Exchangeability of tissue potassium in skeletal muscle

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RENKIN, EUGENE M. *Exchangeability of tissue potassium in skeletal muscle.* Am. J. Physiol. 197(6): 1211–1215. 1959.—The quantity of 'rapidly exchanging' K^+ in isolated, skeletal muscles of dogs was determined by measuring the back-diffusion of K^{42} during perfusion with blood containing this isotopic tracer. This quantity was not constant, but varied from 18% to 88% of the total tissue K^+. The rapidly exchanging fraction increased as arterial pressure and blood flow increased, and was greater in preparations with low vascular tone. It is concluded that the rapidly exchanging K^+ represents all tissue K^+ in well circulated regions, and the remaining K^+ (slowly exchangeable) all tissue K^+ in poorly circulated regions. As arterial pressure increases, or vascular tone decreases, the well circulated regions are increased at the expense of the poorly circulated regions due to opening or widening of minute vessels which were originally closed or narrowed.

ALL OR VERY NEARLY ALL of the potassium in mammalian skeletal muscle is ultimately exchangeable with plasma potassium. However, Ginsburg and Wilde showed that 24–48 hours were required for a close approach to exchange equilibrium *in vivo* (1). A part or parts of the total appeared to equilibrate more rapidly than the rest, but the experiments gave little or no indication of the mechanisms responsible for the inhomogeneity of muscle potassium. An earlier publication (2) showed that diffusion of antipyrine, urea and sucrose from blood to tissues in isolated legs of cats took place at two rates, designated fast and slow, and that the fraction of the total distribution volume of each substance exchanging rapidly or slowly was dependent on the rate of blood flow and on the state of dilatation or constriction of the blood vessels. Accordingly, it seemed most reasonable to interpret the two divisions of tissue mass as representing well-circulated and poorly-circulated regions of tissue. If this hypothesis of a double (possibly multiple) local circulation is correct, the partition of tissue potassium

into rapidly exchanging and slowly exchanging fractions might be expected to follow along the same lines. Since analysis of exchange kinetics in intact animals and even in isolated, perfused legs is complicated by a multiplicity of organs and tissues, it seemed best to use a single skeletal muscle for study of the exchangeability of muscle potassium.

THEORY

In the preceding paper (3), the observed, or apparent extraction of K^{42} from the bloodstream by a perfused muscle was defined in relation to arterial blood radioactivity:

$$E = \frac{A(a) - A(v)}{A(a)} \qquad (1)$$

$A(a)$ and $A(v)$ represent the radioactivity due to K^{42} in arterial and venous blood, respectively. Since the concentrations of carrier K^+ in arterial and venous plasma are the same, these terms are proportional to arterial and venous K^+ specific activity. It was shown that E did not remain perfectly constant over a period of time, but decreased slowly as K^{42} accumulated in the tissues. Only the value measured at zero time (E_0) was a true measure of extraction, although for short periods, relatively small errors were introduced by ignoring this fact. To obtain an expression for K^{42} extraction constant for all values of time (true extraction, E') it is necessary to express extraction in relation to the difference in tracer specific activity between arterial blood and the tissues:

$$E' = \frac{A(a) - A(v)}{A(a) - A(t)} \qquad (2)$$

where $A(t)$ represents the specific activity of K^{42} in the tissues. Thus the relations between apparent extraction (E) and true extraction (E') are as follows: at zero time,

$$A(t) = 0, \quad \text{and} \quad E_0 = E' \qquad (3)$$
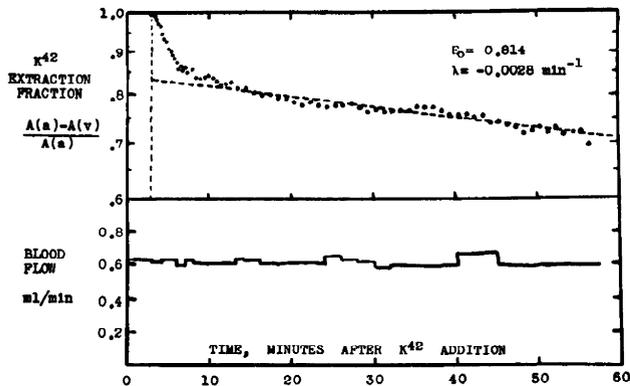
FIG. 1. Equilibration of K^{42} between blood and tissue in an isolated, perfused skeletal muscle. *Exp. P-26*, gracilis, 11.2 gm. Perfusion fluid: blood, hct. 0.47, $[K^+]_p$ 4.28 μEq/ml. Perfusion pressure (mean) 41 mm Hg, mean blood flow 0.62 ml/min. Fat-free muscle weight was 9.68 gm, total K^+ 92.7 μEq/gm or 898 μEq in the whole muscle.

at all other times

$$E = E' \frac{A(a) - A(t)}{A(a)} = E_0 \frac{A(a) - A(t)}{A(a)} \qquad (4)$$

For a homogeneous mass of tissue carrier equilibrating with a tracer supplied at constant specific activity in the blood, the ratio between tissue and blood specific activity of tracer is described by the following relation (see *eq. 8*, ref. 4);

$$\frac{A(t)}{A(a)} = 1 - e^{-(R/K)t} \qquad (5)$$

R is the rate of influx of K^+ into the tissue and K the quantity of exchangeable potassium there. The symbol e is the base of natural logarithms and the t in the exponent signifies time. By rearranging terms in the above,

$$1 - \frac{A(t)}{A(a)} = \frac{A(a) - A(t)}{A(a)} = e^{-(R/K)t} \qquad (6)$$

Combining this with *equation 4* leads to the final result:

$$E = E_0 e^{-(R/K)t} \qquad (7)$$

This equation describes theoretically the fall of apparent K^{42} extraction with time in the course of an experiment such as that illustrated in figure 1A of the preceding paper (3). If E is plotted on a logarithmic scale against time, the slow phase of the curve which follows the vascular washout period should be a straight line with an intercept on the ordinate equal to E_0 and a negative slope equal to R/K. As was shown in the previous article, the influx rate R can be determined from E_0, the blood flow and the blood content of exchangeable K^+ (*eq. 3*, ref. 3), and therefore the quantity of potassium which exchanges at this rate (K) can be determined.

A method similar in principle to this was used by

Conn and Robertson (5) in their study of K^+ exchange in the mammalian heart *in situ*. Their analysis of the exchange kinetics is more refined than that presented here in that it takes into account possible interactions between the initial rapid component (which I designated vascular washout) and the ultimate slow component of the exchange process. Application of their equations to the present experimental data yields almost exactly the same values for exchangeable K^+, and therefore only the simpler method is described here. For reasons presented in an appendix to this paper their method for computing the cell membrane exchange rates of K^+ is not applicable to skeletal muscle.

METHODS

Isolated gracilis or gastrocnemius muscles of dogs were perfused with blood to which trace amounts of K^{42} were added, as described previously (3). Blood flow and arterial and venous blood radioactivity were measured continuously, and blood hematocrit and plasma $[K^+]$ at intervals in order to permit calculation of K^+ influx as described there. In several experiments, a perfusion fluid free of cellular elements was prepared by mixing dog plasma with a solution of purified human hemoglobin, the final HbO_2 concentrations being 4–5 gm/100 ml and the plasma protein concentrations 2–3 gm/100 ml. The concentrations of K^+, Na^+, HCO_3^- and glucose in such artificial perfusates were adjusted to nearly normal values. The hemoglobin was prepared by the method of Hamilton *et al.* (6) and was dialyzed against mammalian Ringer's fluid before use. These artificial perfusates appeared to be adequate with respect to protein osmotic pressure and oxygen transport in experiments on resting muscles of 2–4 hours duration. The reason for their use was to test the possibility that exchange of K^{42} between plasma and erythrocytes might introduce errors into the measurement of K^+ exchange between plasma and muscle cells.

In the present series of experiments, blood flow was kept within a small percentage of a fixed value for periods of 30 minutes to 3 hours, to permit careful measurement of the fall in apparent K^{42} extraction from the blood. Total muscle K^+ was measured at the end of each experiment by digestion of the entire muscle or several representative pieces of it in dilute HNO_3, followed by flame photometry with Li^+ as an internal standard.

RESULTS

I. Quantity of Exchangeable Potassium in Perfused Skeletal Muscles

Figure 1 illustrates the determination of exchangeable K^+ in a typical experiment at constant blood flow. The rate of flow in this particular experiment was low, in consequence of selection of a low arterial perfusion pressure, and K^{42} extraction relatively high. Apparent extraction is plotted on a logarithmic scale against time. After the initial vascular washout (10 min. at this low
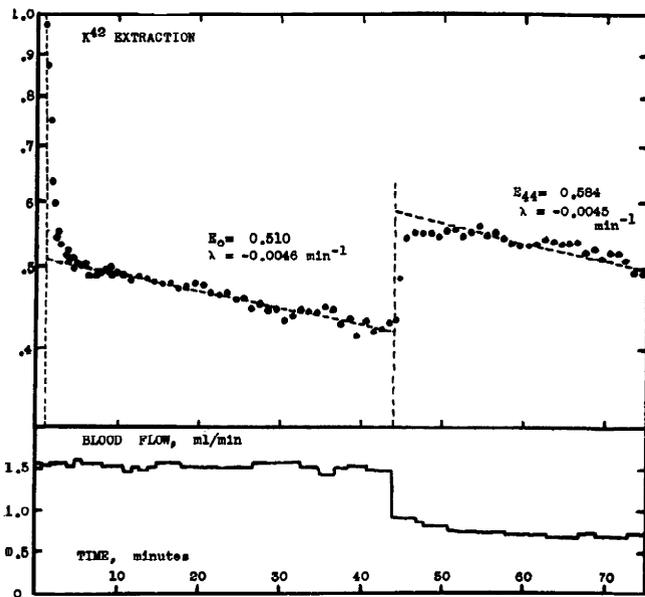
FIG. 2. Effect of suddenly changing blood flow on equilibration of K^{42} between blood and tissue. *Exp. P-28*, gracilis 17.3 gm. Perfusion fluid a cell-free solution of HbO_2 (4.5 gm/100 ml) and plasma proteins (2.5 gm/100 ml) in HCO_3^- buffered Ringer's fluid, $[K^+]_p$ 4.30 μEq/ml. Total muscle K^+ 1380 μEq. *1st period* (0–44 min.): mean blood flow 1.70 ml/min., E_0 0.510, R 3.74 μEq/min., K 810 μEq, or 0.59 of the total. *2nd period* (44–78 min.): mean blood flow (54–78 min.) 0.73 ml/min., E at 44 min. by extrapolation 0.584 E' (0.584 ÷ 0.815) or 0.718 (see text), R 2.25 μEq/min., K 505 μEq, or 0.37 of the total present.

flow), the curve follows a very nearly linear course for 50 minutes. Linear relations were observed in all experiments, providing blood flow was maintained with sufficient constancy, and there were no changes in vascular tone. Extrapolation of the straight line back to 3 minutes (blood transit time through the vascular bed, indicated by the first appearance of radioactivity in the venous blood), yields $E_0 = 0.814$. K^{42} clearance was thus 0.62 ml/min. $\times$ 0.814 $= 0.505$ ml/min. K^+ influx from blood to tissue was 0.505 ml(blood)/min. $\times$ 4.28 μEq K/ml plasma $\times$ (1 − 0.47) ml plasma/ml blood $= 1.14$ μEq/min. The slope of the exponential part of the curve is −0.0028/min., and therefore (*eq. 7*):

$$\text{Exchangeable } K^+ = \frac{K^+ \text{ influx}}{- \text{ slope}} = \frac{1.14 \,\mu\text{Eq./min.}}{0.0028/\text{min.}} = 520 \,\mu\text{Eq.}$$

Since total tissue K^+ measured directly was 898 μEq in this muscle, only 0.58 of the total appeared to be exchangeable with blood K^+. In 17 determinations on nine perfused muscles, ratios of 0.18–0.88 were observed (table 1).

Discussion. The experimental results conform to theoretical prediction within the capacity of the present methods to measure. However, only a fraction of the total tissue K^+ appears to exchange with the blood at the rate measured. This cannot result from trapping of plasma K^+ by slow exchange with that in circulating erythro-

cytes, for the exchangeable fractions measured with erythrocyte-free perfusion fluids are quite comparable with those obtained with whole blood. Nor does it appear likely that the unaccounted for K^+ represents K^+ trapped in nonperfused parts of the experimental muscles. In earlier studies (2) using similar perfusion techniques on the entire hind legs of animals, it was shown that distribution of antipyrine and urea conformed closely to the total tissue water, and in a few experiments on the present preparation (unpublished), antipyrine and Na^{24} were observed to equilibrate almost completely with total tissue water and with extracellular water, respectively, in the course of an hour. In the experiments of Ginsburg and Wilde on intact animals, all muscle K^+ was ultimately exchangeable (1), and there is no reason to believe that all tissue K^+ in these isolated muscles would not equilibrate with plasma K^+ if the perfusion experiments could be run as long as the 24–48 hours required *in vivo*. It seems most reasonable to conclude that the tissue K^+ presently unaccounted for is not inexchangeable, but exchanges with the blood at a rate much slower

TABLE 1. *Summary of Experimental Data*

Hct.	$[K^+]_p$, μEq/ml	pA, mm Hg	Q, ml/min. 100 gm	E',	R, μEq/min. 100 gm	$\frac{K}{K_{tot.}}$	PS,† ml/min. 100 gm
Experiment P-5							
0.00	4.60	70.	4.3	0.608	12.0	0.47	4.0
0.00	4.60	120.	7.2	0.440	14.5	0.67	4.2
Experiment P-7							
0.00	5.23	60.	6.5	0.534	18.1	0.82	4.9
Experiment P-8							
0.47	3.60	125.	3.3	0.706	4.5	0.39	4.1
0.47	3.60	180.	7.4	0.487	6.9	0.66	4.9
Experiment P-10							
0.33	5.38	87.	11.2	0.482	19.4	0.58	7.4
Experiment P-11							
0.48	4.00	80.	4.6	0.728	7.0	0.24	6.0
0.48	4.00	50.	2.3	0.928	4.4	0.18	6.1
0.48	4.00	110.	5.7	0.688	8.2	0.33	6.7
*Experiment P-19**							
0.55	5.22	131.	19.5	0.458	21.0	0.74	11.9
Experiment P-26							
0.53	4.28	41.	5.5	0.814	9.1	0.51	9.2
0.51	4.58	82.	12.3	0.597	16.5	0.88	11.2
Experiment P-27							
0.47	5.27	85.	8.5	0.492	11.7	0.39	5.8
Experiment P-28							
0.00	4.30	35.	9.9	0.510	21.6	0.59	7.0
0.00	4.30	19.	4.2	0.718	13.0	0.37	5.3
0.58	4.18	113.	8.9	0.505	7.8	0.28	6.2
0.58	4.18	72.	4.8	0.621	5.2	0.24	4.7

Note. It will be observed here and also in fig. 3 that K^+ influx rates in red cell-free perfusion experiments tend to be higher at comparable rates of flow than in perfusions with whole blood. However, K^{42} clearances in these experiments do not differ appreciably from similar blood perfusions. The greater K^+ influxes are due entirely to the greater supply of plasma K^+ by cell-free perfusates, since the 'plasma' makes up the whole bulk of these fluids in contrast to only about half of the bulk of blood. This effect illustrates the supply dependence of K^+ transport in perfused muscles. * *Exp. P-19* was on a gastrocnemius muscle; all the others are graciles. † *PS* was calculated from the following relation (3): $E' = 1 - e^{-(PS/Q)}$.
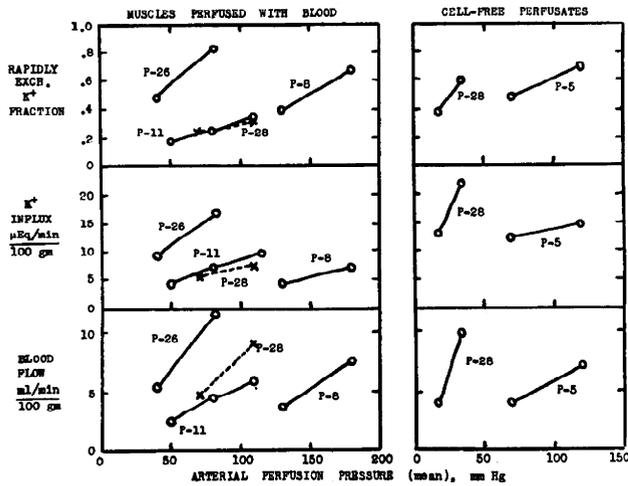
FIG. 3. Relation of rapidly exchanging K+, K+ influx (blood to tissues) and blood flow in perfused skeletal muscles.

than the rate measured. Therefore, the fraction of tissue K+ exchanging at the rate measured in these experiments will be designated rapidly exchanging, and the remainder, slowly exchanging. Whether the latter fraction comprises a single part, or a series of parts exchanging at successively slower rates cannot be determined by experiments of the present type.

II. Effects of Changes in Blood Flow on the Relative Fractions of Rapidly and Slowly Exchangeable Potassium

In five experiments, the measurements described above were made at two or more different rates of blood flow, produced by suitable adjustment of arterial perfusion pressure. The results obtained are included in table 1. In *experiment P-26*, two separate equilibrations of the kind illustrated in figure 1 were run, between which part of the K^{42} accumulated in the tissues was removed by washing out with nonradioactive blood. In other experiments, a simpler procedure was followed. After perfusion at the initial rate had gone on long enough for accurate measurement of the slope of the extraction curve, arterial pressure was suddenly changed, bringing blood flow to a new level. An example of this procedure is shown in figure 2. After flow was lowered, K^{42} extraction rose to a higher value, and when the flow became steady, the curve fell exponentially much as before. K+ influx and rapidly exchangeable K+ are computed as before, except that in order to determine E' for the second flow rate, allowance must be made for the accumulation of K^{42} in the tissues during the first period. From *equations 7* and *4*, the relation between E' and E at any time may be expressed as follows:

$$E' = E \div e^{-(R/K)t} \qquad (8)$$

At the end of the initial 44 minutes,

$$E' = E \div e^{-0.0046/min. \times 44min.} = E \div 0.815.$$

Extrapolation of the second part of the curve back to the time of change (using only the linear part of the curve

obtained during constant blood flow) yields an apparent E of 0.584, and $E' = 0.584 \div 0.815 = 0.718$. The rest of the computations are given in the legend to the figure. The fraction of exchangeable K+ at the lower flow is distinctly less than at the higher. In all experiments, the fraction of rapidly exchangeable K+ was greater at the higher blood flow rates. K+ influx rates were also greater at higher flows, as described in the previous article (3).

Discussion. In individual muscle preparations, the fraction of rapidly exchanging tissue K+ change with the blood flow in a consistent fashion. Qualitatively, at least, they parallel the increased K^{42} clearance and K+ influx which accompany increased blood flow (3). However, the widely disparate fractions observed from preparation to preparation suggest other factors contributing to the partition of total tissue K+. Again consistent with previous observations on the exchange rate, a large part of the individual variation is associated with differences in spontaneous vascular tone. To demonstrate this relation, figure 3 depicts in parallel graphs the simultaneous relations of rapidly exchangeable K+ fraction, K+ influx rate and blood flow to the arterial perfusion pressure in the five experiments cited above. The positions of the blood flow-pressure curves define the vascular resistance of each preparation. The muscles perfused with cell-free perfusates are plotted separately, since due to the low viscosity of these fluids (approx. 0.4 that of blood), they are not directly comparable with the others. The more vasodilated muscles have higher influx rates and larger fractions of rapidly exchangeable K+ for given perfusion pressures. The parallelism is not perfect, however, and just as in the case of the effects of vascular tone on K^{42} clearance and K+ influx, other factors must be operative.

GENERAL DISCUSSION

In view of the evidence presented in this paper and in the preceding one (3), it is concluded that the partition of tissue potassium is largely the result of nonuniform distribution of blood flow throughout the vascular bed. The rapidly and slowly exchanging fractions correspond to well circulated and poorly circulated regions in the muscle. On this basis, the observations presented above can be used to differentiate among the three alternative patterns of non-uniform capillary circulation described in the preceding paper (3).

a) If the nonuniformity took the form of arteriovenous shunting of part of the total blood flow, rapidly exchangeable K+ should equal total tissue K+, and remain constant despite variations in the rate of K+ exchange with blood flow. Thus arteriovenous shunting is eliminated as the principal form of circulatory inhomogeneity.

b) If the vascular bed were partitioned into fixed well perfused and poorly perfused regions, there would be no reason to expect an increase in the size of the rapidly exchanging K+ fraction with an increase in blood flow. The presence of invariant regions of well circulated and poorly circulated tissue in the perfused muscles cannot

be ruled out, but it is clear that such partition cannot be responsible for the variability of exchangeable K+.

c) The only pattern on nonuniformity consistent with the present observations resembles that above, but with the ratio between the well perfused and poorly perfused compartments variable, as a consequence of changes in minute vessel hemodynamics. As arterial pressure increases, blood flow through the initially poorly perfused capillaries must increase more rapidly, in proportion, than the flow through the already well perfused capillaries. This may be brought about by opening or widening of arterioles which were partially or completely closed, due to the increase of pressure within them. As a result, poorly circulated regions would be converted into well circulated regions. The effects of changes in vascular tone on the fraction of rapidly exchanging tissue K+ can be explained by exactly the same mechanism, with contraction or relaxation of vascular smooth muscle initiating the change in blood flow distribution. The ratio of rapidly exchanging to total tissue K+ would be a measure of the relative size of the well perfused compartment in terms of tissue mass, and the *PS* product largely a measure of the functional capillary surface per unit mass of tissue.

The relation of the heterogeneously circulated tissue regions to the microscopic anatomy of the vascular bed remains unknown, nor do the present observations provide any information concerning the significance of nonuniformity of transcapillary exchange to local metabolic processes. Both questions appear to merit attention. The possible role of vasomotor control of transcapillary exhange through modification of the nonuniform circulatory pattern also deserves study.

APPENDIX

Determination of Cell-Membrane K+ Exchange Rates

Exchange of K+ between plasma and cells does not take place directly, but through the medium of the interstitial fluid compartment; and it is not generally possible to determine the rate of cell-membrane transport from measurements of over-all blood-tissue transport. The relation between these two quantities may be developed as follows. Since there is relatively little K+ in the interstitial fluid in comparison with the intracellular fluid, it appears reasonable to assume that after the first few minutes of diffusion, the interstitial fluid compartment is in a steady state with respect to movement of tracer:

$$R_{12}^* = R_{23}^* = Q_p E A(a)[K^+]_p \qquad (a1)$$

R_{12}^* and R_{23}^* are the tracer fluxes between plasma and interstitial fluid and between interstitial fluid and cells, respectively. Q_p is the plasma flow, E the K^{42} extraction, $A(a)$ is the specific activity of K^{42} in the arterial plasma and $[K^+]_p$ the carrier K+ concentration. The relation of the carrier fluxes (R_{12} and R_{23}) to their respective tracer fluxes is:

$$R_{12} = R_{12}^* \div A(a) \qquad (a2)$$

$$R_{23} = R_{23}^* \div A(i) \qquad (a3)$$

R_{12} is the over-all blood-tissue exchange rate described in the body of this paper. In order to determine R_{23}, the cell-membrane exchange rate, it is necessary to know $A(i)$, the specific activity of tracer in the interstitial fluid. Unfortunately, this is generally unobtainable.

In their study of K+ exchange in the heart, Conn and Robertson (5) calculated cell membrane exchange of K+ on the assumption that $A(i) = A(v)$, where the latter term represents the specific activity of K^{42} in venous blood. This assumption implies that coronary blood reaches complete diffusion equilibrium with cardiac interstitial fluid in a single transit of the capillaries, and therefore that K+ exchange between plasma and interstitial fluid in the heart is entirely blood flow limited. This may be a reasonable assumption for cardiac muscle, but in view of the present observations on skeletal muscle, it is clearly not valid for this tissue (3). Complete equilibration is not attained in a single transit, and $A(i)$ must be less than $A(v)$ by a variable amount. If the attempt is made to calculate cell membrane exchange of K+ by this method, even as a minimal estimate of the true exchange rate, the results vary widely and show no uniform tendency to approach an upper limit either at very low blood flows (where $A(v)$ might be expected to approach $A(i)$) or at very high flows (where R_{12} might be expected to approach R_{23}).

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