J. Physiol. (1959) 146, 392–409

ACETYLCHOLINE AND POTASSIUM MOVEMENTS IN RABBIT AURICLES

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(*Received* 5 *December* 1958)

Numerous measurements have been made of the resting and action potentials of atrial tissue from various mammals, and inferences have been drawn about the concurrent ionic movements, but direct measurements of the fluxes appear to be rare. Estimates based on net changes in the composition of the tissue in special circumstances (Holland, 1954, 1957) are well known not to give a measure of the total influx and efflux, and even estimates with the use of tracer are subject to limitations particularly obvious in beating tissues in which the fluxes are probably different at different stages of each contractile cycle. Rayner & Weatherall (1957) estimated that the potassium influx and efflux of isolated quiescent left auricles were about 5·8 pmole/cm^2.sec, and showed that part of the tissue potassium of right auricles exchanged more rapidly. Klein & Holland (1958) give a figure of 3·45 pmole/cm^2.sec for beating pairs of auricles, but this figure is based only on the slow component of the efflux curve, which deviates considerably from a simple exponential, and includes no allowance for delays due to diffusion (Harris & Burn, 1949), and is therefore an underestimate.

In view of the complex actions of actylcholine on auricles, its effect on potassium movements is particularly interesting. It has been inferred from observed electrical changes that acetylcholine increases the permeability of atrial cells to potassium (Burgen & Terroux, 1953; Marshall & Vaughan Williams, 1956; Trautwein & Dudel, 1958) and it is known that vagal stimulation (Howell & Duke, 1908) and acetylcholine (Lehnartz, 1936) causes liberation of potassium from auricles; but it is not clear from such observations whether the net loss is purely due to increased potassium efflux or whether movement against the concentration gradient is also accelerated. Harris & Hutter (1956) have shown that in frog auricles and sinus venosus ^{42}K movements are accelerated in both directions by acetylcholine. Anticholinesterases might be expected to act in a similar way to acetylcholine, although

reduced potassium loss in the presence of physostigmine has been described by Holland (1954).

The present paper reports observations on the uptake and loss of ^{42}K and the total potassium content of inactive and beating rabbit auricles without treatment, or exposed to acetylcholine, carbachol, eserine and electrical stimulation. The observations show that influx and efflux of potassium are increased by all these procedures, and that part of the potassium, at least in beating auricles, exchanges less quickly than the rest.

METHODS

The methods of Rayner & Weatherall (1957) were used with minor modifications, as follows: The salt solution used contained (mM): Na 145, K 5·8, Ca 1·7, Mg 1·2, Cl 128, HCO_3 25, SO_4 1·2, H_2PO_4 1·2, dextrose 11, and was equilibrated before and during use with 95% O_2 + 5% CO_2. Auricles were prepared and stretched on frames as previously described, so that they formed triangular or quadrilateral sheets with a mean surface area of $2{\cdot}08 \pm 0{\cdot}98$ (S.D.) cm^2 and a mean thickness (determined from the ratio of volume, taken from the weight and an assumed specific gravity of 1·06, to area) of $0{\cdot}044 \pm 0{\cdot}015$ (S.D.) cm. They were placed in a bath of flowing oxygenated saline at 37° C so that the auricle lay over the end window of a Geiger–Müller tube. The bath was modified from that previously used (Creese, 1954) in various ways, directed to maintaining a more regular flow, a more constant depth and a more rapid clearance of materials from the bath. The principal adaptations consisted of introducing fluid through a number of small holes spaced across one end of the bath, instead of through one large hole, tapering the opposite end of the bath and leading the outflow to a constant-level device. The mechanical contractions of the auricles were recorded isometrically and transmitted by a rigid lever to a piezo-electric crystal, fed through a single stage amplifier to an oscilloscope (Cossor model 1049, Mark III) and photographed. The tissues were stimulated in some experiments with square-wave pulses (duration 2 msec) from a generator (Bernstein, 1950) through two platinum electrodes: the output of the stimulator was fed also to the second beam of the oscilloscope. To avoid changes in the potassium fluxes consequent on dissection at room temperature and immersion in a new medium at 37° C, auricles were left for at least 1 hr in medium not containing tracer before their ^{42}K uptake began. The rate of uptake of ^{42}K was sometimes followed as suggested by Persoff (1958), by passing a radioactive medium through the bath containing the auricles over the end window of a Geiger counter, as well as using the apparatus with inactive medium for determining the rate of efflux. The radioactivity in the bath was necessarily high, but could be reduced by over 99% to within double the original background by flushing for 40 sec with inactive medium. When an auricle on its supporting frame was immersed in the active medium, the count of the contents of the bath fell by up to 12%, presumably because part of the active medium was displaced by tissue containing no radioactive material; subsequently the count rose, reaching values 30–50% above the original count of the active medium in the bath after ½–1 hr, as expected while the tissue concentrated ^{42}K. In order to estimate the count due to the tissue alone, the bath was flushed with inactive medium and the efflux of ^{42}K from the tissue was followed for 20 min: the counts in the last 15 min were extrapolated to give the auricle count at the moment of changing from active to inactive medium, and the count so estimated was deducted from the final count observed during the influx period to give the count due to the active medium. The apparatus was calibrated by ashing the auricle as described below, dissolving the ash in a standard volume, and estimating its radioactivity in a liquid counter (Type M6, 20th Century Electronics). A suitable dilution of the soak-in medium was estimated similarly, and the amount of potassium taken up from the medium and remaining in the auricle was calculated from the ratio of these counts and from the concentration of potassium in the medium. The validity of this procedure was checked in two ways. First, the uptake of ^{42}K so estimated after periods of immersion in active medium was compared with uptakes in other auricles determined by ashing

and estimating in a liquid counter without any period of efflux; agreement was within the limits of experimental error. Secondly, however long the immersion, the total uptake of ^{42}K should in no case have led to an estimated specific activity of the tissue greater than that of the medium. Apart from experiments in which treatment during efflux probably caused loss of potassium and the final potassium content of the auricle was abnormally low, this criterion was consistently fulfilled. Drugs were administered either by changing to perfusion with medium in which drug was dissolved or by leaving the main perfusion undisturbed and infusing a separate solution from a mechanically driven syringe through a polythene tube into the bath upstream from the tissue: the rate of infusion was approximately 0·3 ml./min and the rate of flow of medium was about 30 ml./min. The final concentration of drug when this technique was used was therefore approximately 1% of the solution injected, though the figures so obtained may have an error of 10%. No conclusions are reached which depend on differences of this magnitude. Each auricle was ashed for 90 min at 120° C in 1 ml. of a mixture of nitric and perchloric acids (10:1, v/v) and the acid was removed by evaporating at 180° C. The ash so obtained was dissolved in water and made up to 25 ml. for estimation of radioactivity, sodium and potassium. Clear neutral solutions were consistently obtained in this way, whereas ashing in nitric acid alone as previously used sometimes failed to remove traces of fat. Recoveries of added potassium by the new method ranged from 94·8% to 100·8% (7 estimations, mean 99·6%).

RESULTS

Inward movement of ^{42}K

If the tissue potassium exchanged uniformly, and the fluxes in each direction were either equal or proportional to the concentrations of potassium inside and outside the cells, the ^{42}K content of the tissue would approach exponentially

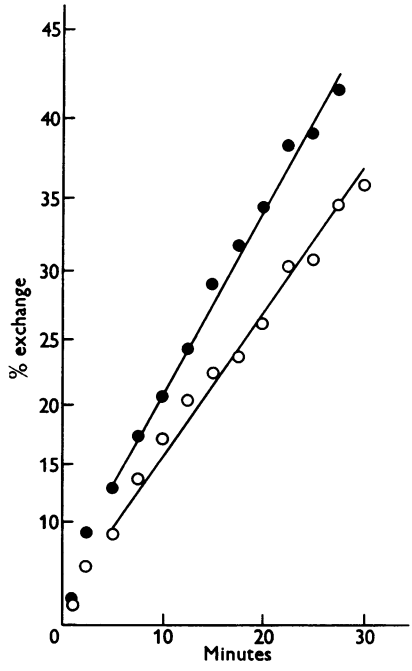

Fig. 1. Uptake of ^{42}K by left (○) and right (●) auricles. Expts. 3*L* and 3*R*.

a value such that the specific activities of tissue and medium were equal. These conditions are not exactly realized, but it is convenient to describe the rate of uptake over short periods by the slope of the line best relating ln $(1-f)$ to time, where f is the fraction of the total tissue potassium which

TABLE 1. Uptake of ^{42}K by unstretched auricles

Treatment	Duration (min)	No. of expts.	$[K]_e$ (m-equiv/l.)	K content (m-equiv/kg dry; mean ± S.E.)	Specific activity (% of medium mean ± S.E.)
Left auricles					
Control	20	3	4·5	277 ± 2	28·3 ± 1·4
Carbachol 1 μM	20	3	4·5	330 ± 27	34·7 ± 2·8
Control	20	3	5·3	311 ± 13	21·9 ± 1·0
Carbachol 1 μM	20	3	5·3	339 ± 21	30·9 ± 2·2
Right auricles					
Control	20	3	4·5	283 ± 8	35·0 ± 1·5
Carbachol 1 μM	20	3	4·5	294 ± 28	36·5 ± 6·3
Control	20	3	5·3	318 ± 8	33·8 ± 2·6
Carbachol 1 μM	20	3	5·3	309 ± 14	30·9 ± 1·8

TABLE 2. Rate of entry of ^{42}K in left auricles

Expt.	Treatment	Dry weight (mg)	Cell volume (μl.)	Final [K] (mM)	Influx rate constant (min^{-1}) Period (1)	Period (2)	Period (3)
1*L*	Control throughout	13·2	27	152	0·015	0·014	—
2*L*	Control throughout	12·4	21	160	0·016	0·016	—
3*L*	Control throughout	12·6	26	166	0·014	0·014	—
4*L*	Control throughout	17·1	30	174	0·013	0·013	—
5*L*	(1) Control; (2) ACh 1 μM	13·5	28	127	0·015	0·015	—
6*L*	(1) Control; (2) ACh 1 μM	14·3	31	150	0·012	0·112	—
7*L*	(1), (3) Control; (2) ACh 5 μM	15·0	39	114	0·011	0·015	0·008
8*L*	(1), (3) Control; (2) ACh 36 μM	9·9	22	150	0·020	0·028	0·016
9*L*	(1), (3) Control; (2) CCh 1 μM	22·0	47	136	0·013	0·021	0·012
10*L*	(1), (3) Control; (2) CCh 1 μM	32·8	56	169	0·011	0·015	0·013
11*L*	ACh 28 μM	13·2	29	142	0·018	—	—
12*L*	CCh 1 μM	19·4	33	181	0·024	—	—
13*L*	CCh 1 μM	22·5	39	157	0·030	—	—
14*L*	CCh 1 μM	20·2	46	123	0·030	—	—
15*L*	Stim. 4/sec throughout; (2) ACh 1 μM	15·6	30	146	0·025	0·025	—
16*L*	Stim. 2/sec throughout; (2) ACh 4 μM	6·9	26	108	0·025	0·027	0·021
17*L*	Stim. 2/sec throughout; (2) ACh 16 μM	6·4	25	108	0·024	0·015	0·029
18*L*	Eserine 10 μM throughout; (2) ACh 1 μM	23·8	62	120	0·015	0·028	0·013
19*L*	Eserine 10 μM throughout; (2) ACh 1 μM	22·3	40	142	0·019	0·021	0·012
20*L*	Eserine 10 μM + stim. 2/sec throughout; (2) ACh 1 μM	27·7	53	149	0·019	0·020	0·017

ACh, acetylcholine; CCh, carbachol; periods (1), (2), (3), successive periods of 15 min, beginning 5, 20 and 35 min respectively after exposure of auricles to radioactive medium.

has exchanged. When ln $(1-f)$ was plotted against time for normal auricles, lines were obtained with no consistent departures from rectilinearity in the first 30 min (Fig. 1) and mean slope $0{\cdot}0140 \pm 0{\cdot}0027$ min^{-1} (S.D. for 10 observations) for left auricles and mean slope $0{\cdot}0214 \pm 0{\cdot}0035$ min^{-1} (S.D. for 10 observations) for right auricles. These figures correspond to an exchange of 25% and 35% respectively of the tissue potassium in 20 min, and agree closely with those found for unstretched auricles immersed free in oxygenated medium with slightly lower potassium concentration (Table 1; and also Rayner &

TABLE 3. Rate of entry of ^{42}K in right auricles

Expt.	Treatment	Dry weight (mg)	Cell volume (μl.)	Final $[K]_i$ (mM)	Influx rate constant (min^{-1}) Period (1)	Period (2)	Period (3)
1*R*	Control throughout	13·2	26	162	0·021	0·021	—
2*R*	Control throughout	12·8	25	149	0·022	0·022	—
3*R*	Control throughout	16·6	37	133	0·018	0·018	—
4*R*	Control throughout	12·8	22	177	0·019	0·019	—
21*R*	Control throughout	12·4	30	147	0·023	0·018	—
7*R*	(1), (3) Control; (2) ACh 5 μM	14·6	36	117	0·021	0·015	0·009
8*R*	(1), (3) Control; (2) ACh 20 μM	9·5	19	159	0·030	0·026	0·019
22*R*	(1) Control; (2) ACh 30 μM	9·7	21	163	0·018	0·016	—
9*R*	(1), (3) Control; (2) CCh 1 μM	16·4	30	124	0·020	0·013	0·011
10*R*	(1), (3) Control; (2) CCh 1 μM	22·6	36	161	0·022	0·015	0·018
23*R*	ACh 2 μM	11·0	38	97	0·022	—	—
11*R*	ACh 30 μM	11·6	29	127	0·037	—	—
12*R*	CCh 1 μM	19·7	39	151	0·028	—	—
13*R*	CCh 1 μM	19·5	36	129	0·029	—	—
14*R*	CCh 1 μM	11·8	19	161	0·030	—	—
24*R*	Stim. 2·6/sec throughout	14·6	38	129	0·028	0·028	—
15*R*	Stim. 4/sec throughout; (2) ACh 1 μM	14·2	31	141	0·026	0·026	—
17*R*	Stim. 2/sec throughout; (2) ACh 4 μM	8·6	27	111	0·032	0·032	0·011
25*R*	Stim. 2/sec throughout; (2) ACh 10 μM	9·0	14	158	0·026	0·025	—
26*R*	Stim. 2/sec; ACh 5 μM	12·8	32	134	0·028	—	—
18*R*	Eserine 10 μM throughout; (2) ACh 1 μM	18·3	45	113	0·025	0·028	0·021
20*R*	Eserine 10 μM + stim. 2/sec throughout; (2) ACh 1 μM	16·8	34	132	0·026	0·026	0·018

Abbreviations as in Table 2.

Weatherall, 1957). It therefore appears that stretching the auricles and the additional handling involved in setting up the stretched preparation do not greatly influence the potassium influx an hour later, and the results obtained by the two methods may be regarded as comparable. Observations on influx were sometimes extended to 50 min without showing appreciable changes in slope, at least in left auricles. After 50 min the rate of change of the tissue count was becoming small compared with the random fluctuations, and estimates of the slope were unreliable. Results of individual experiments are shown in Tables 2 and 3, with details of the tissue potassium concentrations. A large part of the variation between individual auricles is related to the size

of the tissue. As Fig. 2 shows, the rate constants are on the whole greater in small auricles. It is likely that the slower rates in larger auricles are due to delay in exchange by diffusion through the thicker tissues.

Outward movement of ^{42}K

Typical curves showing the efflux of ^{42}K, estimated by direct counting, from untreated left and right auricles are plotted semilogarithmically in Fig. 3. The decline in count of left auricles is nearly linear, with an initial rate constant (based on the efflux 5–20 min after transfer to inactive medium) of $0{\cdot}0168 \pm 0{\cdot}0029$ min^{-1} (S.D. of 14 observations on separate auricles); there was,

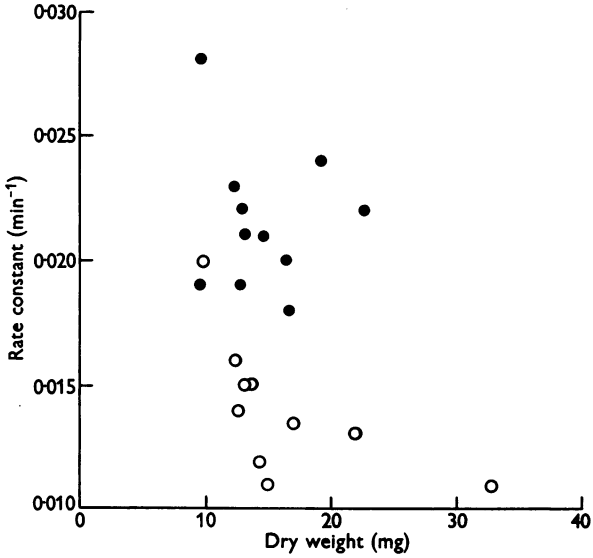

Fig. 2. Relation of influx rate constants to dry weight of left (○) and right (●) auricles.

however, always some decrease in the slope as the experiment proceeded, so that the apparent rate constant declined to $0{\cdot}0125 \pm 0{\cdot}0020$ min^{-1} (S.D. of 4 observations on separate auricles) during the fourth hour of efflux. In those experiments in which the rate constants for influx and efflux were measured successively on the same auricles between 5 and 20 min after applying first active and then inactive medium, the efflux rate constant over this period was consistently higher (by 20–40%) than the influx rate constant. In right auricles curvature of the semilogarithmic plot was consistently greater; the initial slope was steeper than that for left auricles with a mean rate constant of about 0·032 min^{-1} and the late part parallel to or at times slightly flatter than that observed in left auricles during the fourth hour. As in left auricles the rate constant early in efflux was consistently greater than that early in influx: the

TABLE 4. Rate of loss of ^{42}K in left auricles

Expt.	Uptake of radioactivity: Duration (min)	Uptake of radioactivity: Conditions	Treatment during loss of ^{42}K	Dry weight (mg)	Cell volume (μl.)	Final $[K]_i$ (mM)	Efflux rate constant (min^{-1}): Period (1)	Period (2)	Period (3)
27L	80	Control	Control	27·9	63	133	0·012	—	—
28L	4	Control	Control	23·1	47	156	0·017	—	—
29L	80	Control	Control	32·3	64	141	0·014	—	—
4L	31	Control	Control	17·1	30	174	0·024	—	—
9L	51	CCh 1 μM 20–35 min	Control	22·0	47	136	0·017	—	—
10L	51	CCh 1 μM 20–35 min	Control	32·8	56	169	0·016	—	—
7L	51	ACh 5 μM 20–35 min	Control	15·0	39	114	0·017	—	—
30L	51	Stim. 2/sec	Control	25·8	54	112	0·018	—	—
31L	51	Stim. 2/sec	Control	13·4	28	127	0·026*	—	—
32L	60	Control	ACh 1 μM	22·0	47	140	0·017	—	—
33L	58	Control	(1) Control; (2) ACh 4 μM	13·2	30	155	0·014	0·023	—
34L	58	Control	(1) Control; (2) ACh 5 μM	11·2	25	135	0·015	0·029	—
8L	51	ACh 36 μM 20–35 min	(1), (3) Control; (2) ACh 20 μM	9·9	22	150	0·020	0·028	0·016
35L	95	Control	(1) Control; (2) CCh 1 μM	25·0	56	140	0·018	0·029	—
36L	60	Control	(1) Control; (2) CCh 1 μM	23·6	59	138	0·016	0·028	—
37L	51	Stim. 2/sec	Stim. 2/sec	25·0	59	130	0·027	—	—
38L	51	Stim. 2/sec	Stim. 2/sec	23·6	54	143	0·030	—	—
39L	30	Stim. 2/sec throughout; ACh 3 μM 15–30 min	Stim. 2/sec	14·8	31	140	0·029	—	—
16L	46	Stim. 2/sec. throughout; ACh 4 μM 20–35 min	Stim. 2/sec	6·9	26	108	0·027	—	—
17L	51	Stim. 2/sec throughout; ACh 16 μM 20–35 min	Stim. 2/sec	6·4	25	108	0·034	—	—
40L	66	Control	Stim. 2/sec; (2) ACh 3 μM	10·3	35	119	0·024	0·024	—
18L	51	Eserine 10 μM throughout; ACh 1 μM 20–35 min	Eserine 10 μM	23·8	62	120	0·022	—	—
19L	51	Eserine 10 μM throughout; AChl 1 μM 20–35 min	Eserine 10 μM	22·3	40	142	0·021	—	—
20L	51	Eserine 10 μM + stim. 2/sec throughout; ACh 1 μM 20–35 min	Eserine 10 μM + stim. 2/sec	27·7	53	149	0·024	—	—

Abbreviations as in Table 2, except that the periods (1), (2) and (3) began 5, 20 and 35 min respectively after transfer of auricles to inactive medium.
* Auricle beating spontaneously.

TABLE 5. Rate of loss of ^{42}K in right auricles

Expt.	Uptake of radioactivity: Duration (min)	Uptake of radioactivity: Conditions	Treatment during loss of ^{42}K	Dry weight (mg)	Cell volume (μl.)	Final $[K]_i$ (mM)	Efflux rate constant (min^{-1}): Period (1)	Period (2)	Period (3)
4 *R*	31	Control	Control	12·8	22	177	0·024	—	—
21 *R*	31	Control	Control	12·4	30	147	0·030	—	—
27 *R*	80	Control	Control	16·6	26	177	0·039	0·035	0·034
28 *R*	80	Control	Control	23·6	61	115	0·025	0·022	0·022
9 *R*	51	CCh 1 μM 20–35 min	Control	16·4	30	124	0·040	—	—
10 *R*	51	CCh 1 μM 20–35 min	Control	22·6	36	161	0·034	—	—
33 *R*	58	Control	(1) Control; (2) ACh 5 μM	9·6	15	178	0·031	0·034	—
34 *R*	58	Control	(1) Control; (2) ACh 13 μM	9·3	16	145	0·032	0·032	—
8 *R*	51	ACh 20 μM 20–35 min	(1), (3) Control; (2) ACh 20 μM	9·5	19	159	0·030	0·036	0·024
41 *R*	90	Control	(1), (3) Control; (2) CCh 1 μM	8·3*	17*	181*	0·033	0·035	0·021
7 *R*	51	ACh 5 μM 20–35 min	Stim. 2/sec	14·6	36	117	0·029	—	—
17 *R*	51	Stim. 2/sec throughout; ACh 4 μM 20–35 min	Stim. 2/sec	8·6	27	111	0·035	—	—
24 *R*	31	Stim. 2·6/sec	Stim. 2·6/sec throughout; (2) ACh 6 μM	14·6	38	129	0·037	0·041	0·025
40 *R*	61	Stim. 3/sec	Stim. 3/sec throughout; (2) ACh 4 μM	7·4	22	128	0·038	0·038	—
18 *R*	49	Eserine 10 μM throughout; ACh 1 μM 20–35 min	Eserine 10 μM	18·3	25	113	0·036	—	—
20 *R*	51	Eserine 10 μM + stim. 2/sec throughout; ACh 1 μM 20–35 min	Eserine 10 μM + stim. 2/sec	16·8	34	132	0·035	—	—

* Dry weight not determined; estimate based on wet weight and assumed water content of 83%.

difference was greater than on the left, being usually by a factor between 1·5 and 2·0. Results of individual experiments are summarized in Tables 4 and 5.

Estimation of potassium fluxes in quiescent auricles

In left auricles the exchange of potassium is sufficiently nearly uniform to justify estimating potassium fluxes in the conventional way (Harris & Burn, 1949; Keynes & Lewis, 1951). Application of the procedure, including esti-

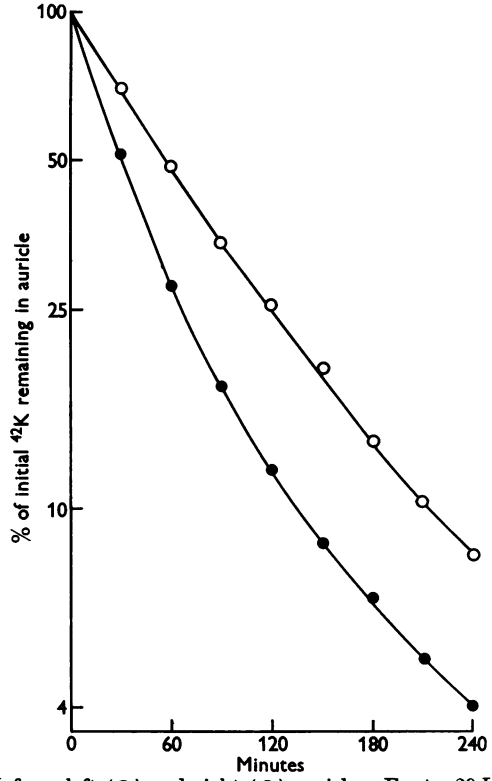

Fig. 3. Efflux of ^{42}K from left (○) and right (●) auricles. Expts. 29*L* and 28*R*. The curve through the points for the left auricle is that expected for a tissue exchanging with mean rate constant 0·0115 min^{-1} and S.D. the natural logarithm of the rate constants ±0·6. The curve through the points for the right auricle is given by the expression $Y = 0{\cdot}6(1 - e^{-0{\cdot}031t}) + 0{\cdot}4(1 - e^{-0{\cdot}008t})$. Some of the counts taken have not been plotted, in order to avoid overcrowding the graph.

mates of necessary tissue dimensions and correction for diffusion (Keynes, 1954), has been described previously (Rayner & Weatherall, 1957) and need not be repeated. The potassium concentrations of the auricles at the ends of the experiments, whether treated with drugs or not, were within the range observed for fresh auricles (Rayner & Weatherall, 1957), and no adjustment has been made for the small losses of potassium which probably occurred.

All estimates are based on the final quantity of potassium in the tissue. As the inward and outward rate constants in the present experiments are somewhat higher than those previously observed by us, probably because of the raised external potassium concentration (D. A. Persoff, unpublished), the ratio of apparent to 'true' fluxes is larger than before, being usually of the order of 0·4 or 0·5. Some doubt may be felt about the value of results involving such a large correction, but mean values so calculated are given in Table 6 as provisional estimates.

The moderate departure from a strictly exponential relationship may be accounted for in various ways. The addition of a second exponential term with a slightly slower rate constant to the equations naturally improves the description of the influx and efflux curves. Such a double exponential process implies division of the tissue potassium into more than one part and this

TABLE 6. Estimated K fluxes in left auricles

Treatment	Influx No. of expts.	Influx (pmole/cm². sec; mean ± S.D.)	Efflux No of expts.	Efflux (pmole/cm². sec; mean ± S.D.)
Control	4	11·4 ± 3·5	5	16·9 ± 3·1
Carbachol 1 μM	5	32·4 ± 15·5	2	36·1 ± 1·3

possibility receives further attention later. A somewhat more complex combination of exponential terms has been derived by Creese, Neil & Stephenson (1956) to account for the influx and efflux curves of ^{42}K exchange in rat diaphragm on the basis of a log-normal distribution of individual rate constants. A theoretical curve of the type described by these authors is used in Fig. 3, and the fit with observed points is very good. The value adopted (0·6) for λ (the S.D. of the natural logarithms of the individual rate constants) is larger than that in diaphragms and implies considerable variation between individual cells in the tissue; on the other hand there are advantages in using this method of analysis because the initial inward rate constant gives an accurate estimate of the mean rate of exchange. Whatever interpretation is used, the difference between inward and outward rate constants can be attributed to differences in the degree of labelling of different parts of the tissue potassium at the beginning of efflux. The duration of influx was never sufficient for the tissue potassium to exchange fully, so the more rapidly exchanging potassium will have been more heavily labelled than the slower part and had the effect of making the apparent efflux rate an exaggerated estimate. It follows that the efflux rates given in Table 6 are too high, and the true effluxes are on this assumption only slightly greater than the influxes.

Treatment of the results with right auricles in the way described by Creese *et al.* (1956) requires a much larger value for λ, implying a variation in individual rate constants of over $\pm 80\%$: moreover, curves of this type fit the results less well than on the left. Right efflux curves in the range studied, i.e.

up to 4 hr after beginning the wash-out period, are well fitted by curves which are the sum of two exponential processes, i.e.

$$Y = Y_0\{ae^{-k_1 t} + (1-a)e^{-k_2 t}\}$$

with values of k_1 between 0·033 and 0·050 min^{-1}, of k_2 between 0·008 and 0·012 min^{-1}, and of a about 0·8. If it is assumed that the influx of ^{42}K followed a similar double exponential course, as is consistent with the available observations, then the fast component would be nearly 100% labelled after the 80–90 min duration of ^{42}K uptake in these experiments, while the slow component would not be more than 50–70% labelled; and it is reasonable to suppose that a would have been somewhat smaller, probably about 0·7, if the tissue had been soaked in the ^{42}K medium until all the potassium was equally labelled. The possible significance of these values is discussed later.

Effect of electrical stimulation

Electrical stimulation for periods of 15 min, generally at rates between 2 and 4 per second, increased the inward and outward movements of ^{42}K (Fig. 4). The effect was greater in left than in right auricles, and about equal on inward and outward movements. In auricles driven at 2–4 per sec the rate constants for ^{42}K entry were 0·0247 ± 0·0003 (S.E.) min^{-1} for left and 0·0273 ± 0·0007 min^{-1} for right auricles, with less difference between left and right auricles than when they were not stimulated. Similarly for ^{42}K efflux the constants were closer than before, being 0·0285 ± 0·0014 min^{-1} (left) and 0·0320 ± 0·0030 min^{-1} (right) (see Tables 2–5). Stimulation at 8 per second gave values in the same range, but the auricles failed to follow, showing instead frequent irregular and weak contractions. When left auricles were stimulated continuously throughout 50 min of uptake of ^{42}K followed by 3 hr of efflux into an inactive medium, the rate of ^{42}K efflux declined more than usual for left auricles (Fig. 5), though the departure from linearity was not as great as in normal right auricles. Similar observations have been made on left auricles stimulated only throughout ^{42}K efflux (Persoff, 1958). The efflux curve of left auricles stimulated during 50 min of uptake of ^{42}K but not during efflux did not differ appreciably from the usual efflux curve of left auricles (Fig. 5).

Estimation of the increased potassium flux due to stimulation

If all the uncertainties suggested by departures from a simple exponential loss of ^{42}K are ignored, it is possible to estimate the extra efflux of potassium during stimulation in experiments such as that shown in Fig. 4. The mean flux before and after each period of stimulation has been calculated and corrected for diffusion as before, and deducted from the similarly calculated flux during the period of stimulation. From Expt. 43*L* (Fig. 4) the value so obtained is

$1{\cdot}58 \pm 0{\cdot}47$ (S.E.) pmole/cm^2.impulse. Other experiments give values of the same order, but so many assumptions are involved in reaching this figure that it must be regarded with much caution.

Effects of choline esters and eserine

Acetylcholine accelerated the loss (Table 4), and in sufficient concentration (5μM) accelerated also the entry of ^{42}K in quiescent left auricles (Table 2, Expts. 7*L*, 8*L*; Fig. 6). In right auricles and in electrically driven left auricles its effects were less simple. The concentrations of acetylcholine used all

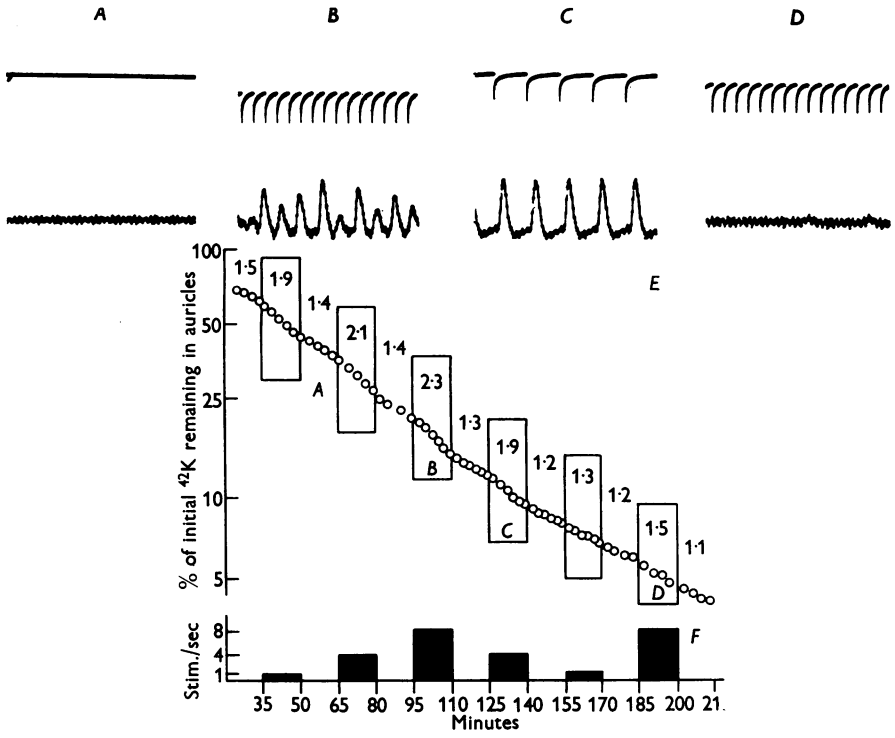

Fig. 4. Efflux of ^{42}K from left auricle at different rates of stimulation. *A*, mechanical response of unstimulated auricle 57 min from start of wash-out period. *B*, *C*, *D* stimulus (upper line) and mechanical response (lower line) of auricle at 107, 137 and 195 min from start of wash-out period. *E*, ^{42}K count of auricle. The numerals above each group of points give the rate constants (in 10^{-2} min^{-1}) and the letters below refer to the records at the top of the figure. *F*, rate of stimulation. Expt. 43*L*.

weakened the contractions and in spontaneously beating auricles reduced the rate of beating by 10–90%. Whatever the effect on the tissue, efflux of ^{42}K was usually accelerated, but the rate of uptake of ^{42}K was affected rather variably (Table 3). Carbachol ($1\,\mu$M) acted like acetylcholine, consistently accelerating inward and outward movement of ^{42}K in left auricles (Tables 1–3), but affecting ^{42}K influx in right auricles more variably. During ^{42}K

efflux, carbachol always increased the efflux rate, even though the auricles were slowed or stopped.

In order to minimize possible losses of acetylcholine by destruction in the tissue, some experiments were made in the presence of eserine (10 μM). This concentration of eserine is much lower than that (5 mM) used by Holland (1954). In its presence, the inward and outward rate constants in left and right

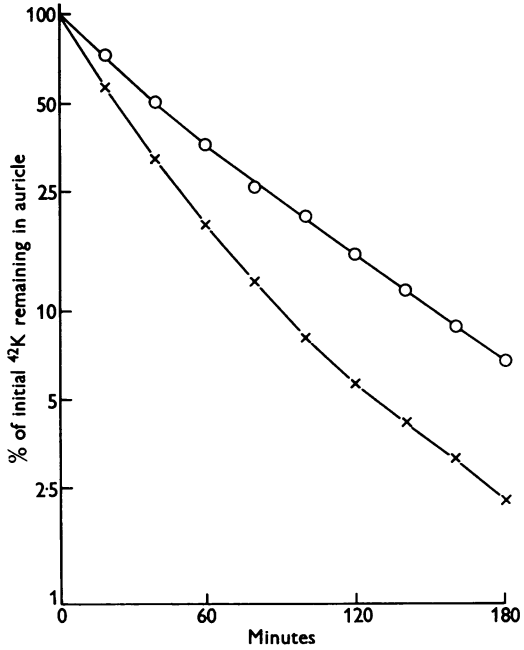

Fig. 5. Efflux of ^{42}K from left auricles, unstimulated (○) and stimulated at 2/sec (×) during efflux. Both auricles were stimulated at 2/sec during uptake of ^{42}K, in the 51 min before zero time. Expts. 30*L* and 38*L*. The curves indicate the probable course of efflux and have no mathematical significance.

auricles were always higher (Tables 2–5) than the mean values for controls given above. The number of experiments is small, and the difference significant only for the left efflux (control mean $0{\cdot}0168 \pm 0{\cdot}0008$ (S.E.) min^{-1}: eserine mean $0{\cdot}0215 \pm 0{\cdot}0005\ \text{min}^{-1}$: $t = 5{\cdot}1$; $P < 0{\cdot}001$: for the left influx the figures are control $0{\cdot}0140 \pm 0{\cdot}0009\ \text{min}^{-1}$, eserine $0{\cdot}0170 \pm 0{\cdot}0020\ \text{min}^{-1}$; $t = 1{\cdot}38$; $P \simeq 0{\cdot}2$). Eserine had no striking effect on the contractions of the tissue, but when it was used it was applied throughout the experiments, and small effects would not have been apparent in comparison with variation between preparations. As auricles are known to synthesize acetylcholine as well as to hydrolyse it (Bülbring & Burn, 1949) these increased movements of potassium were probably due to protection of the acetylcholine formed in the tissue, though a direct effect of eserine is not excluded. The presence of eserine (10 μM)

augmented the effects of small concentrations of acetylcholine (1 μM) on influx in left auricles: as before, the effect on influx in right auricles was equivocal.

In some experiments, with or without eserine, auricles were driven at 2 per second and the effect of acetylcholine was observed on the influx. Stimulation at this rate gave rate constants of 0·020–0·025 min^{-1} and 0·025–0·030 min^{-1} in left and right auricles respectively; these are values such as might be produced by acetylcholine alone, and acetylcholine administered in these conditions had no consistent extra effect on the rate constants, which remained on the whole unchanged.

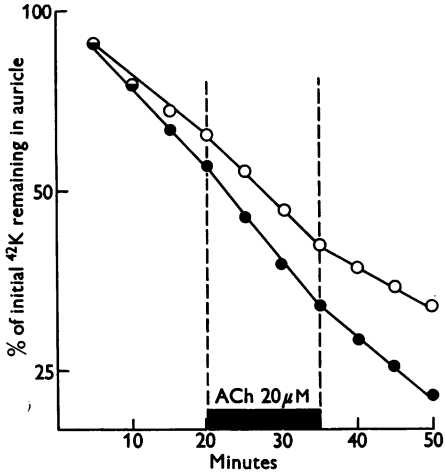

Fig. 6. Influence of acetylcholine on efflux of ^{42}K in left (○) and right (●) auricles. Acetylcholine was infused to give a final concentration of 20 μM during the middle period. Expts. 8*L* and 8*R*.

DISCUSSION

The results with quiescent auricles are at first sight straightforward. The potassium of the tissue appears to exchange fairly uniformly, though the variation between different cells is rather large. As there is some variation, and as efflux measurements were made on tissues which had not reached the same specific activity as the soak-in medium, the more rapidly exchanging part of the tissue potassium will make an exaggerated contribution to the efflux rate constants and the estimates of efflux in Table 6 are probably a little high. There is sufficient variation in the potassium concentration in different auricles to make estimates of gain or loss of potassium in individual cases unsatisfactory, but the mean level in control auricles and our previous observations suggest that there were slight losses of potassium during all experiments, though not as large as the flux differences of Table 6 suggest.

Acetylcholine and carbachol accelerated movements in both directions. Efflux appeared to be sensitive at lower concentrations than influx, but this may indicate only that an effect on influx was too small to detect at

these concentrations. This is to be expected if there is an increase in the permeability of the cell membrane to potassium. All the efflux of potassium is presumably regulated by the passive permeability of the membrane, whereas a large part of the influx may involve active transport which is not necessarily affected by choline esters.

In active auricles the exchange of potassium is less uniform and approximates closely to what would occur in a system consisting of one part exchanging with a rate constant of about 0·04 min^{-1} and a smaller part exchanging at a little more than 0·01 min^{-1}. The fast part is not due to the anatomical pacemaker, as we previously suggested (Rayner & Weatherall, 1957), because a similar fraction appears in left auricles when they are driven electrically. Earlier experiments (Rayner & Weatherall, 1957, Table 3) suggested that there was no fast uptake by left auricles which were beating in undivided pairs, but the results could have been vitiated by inadequate allowance for the effects of cutting the bridge of tissue between the two auricles. If it is assumed that exchange of a little potassium (2–4 %) of each auricle is accelerated by such a cut (as it may be by end effects and increased surface area) the typical difference between active and inactive tissue is apparent and the previously unexplained discrepancy in the faster uptake of separated than undivided right auricles is accounted for.

It is evident that the fast part of the tissue potassium is related to beating and does not completely exchange with the remainder as long as beating continues: if it did, there would be no slow component at all in the exchange. On the other hand, the separation between fast and slow parts of the tissue potassium does not persist in any obvious way after activity ceases; as Fig. 5 shows, after stimulation throughout influx of ^{42}K, the efflux follows a typical, simple course in an inactive left auricle.

The distinction might result entirely from differences between cells in different parts of the tissue. For instance, only some of the cells in the tissue may be active while the rest are either not being excited or are inactive or dead for lack of oxygen or other essential metabolites. It seems rather unlikely that excitation is not spreading freely in a syncytial tissue such as the auricle, but localized injury might abolish activity in some of the cells. Persistent depolarization of the central cells of the tissue has been observed in stimulated rat diaphragm (Creese, Scholes & Whalen, 1958), but the tissues which they used were thicker than the auricles used here and only the central third of their tissue was seriously affected. The possibility cannot be entirely excluded, but we have seen no histological differences between sections of fresh auricles and of auricles which have been beating for 3 hr, and have found no correlation between the size of the slow fraction and the thickness of the tissue, such as would be expected if the centre of the tissue was damaged by anoxia.

It may also be supposed that interference with free movement of potassium

between the medium and the cell water has more complex effects in active than in quiescent tissues. A number of events may affect this movement, including extrusion of extracellular fluid at each mechanical contraction, and sudden local changes of K or ^{42}K concentration at one or other side of the cell wall, and restricted diffusion within cells of the kind suggested by Harris (1957) in skeletal muscle. There is evidence that ^{42}K leaves beating hearts at different rates in different parts of the cardiac cycle (Wilde, O'Brien & Bay, 1955). Diffusion of inulin into the extracellular fluid of rat diaphragms stimulated at 2 per second is not much altered in rate from diffusion into resting diaphragm (Creese, Hashish & Scholes, 1958), so the effect of mechanical disturbances appears to be unimportant and the extra output of ^{42}K is more probably related to changes at the cell membrane. There is a considerable difference between the value given by Wilde *et al.* (1955) of 44·9 pmole/cm^2.impulse for turtle ventricle and the excess output of 1·6 pmole/cm^2.impulse in the present experiments, which does not appear to be wholly due to the different experimental and theoretical procedures used. R. D. Keynes (personal communication) has pointed out that Weidmann's (1952) figures for the electrical characteristics of Purkinje fibres suggest that the size of the outward movement of a monovalent cation necessary to account for the falling phase of the spike in these fibres is not less than about 15 pmole/cm^2.impulse, and that a reliable estimate of this quantity is desirable to establish whether repolarization is due to efflux of potassium or to some other process. If ^{42}K accumulated in close proximity to the cell membrane faster than it was removed by diffusion, and so were involved in to-and-fro movements in the beating or stimulated heart, the calculations here would underestimate the true fluxes. This applies also to the observations of Wilde *et al.* (1955) and does not affect the difference between their findings and those presented here, but it might be involved in discrepancies between electrical and ionic measurements. It is also perhaps relevant that the three figures are obtained from different species and different types of cardiac muscle, and that much of the difference may arise in this way.

No effects directly related to beating appear likely to account for the appearance of fast and slow phases in the exchange of the beating tissue, and a more definite physical separation between fast and slow fractions appears more likely. The distinction may be sharp, or it may be gradual: the efflux curves for two such dissimilar systems as the pure double exponential (bimodal without variation at each mode) and the type described by Creese *et al.* (1956) (unimodal with log-normal variation) differ almost undetectably within the useful working range if appropriate constants consistent with the present results are chosen. The rapidly exchanging potassium may therefore be a distinct entity or only the most accessible part of a continuum. From the point of view of membrane potential measurements there is some ground for expecting part of the tissue potassium to be separate from the rest, since the

resting potential is generally found to be about two-thirds of that expected from the relative potassium concentrations (Burgen & Terroux, 1953; Furchgott & Sleator, 1954; Marshall & Vaughan Williams, 1956; Trautwein & Dudel, 1958). If so, it is necessary to expect the potassium of the left auricles to be similarly divided, because the membrane potential is also lower than the theoretical potassium potential in quiescent left auricles. No difficulty arises in this respect, because the rate of exchange of the slow fraction is so near that of the whole left auricle that it would have little influence on the ^{42}K uptake and efflux curves, which anyway deviate somewhat from a single exponential process. In view of the complex structure of cardiac muscle and the known high concentration of potassium which may occur in subcellular particles (Bartley, Davies & Krebs, 1954; Auditore & Holland, 1956), the isolation of part of the potassium in this way is a distinct possibility. It is worth observing that three independent approaches (membrane potential measurements, separation of subcellular particles by differential centrifugation, and radioactive tracer exchanges) give values between 15 and 35% for the size of the small, less accessible fraction, and that the lowest figures (15%; Auditore & Holland, 1956) are obtained by the method (physical separation) most likely to lead to losses of potassium from this fraction.

SUMMARY

1. Inward and outward movement of ^{42}K has been followed in quiescent, spontaneously beating, and electrically driven auricles at 37° C.

2. In non-beating left auricles the exchange of potassium deviates only slightly from a simple exponential process. The rate of influx was estimated as $11{\cdot}4 \pm 3{\cdot}5$ pmole/cm^2.sec (S.D. of 4 observations) and of efflux $16{\cdot}9 \pm 3{\cdot}1$ pmole/cm^2.sec. The potassium contents of individual auricles kept under standard conditions for various times showed an average net loss of potassium corresponding to a smaller difference, and possible sources of this discrepancy are discussed.

3. In beating right auricles, the ^{42}K exchange required at least two exponential terms for its description, the early part of the exchange being faster than in quiescent left auricles.

4. Stimulation of inactive auricles initially accelerated the ^{42}K influx and efflux. Stimulation continued throughout efflux produced an initially fast and later slow loss of ^{42}K resembling that in spontaneously beating tissues.

5. Acetylcholine (4–30 μM) accelerated influx and efflux of potassium in left auricles. Efflux was more affected and the net loss of potassium from the tissue was increased. Carbachol (1 μM) acted similarly.

6. Acetylcholine accelerated efflux of potassium in right auricles. Its effect on influx in right auricles was variable.

7. It is concluded that only part of the tissue potassium is involved in the exchanges which occur with each beat of the heart, and the significance of this division is discussed.

The expenses of this work have been met by grants from the Medical Research Council and the Central Research Fund of the University of London.

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