresponding to a flux of 4.43 p.mole. cm.$^{-2}$ sec.$^{-1}$

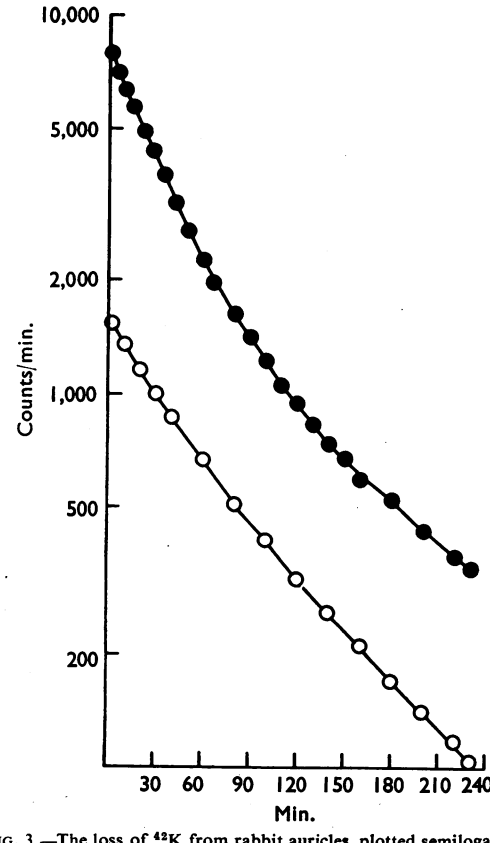

FIG. 3.—The loss of ^{42}K from rabbit auricles, plotted semilogarithmically. ●, Right auricle. O, Left auricle.

Efflux can be calculated from the expression

$$m_0 = k\,\frac{V}{A}\,C_i \qquad (2)$$

(Keynes and Lewis, 1951), where V is the volume of the tissue cells, C_i the concentration of potassium in them, and A the surface area of the cells. For the whole tissue the outward movement is $k.V.C_i$ which in this experiment gives the value 0.0962 micromoles/min. These figures require correction for diffusion, as discussed below, and they involve such approximations so that the net loss of potassium as estimated by the difference between influx and efflux is very unreliable: however, its value 0.0007 micromoles/min. or about 0.5%/hr. is as near to the average loss of 2%/hr. observed throughout these experiments as can be expected.

The correction for delays due to diffusion has been discussed by Harris and Burn (1949), Keynes (1954) and Creese (1954). All these authors have found apparent diffusion coefficients of potassium in the extracellular spaces to be about one-quarter of those for the ion in free solution, namely of the order of 5×10^{-6} cm.$^{-2}$ sec.$^{-1}$ This value may be used for D' in the expressions of Keynes (1954).

$$\frac{M'}{M} = \frac{\lambda}{b}\tanh\frac{b}{\lambda} \qquad (3)$$

and

$$\lambda^2 = \frac{\epsilon}{1-\epsilon}\,\frac{D'V.C_0}{M.A} \qquad (4)$$

where ε is the fraction of the volume of tissue occupied by extracellular fluid, C_0 the concentration of ions in the extracellular fluid (taken as equal to that in the medium), M is the true flux and M' the apparent one, and b is half the thickness of the tissue. In view of the uncertainties attaching to a number of these estimates, it is hardly worth calculating the correction afresh for every separate auricle, and á value of 1.3 has been used for M'/M as representing the median of a number of observations on auricles of approximately average size. The corrected fluxes then

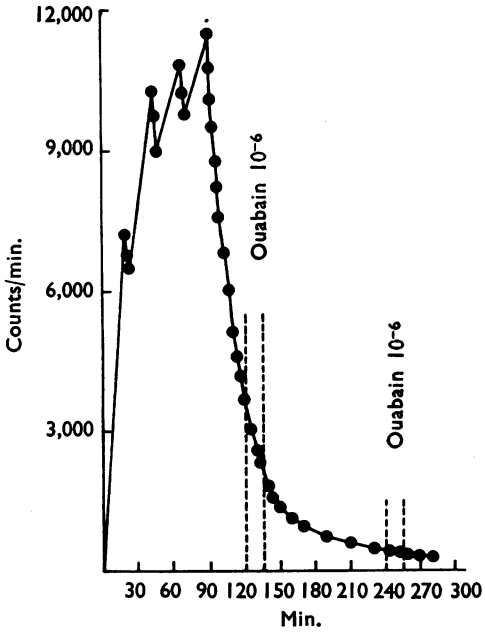

FIG. 4.—The uptake and loss of ^{42}K in a right auricle. Ouabain (10^{-6}) was included in the perfusion medium for two periods of 15 min. during the efflux, at the times shown by the dotted lines.

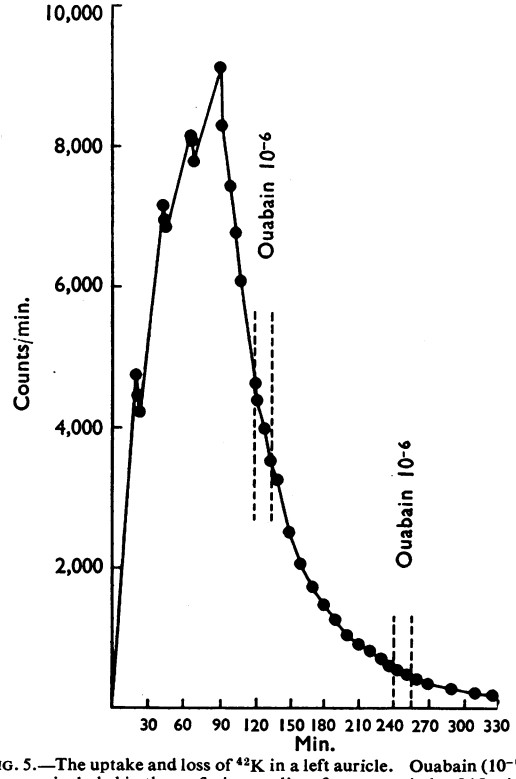

FIG. 5.—The uptake and loss of ^{42}K in a left auricle. Ouabain (10^{-6}) was included in the perfusion medium for two periods of 15 min. during the efflux, at the times shown by the dotted lines.

become 5.75 and 5.81/mole cm.$^{-2}$ sec.$^{-1}$ respectively.

Potassium Fluxes in Normal Right Auricles.—The application of similar arguments to the results with right auricles is hampered by the evident inhomogeneity of the potassium exchange and the presumable fluctuations in the influx and efflux with each contraction. It is, at first sight, unlikely that beating alone accounts for the non-exponential uptake and output of ^{42}K, and the similarity of exchange in left auricles whether they are driven by remaining connected to right ones or are independent and inactive (Table III) confirms this point. It seems more likely that some part of the tissue has a more rapid turnover than the rest: the pacemaker might so function, as it is known to show electrical activity independently of the rest of the auricle (Marshall and Vaughan Williams, 1956). Whether there are two distinct fractions or a gradation of cells exchanging at different rates is not evident from the results: the progressive curvature of the semi-logarithmic plot may indicate such a gradation (Creese, Neil, and Stephenson, 1956), but it could also result if there were two distinct fractions of which the exchange rates were gradually decreasing during the time in which the preparation was studied. In the circumstances it appears undesirable to attempt a more quantitative interpretation of the results.

Potassium Movements in the First Hour After Immersion.—It is also possible to estimate the influx of potassium in the early part of the time after immersion in saline from observations such as those shown in Fig. 2, by using expression (1) above. Appreciable errors arise if the total exchange of the tissue is less than about a quarter, because the extracellular fluid, which must exchange more rapidly than the bulk of the tissue, then makes an appreciable contribution to the total. Also, in the initial period the rate of efflux is uncertain and so there is some doubt about the appropriate value for k. It is possible that the rate of efflux declined rapidly *pari passu* with the influx: it is also possible that the rate of efflux did not change, and that the initial rapid influx involved a net gain of potassium by the tissue, perhaps recovering losses which occurred during dissection. There was too much variation in the estimated potassium content of individual auricles to distinguish these possibilities, and it seemed reasonable to adopt the simpler assumption that the

TABLE IV
ESTIMATED INFLUX OF POTASSIUM IN RABBIT AURICLES

Treatment	Period of Immersion Before Measuring Uptake (min.)	Duration of Measurement (min.)	Mean Influx During Period (mEq./kg. Dry Wt. of Tissue/min.)	
			Left	Right
Control	0	10	8·3	12·1
Ouabain 10^{-6} ..	0	10	7·7	10·5
,, 4×10^{-6}	0	10	5·7	6·7
,, 10^{-5} ..	0	10	5·1	5·5
Digoxin 10^{-5} ..	0	10	6·1	7·1
Control	20	20	6·3	11·0
Ouabain 10^{-7} ..	20	40	4·4	6·8
,, 10^{-6} ..	20	40	3·3	3·3
,, 10^{-5} ..	20	20	2·2	1·4
Control	90	20	5·0	8·4
Ouabain 10^{-5} ..	90	20	1·6	1·5

efflux was constant at a rate of -0.015 min.$^{-1}$ for left and -0.035 min.$^{-1}$ for right auricles. Even if the true rates were twice this magnitude it would increase the inward movements estimated on the influx for 20 min. by only 10 to 20%, so the error is unlikely greatly to exaggerate those which arise from variation between individual tissues. Some values obtained in this way are shown in Table IV, for auricles immersed in active saline for varying periods either immediately or 20 or 90 min. after dissection. The correction for diffusion varies somewhat for different auricles, because they varied somewhat in size, but in view of the other approximations a uniform correction $\times 1.3$ has been applied as before to all the estimations for this effect.

Effects of Digoxin and Ouabain

Gross Effects.—When digoxin or ouabain was added to the medium in sufficient concentration, right auricles or pairs of auricles initially beat more forcibly and more rapidly. Irregularities both of rate and magnitude appeared, transiently or persistently, and after a time the auricles ceased to beat. Sporadic contractions or periods of contraction sometimes occurred later, especially with digoxin.

Once irregularities had begun, the size of the beat appeared to depend mainly on the previous activity of the tissue, being largest after a quiescent spell: that is, there was a negative staircase effect. The highest concentrations of drug acted more rapidly: as a measure of the effect, the time from exposure to the drug till there was no visible contraction for more than 30 sec. was measured in a number of pairs of auricles (Table V). The results shown there are consistent with a large number of other experiments in which the tissues were not observed continuously and the time to arrest was noted only approximately. The electrical activity of the auricles as a rule showed no augmentation except in frequency: the spikes became lower with little change in the steepness of the rising phase, but considerable flattening of the descending part. Later the form of individual pulses became increasingly irregular. Detectable electrical activity persisted after visible mechanical activity had ceased.

TABLE V
TIME OF ARREST OF BEAT AFTER EXPOSURE TO DIGOXIN AND OUABAIN

Drug	Concentration	Duration of Exposure Causing Arrest (min.)
Digoxin	10^{-6}	>150
	10^{-5}	16, 28, 30, 45
Ouabain	10^{-7}	>150
	10^{-6}	32, 40, 45, 45, 48
	10^{-5}	4,10

Net Changes in Composition.—Loss of potassium occurred consistently from auricles immersed in media containing digoxin or ouabain (Fig. 6).

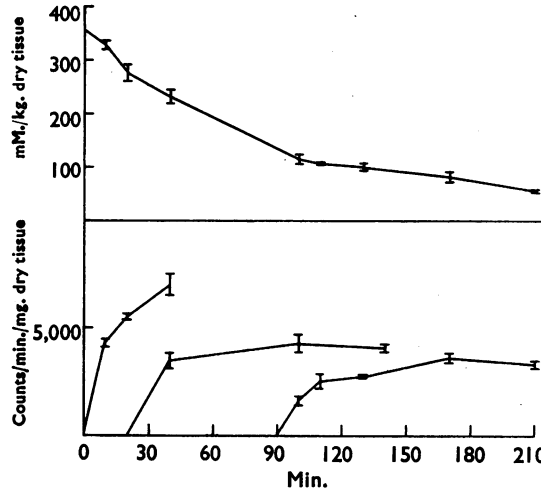

FIG. 6.—The K loss (upper graph) and ^{42}K uptake (lower graph) in single auricles in a medium containing ouabain (10^{-5}). All values are the means for groups of 3 to 6 auricles: results of left and right auricles have been combined. The range marks show the standard error of the mean. Some auricles were immersed in inactive, ouabain-containing saline for 20 or 90 min. before transfer to an active medium.

Right auricles were sensitive to lower concentrations and in a given concentration lost more rapidly than left auricles. On both sides, the rate of loss of potassium was greater in higher concentrations of ouabain or digoxin. In all the conditions examined, the mean amount of potassium in the right auricle had fallen to about 280 mEq./kg. dry weight at the time when beating ceased, as defined above. There were no significant changes

in the sodium and water content of the auricles except in 10^{-5} ouabain in which there was a considerable gain of sodium. However, it must be remembered that the large and moderately variable extracellular volume makes the total tissue sodium a poor indication of the quantity present in the cells, and the observations do not exclude an increase of intracellular sodium comparable in magnitude to the loss of potassium in 10^{-6} ouabain and 10^{-5} digoxin.

^{42}K Influx.—The loss of potassium was accompanied by a reduced entry of ^{42}K to the auricles (Fig. 6). Ouabain was again more active than digoxin in the same concentration, and right auricles were more sensitive than left. Thus, the rapid component of the uptake of right auricles was practically abolished by 10^{-5} digoxin, which had only a small and delayed effect on the left, and 10^{-6} ouabain reduced the uptake on the right without significant effect on the left. These effects were observed when the auricles were exposed to the cardiac glycoside and ^{42}K simultaneously, and since some time is necessary for the drug to reach and act on the cells which it affects, they are not maximal effects. The rate of entry of ^{42}K was further reduced in auricles which had been in saline containing 10^{-5} ouabain for 20 min. before transfer to an active saline with the same concentration of ouabain (Fig. 6), and with 90 min. initial exposure the rate of entry was still lower. The auricles by this time had lost three-quarters of their total potassium, had gained much sodium, and had been inactive probably for over 80 min., so many factors may have affected the result. In these conditions the potassium remaining in the auricles was still completely exchangeable, and somewhat more rapidly (within 160 min.) than in normal conditions.

^{42}K Efflux.—The simplest observations were made by applying media containing ouabain or digoxin for periods of 15 min. to auricles while ^{42}K efflux was being followed, and then reverting to a normal perfusion medium. In these conditions there was no detectable effect from ouabain (10^{-6}) either on the rapid or the slow phases of efflux from right auricles (Fig. 4), although gross mechanical and electrical changes occurred during each exposure and were reversed on restoring a normal medium.

Somewhat different results, which at first suggested a decreased rate of efflux, were obtained in experiments in which pairs of auricles were immersed in active saline containing 10^{-5} digoxin for 30 min. and then allowed to lose their ^{42}K in an inactive medium, still containing digoxin. Usually, two such sequences were performed with the same pair of auricles, once with and once without digoxin, and sometimes the digoxin was used in the first cycle and sometimes in the second. These experiments were performed in conditions commonly used for studying this tissue, namely contracting isotonically in a conventional organ bath at 28°, and loss of radioactivity was followed by removing the medium for counting and replacing it every 5 or 10 min. The results gave an average value for the loss from the whole tissue. After the first half hour, the rate of loss of ^{42}K declined steadily, with a half time of 50 min. for normal auricles and 64 min. for those in 10^{-5} digoxin. In the light of the observations on influx in left and right auricles separately, it is probable that in the presence of digoxin less ^{42}K had entered the rapidly exchanging fraction of the right auricle and the apparently slower rate of efflux was due to the absence of a rapid component and not to a general reduction in the rate of efflux.

Calculation of the Rates of Movement in the Presence of Ouabain.—The rates of outward movement of potassium in the presence of ouabain can be calculated either by assuming that there is no change in efflux at any time and using the observed efflux rate and the observed net loss of potassium: or by using the observed influx of tracer and assuming that the change in the efflux is not large enough seriously to alter the value of $kT/(1-e^{-kT})$. The results of the two methods agreed in all the conditions examined to within about 25%, which is as good as the various approximations used are likely to allow: and this degree of agreement between independent measurements gives some confidence that their magnitude is correct. As shown in Table IV, the cardiac glycosides exert a larger effect when they are allowed to act for some time, though this is complicated by the decline in the rate of potassium influx which occurs in control auricles. With a sufficiently high concentration of ouabain (10^{-5}) the influx is reduced by one-third even in the first 10 min. and, as noted above, this results in a loss of about one-tenth of the tissue potassium and cessation of the beat. With lower concentrations, the efflux is reduced less rapidly, and it is possible that the failure to observe any effect from concentrations of 10^{-7} or lower is a result of not prolonging the experiments sufficiently.

Discussion

The measurements of potassium movements made on control auricles are of limited significance because they apply to non-beating tissue over

relatively long periods and do not give information about the movement which occurs at each systole and diastole. In tissues of the dimensions of rabbit auricles, it is likely that much of the potassium presumably extruded at each beat (Wilde, O'Brien and Bay, 1955) is promptly returned from the extracellular fluid and only part is involved in the observed exchanges of the tissue as a whole. This is borne out by observations on left auricles, where the influx rate was little affected whether the tissue was separated from the pacemaker in the right auricle, or was driven by it while exchange was measured. The observation that part of the potassium of the right auricles exchanges at a fairly similar rate is also suggestive. If beating *per se* substantially altered the exchange rate of the tissue, it ought to do it for the whole tissue, and not to account for acceleration during only part of the exchange. Indeed, the fact that such tracer as remains in the auricles after more than 2 hr. of efflux exchanges more slowly in the right auricle suggests that beating may diminish the overall exchange of the tissue, by promoting reabsorption of extruded ^{42}K instead of letting it escape from extracellular fluid. However, other factors may also be involved in left-right differences.

The significance of the rapid part of the exchange in right auricles is uncertain, and the evidence is insufficient to distinguish between a rapid fraction in all the cells, and the separate existence of a group of cells with a fast exchange, or a gradation of exchange rates in different cells. It is tempting to associate the faster exchange with the pacemaker. The amount of tissue involved has not been estimated from the present results; it looks as though it is larger than the anatomically defined pacemaker (Keith and Flack, 1907), but wider areas may show spontaneous firing (Marshall and Vaughan Williams, 1956), and so could account for the rapid component. It evidently requires further investigation. Whatever its identity, it is important in understanding the action of cardiac glycosides, since it is more sensitive to their action than the rest of the tissue: this is quite consistent with its identity as a pacemaker.

It may be noted that Schreiber (1956) has described two phases of potassium exchange in frog hearts and finds only the slow fraction sensitive to ouabain. This is at variance with the present observations, but there are considerable differences in conditions and insufficient evidence to relate his components to those described here.

As has been observed before (Hajdu, 1953; Conn, 1956; Schreiber, 1956), cardiac glycosides plainly inhibit the influx of potassium with little or no effect on efflux unless or until there is a considerable reduction in the intracellular concentration of potassium. Their effect on sodium movement has not been examined: potassium loss is accompanied by sodium gain, but whether this is due to decreased extrusion or increased influx is not established. Numerous observations on other tissues suggest that the former is more likely (Matchett and Johnson, 1954).

It seems probable that the loss of potassium from the tissue is connected with some of the effects produced by the glycosides. The failure of contraction in different concentrations of digoxin or ouabain occurred when the intracellular potassium had fallen by about 15%, and it is well known that raising the external potassium concentration delays the onset of digitalis-induced failure. It would be dangerous to try to deduce what changes in membrane potential are occurring, since the loss of potassium from the cells, at least with high concentrations of ouabain, is sufficiently rapid to be likely to increase locally the concentration of potassium in the extracellular fluid and, in any case, the drug may modify the electrical properties of the membrane in quite unknown ways. It is possible that the intermittent beating which occurs in concentrations of digoxin or ouabain which reduce the tissue potassium to but not beyond the critical level occurs in this way. It is likely that most of the potassium efflux is associated with each beat, and that, once beating ceases, the efflux from the cells is reduced. With the inward, active transport impaired but not abolished, this reduction in efflux may well be sufficient for the tissue to regain a little potassium and so restore conditions in which beating is possible. Extrusion at each beat then progressively lowers the internal concentration again, and the characteristic negative staircase and further stoppage follow. The fact that it is the mechanical response which diminishes progressively, and that electrical activity persists when beating has ceased, suggests that disturbance of the membrane potential is not the principal element in failure.

It is somewhat surprising that the concentrations of ouabain and digoxin necessary to produce these effects are much higher than those likely to occur in therapeutic conditions, and also than those which produce detectable effects in erythrocytes (Kahn and Acheson, 1955; Joyce and Weatherall, 1955; Glynn, 1955). The observed results suggested that lower concentrations might be effective if they were allowed to act for longer periods; here no such effects were followed for more than about 2 hr. However, it must be

remembered that other components of the mechanism of the heart, such as isolated actomyosin, are sensitive to the action of ouabain (Robb and Mallow, 1953) and little is yet known about the way in which these various components affect each other in the work of the whole heart.

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