METABOLISM OF TISSUE CULTURES

III. A METHOD FOR MEASURING THE PERMEABILITY OF TISSUE CELLS TO SOLUTES*

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Two important factors concerned in the dynamics of tissue growth are (a) the availability of circulating and extracellular foodstuffs to cells (permeability) and (b) the synthesis of these foodstuffs into protoplasm (metabolism). It is clear that either permeability or metabolism might be a primary factor limiting the growth rate of tissues, and it is important to know whether tumor growth is associated with any specific anomaly in tumor cell permeability. For technical reasons much less is known about the permeability of tissue cells than about their metabolic functions. The present report describes a method by which both of these factors may be quantitatively studied in cultures of normal and tumor cells with the aid of radioactive isotopes. The results obtained by this method will be presented in succeeding papers.

Although many aspects of tissue metabolism can be investigated without the use of intact tissues, as in tissue slices or cell-free extracts, permeability relations between cells and their environment present a more difficult problem. Such is the delicacy of the processes whereby substances enter the cell that any deviation from the normal state of the tissue may cast doubt on the applicability of the results obtained. An understanding of permeability processes is fundamental to our knowledge of over-all cell metabolism, yet for only one type of cell, the erythrocyte, are there direct means for measuring these processes.

The erythrocyte is by no means an ideal cell for studying cell permeability in general, but it has the practical advantage of being accessible in large quantities and easily separable from its extracellular environment. Even with erythrocytes isotope techniques have been of great value, since they alone permit a distinction between static and dynamic equilibria and the measurement of rates (1). With isotope methods the rate of movement of substances across a barrier, as well as of metabolic turnover, can be measured without altering concentrations of the substances being studied or other environmental conditions.

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By tissue culture methods, tissue cells can be obtained in a relatively intact state for observation and analysis. In previous work, it has been possible to calculate permeability of normal and tumor cells to water by measuring the rate of their endosmotic swelling (2). The present method is based on the roller bottle method of culture (3); its adaptation to measurement of radioactivity of the cells in a culture, independently of that in the culture medium, makes possible continuous chemical measurements of the rate of passage of an isotope between medium and cells. Permeability of cells to potassium and phosphate ions has been successfully studied by this method.

Technique

Tissues are cultivated in roller bottles bearing a window on one side to which a thin coverslip is attached. The inner side of the coverslip is moistened with chicken plasma (0.02 to 0.05 ml.) and 10 to 100 mg. of minced tissue is distributed over it. The wet tissue is weighed rapidly to the nearest milligram in a small covered Petri dish. After coagulation of the very thin plasma layer, 4 ml. of a medium not containing the isotope are introduced into the bottle, and rotation is begun. The control period of incubation without radioactive medium (preincubation period) lasts at least 4 to 18 hours to allow the repair of traumatized tissue and the release of intracellular material into the medium as a result of cutting cells. We have found that 30 to 50 per cent of the phosphate originally present in tissue is lost in the process of mincing and explantation; an additional small loss of phosphate takes place within the next hour or two (4).

At the end of the preincubation period, the medium is drawn off as completely as possible and radioactive medium (4 ml.) is substituted. The new medium is made up exactly like the original medium except for the addition of the radioactive substance (potassium or phosphate) in place of the corresponding non-radioactive component, and it is warmed to the temperature of the incubating culture before substitution. It is introduced into the bottle in such a way that it does not come in contact with cells. The bottle is then rotated slowly once or twice to achieve the usual distribution of medium and cells, and the initial radioactivity reading is made. Thus, the small amount of radioactive medium held in the angle where the coverslip is joined to the bottle is included in this control radioactivity reading (C_0) before penetration of the tissue has occurred. The bottle is kept rotating about its axis of symmetry, and readings are made at intervals by means of a Geiger-Müller counter in the incubator. At the conclusion of an experiment the medium is withdrawn, tissue is washed gently with saline solution, and removed for analysis. Total phosphorus (or potassium) is determined in cells and media and radioactivity measurements are made on both.

Radioactivity readings are made with the bottle in the position shown in Fig. 1. In addition to the radioactivity of the cells, a contribution is made to the total reading by the radioactivity of the medium. This contribution is reduced by the position of the bottle to 5 to 10 per cent of the value the same amount of P^{32} would give in the position of the tissue. This is due to the

facts that the beta particles responsible for all or nearly all of the radiation from the isotopes used (1) have a much more restricted solid angle with respect to the counter than those emanating from the tissue, (2) are partially absorbed in the medium, and (3) have to traverse a longer air path between the medium and the counter.

Calculation of True Radioactivity of Cells.—As the radioactive isotope is taken up by the cells of the explanted tissue, there is then a progressive increase in the apparent radioactivity of the bottle read in the position shown in Fig. 1, as the isotope is translocated to a position close to the counter (a correction of each reading being made for radioactive decay, of course). Since a concurrent drop occurs in that contribution which the medium makes to the reading, increments in the reading are not exactly equal to the radioactive

FIG. 1. Diagram illustrating relative positions of culture bottle and Geiger-Müller counter while readings of tissue radioactivity are being made.

uptake, but are proportional to it. At the end of the experiment the radioactivity of medium and tissue is determined by placing ashed samples in porcelain capsules under the counter. In this way the final radioactivity of the cells is determined under the same conditions used for determining radioactivity of media. This is called the true final cell radioactivity (P'_{if}). Using this value, the final reading (R_f), and the control reading (C_0), the true cell radioactivity at any time can be calculated from the reading at that time, according to the following calculation.

The apparent radioactivity of the whole bottle at any time is made up of two components, that from the cells and that from the medium, and is the sum of the two. Let

- R = apparent activity of bottle at time t (reading)
- A = apparent activity of cells at time t
- C = apparent activity of medium at time t

Then

$$R = A + C, \text{ and } R_0 = C_0 \text{ (when } A_0 = 0\text{)}$$

Since C is proportional to the amount of radioactivity remaining in the medium,

$$C = \frac{P' - P'_i}{P'} C_0$$

where P' = true total activity in bottle, and P'_i = true activity in cells. Since the apparent and true cell activities are always proportional,

$$A = \frac{A_f}{P'_{if}} P'_i$$

where A_f = observed final activity of cells (in bottle) and P' = true final activity of the cells.

Whence,

$$P'_i = K(R - C_0), \text{ where } K = \left(\frac{A_f}{P'_{if}} - \frac{C_0}{P'} \right)^{-1}$$

Since K in the last equation is constant independently of time, true cell activity is always proportional to the difference between the reading at any time and the control reading, and in the case of the final reading,

$$P'_{if} = K(R_f - C_0), \text{ or } K = \frac{P'_{if}}{R_f - C_0}$$

Therefore,

$$P'_i = \frac{P'_{if}}{R_f - C_0} (R - C_0)$$

True cell radioactivity is calculated from the readings at each time interval, and a curve of uptake of the isotope is drawn.

Uptake of Radioactive Potassium and Phosphate.—Curves giving the results of typical experiments on the accumulation of K^{42} and P^{32} are shown in Figs. 2a and 3a. These cultures were grown in a peptone medium which, though incomplete, allows excellent peripheral cell growth (4). The curve for K^{42} uptake approaches asymptotically a limiting value where the specific activity (K^{42}/K) of the cells is equal to that of the medium. On the other hand, the turnover of phosphorus is far from completion even after several hours, although a rapid rate is observed at first. If one assumes that only the inorganic phosphate in the cells is capable of exchange with extracellular radioactive phosphorus, which is entirely inorganic in the medium used, it will be seen that a rapid exchange between the medium and the diffusible inorganic phosphate of the cell would result in a rapid partial turnover, followed by a slower rate of P^{32} uptake as the turnover of organic compounds caused further P^{32} exchange. On this basis, the rate of entry of P^{32} into the inorganic phos-

phate of the cells becomes of the same order of magnitude as that of K^{42} into the whole cell potassium.

Proof of these assumptions regarding the behavior of inorganic and organic phosphorus is given by the data in Fig. 4. In this experiment, a group of parallel cultures was run and individual cultures were sacrificed at intervals. The tissues were then extracted *in situ* twice during 24 hours with cold 5 per cent trichloroacetic acid. From this extract of the acid-soluble phosphorus,

FIG. 2. Uptake of K^{42} by chick embryo muscle.

(a) Curve of observed uptake (continuous line), showing calculation of k_d from the initial slope (broken line).

(b) Logarithmic plot used in calculation of k .

The data used in calculation of these constants are the observed values of P'_d , and the following:

$$\begin{aligned} P &= 540 & S_{eo} &= 0.165 \\ P_d &= 140 & S &= 0.122 \\ SP_d &= 17.1 \end{aligned}$$

Derived constants: $k_d = 19.6$, $k = 0.19$, $k_1 = 0.14$.

inorganic phosphate was precipitated by an ammoniacal magnesium mixture. The specific activity (P^{32}/P) of the cell inorganic phosphorus is seen to rise rapidly while that of the remainder of the cell phosphorus (composed of acid-soluble organic, alcohol-soluble, and "residual" P) is always much less than the inorganic phosphorus specific activity. Separate analysis of the acid-soluble organic fraction, and of the alcohol-soluble and alcohol-insoluble components of the acid-insoluble residue, shows that all of these have lower specific activities than the inorganic component. This favors the belief that the inorganic P of the medium penetrates as such and is subsequently converted into organic forms.

Calculation of Rate Constants¹

Potassium.—The curve for accumulation of K^{42} intracellularly at 37°C ., shown in Fig. 2, appears at first sight to indicate that equilibrium between

FIG. 3. Uptake of P^{32} by chick embryo muscle.

(a) Curve of observed uptake (continuous line), showing calculation of k_d from the initial slope (broken line).

(b) Logarithmic plot used in further calculations. Above, calculation of β ; below, calculation of α . Note that the shaded area in the upper diagram is used in deriving the lower curve (see text). The α and β lines meet the origin at $\ln A$ and $\ln B$, respectively.

Data used are the observed values of P'_i , and the following:

$P_e = 50$	$S_{e0} = 0.98$
$P_d = 9$	$S = 0.48$
$P_n = 42$	$SP_i = 24.8$

Derived constants:

$A = 4.5$	$B = 20.3$
$k_d = 1.56$	$k_1 = 0.174$
$k_n = 0.50$	$k_2 = 0.012$

¹ In the subsequent discussion, the following symbols are used:

t = time in hours.

P = mass (micrograms) of the labeled atom (P or K) in the entire culture bottle.

P_e = mass of P or K in the medium.

P_i = mass of P or K in the cells.

P_d = mass of the "diffusible" component in the cells.

P_n = mass of the "indiffusible" component in the cells.

(Footnote continued on following page)

FIG. 4. Specific activity changes in cell inorganic phosphate. Upper curve: specific activity of medium (S_e). Lower curves: calculated theoretical specific activity of the diffusible (inorganic) phosphorus of cells (S_d); (a) corresponds to case *a* (4) in which organic phosphorus exchanges directly with phosphate of the medium, and (b) to case *b* (4) in which medium phosphate first exchanges with cell inorganic phosphate. Observed values are for cultures analyzed at 1, 2, 6, and 26 hours (two cultures in each series). The eight cultures analyzed were of comparable weight and maintained under the same conditions as the culture shown in Fig. 3. The theoretical curves are derived from the constants given there.

Note: It can be shown from equations (5) that S_d should be constant and maximal when $(S_e - S_d)/(S_d - S_n) = k_n/k_d$. In the case illustrated, this is approximately true between 15 and 25 hours.

(In the case of potassium, P_i and P_d are assumed identical; in the case of phosphorus P_d is assumed to be the cell inorganic phosphate.)

Then,

$$P = P_e + P_i = P_e + P_d + P_n$$

P' = amount of radioactive isotope (millimicrocuries) in the culture bottle.

P'_e, P'_i, P'_d, P'_n correspond to fractions with the same subscripts above.

$$S = \text{specific activity of entire culture} = \frac{P'}{P}$$

S_e, S_i, S_d, S_n are specific activities of parts of the culture; e. g., $\frac{P'_e}{P_e}$ etc.

Further subscripts indicate the time of the observation; e. g.,

S_{e0} = specific activity of medium as placed on the culture.

S_{et} = specific activity of medium when removed from the culture.

intra- and extracellular potassium $P_e \rightleftharpoons P_d$ is a monomolecular exchange uncomplicated by any competing or following reactions. Then if k_d represents the rate of passage of potassium into the cell in exchange for potassium coming out,

$$k_d = \frac{dP}{dt} \text{ (}\mu\text{g./hour)} \quad (1)$$

and the accumulation of K^{42} (designated as P') intracellularly should be given by the expression:

$$\frac{dP'_d}{dt} = k_d (S_e - S_d) \quad (2)$$

S_e and S_d being the specific activities of the external and internal (diffusible) phases. Upon integration, this becomes:

$$S_d = \frac{P'_d}{P_d} = S(1 - e^{-kt}), \text{ where } k = \frac{k_d S_{e0}}{P_d S} \quad (3)$$

Thus the initial rate is determined by S_{e0} , the initial specific activity of the medium, and the final equilibrium by S , that of the entire system. It is S_d/S which approaches 1 exponentially and not S_d/S_e , although the latter is customarily termed the relative specific activity.

It will be seen that k_d depends on the size of the explant; to make results on cultures of different sizes comparable, it is necessary to use $k_1 = k_d/P_d$. Since S_{e0} and S are related by the expression:

$$\frac{S}{S_{e0}} = \frac{P_e}{P_e + P_d}, \text{ and } k_1 = k \frac{P_e}{P_e + P_d},$$

k_d is now equal to the "turnover rate" as customarily defined, and k_1 is the reciprocal of the "turnover time" (5) in hours, being the proportion of the intracellular potassium exchanged per hour.²

An approximate estimate of k_d can be made by drawing a line tangent to the uptake curve at its origin as shown in Fig. 2a. According to equation (2) above, the slope of this line will be $k_d \cdot S_{e0}$ at $t = 0$. The constants can better be derived, using the entire series of observations, by plotting $\log_e (SP_d - P'_d)$ against time. If the assumption were correct that the exchange is mono-

² It will be noted that the turnover rate is defined in terms of an absolute amount of potassium, and the turnover time in terms of an amount relative to that present in the cells. At the turnover time, the cell specific activity would, with infinite medium, be equal to $1 - 1/\epsilon$ times its final value. Under the conditions stated above, this value ($S_d = 0.693S$) would be reached at a time equal to the turnover time multiplied by $\frac{P_e}{P_e + P_d}$.

molecular and if the tissue potassium content (P_d) remains constant, the (negative) slope should be a straight line equal to k in equation (3) above, from which k_d and k_1 may be derived. This derivation of the constants from the data is shown in Fig. 2b.

As will be shown in a future paper, potassium penetration at low temperatures, and also probably at 37° , is actually a more complicated process than the one described here.

Phosphate.—The case of phosphate penetration is complicated by additional reactions, since phosphate is converted into organic forms existing in the cells. This is illustrated by a plot of $\log_e(SP_i - P'_i)$, (Fig. 3b). This line shows a steep portion during the first few hours, which is thought to represent the period during which penetration of phosphate into the cell inorganic phosphate occurs ($P_e \rightleftharpoons P_d$). The later decrease in slope then represents metabolic turnover of organic phosphorus within the cell, as this reaction predominates. Since this secondary slope is approximately a straight line in the region with which we are concerned, and since we are primarily interested in the first process (permeability), we have made the simplifying assumption that the much slower metabolic turnover may be expressed by a single rate constant, although it must involve several reactions. We may now assume either (a) that cell organic phosphorus is in equilibrium directly with phosphate in the medium, or (b) that it is in equilibrium only with the small amount of intracellular inorganic phosphate; *i.e.*,

In the first case the calculations would be somewhat simpler, since the final reaction would be a summation of two reactions similar to that discussed above. The second case (b) seems more likely on *a priori* grounds, and this is borne out by the curve for cell inorganic phosphate specific activity, which as will be shown fails to rise promptly to meet that of the medium. If, as suggested by Sacks and Altshuler (6), phosphorus enters the cell through phosphorylation reactions at the cell surface, our data indicate that the rate of this process will still be best described by case (b). The following calculation is based on the equilibrium (b) above, assuming that the net amount of phosphorus in each of the three compartments is unchanged.

If we consider the rate of passage of phosphorus in either direction between medium and inorganic cell phosphate in micrograms per hour as $k_d = \frac{dP_e}{dt}$, and the rate of exchange between cell inorganic and organic phases as $k_n = \frac{dP_n}{dt}$, k_d and k_n are the turnover rates for permeability and metabolic exchange, respectively. As before, $k_1 = k_d/P_d$ is the proportion of the intracellular phosphate turned over (with respect to the external phase) per hour, or the recipro-

cal of its turnover time; $k_2 = k_n/P_n$ is the proportion of the cell organic phosphorus turned over per hour, or the reciprocal of its turnover time. The movement of P^{32} through the phases can be expressed thus, as in (2):

$$\frac{dP'_e}{dt} = -k_d \left(\frac{P'_e}{P_e} - \frac{P'_d}{P_d} \right), \quad \frac{dP'_n}{dt} = k_n \left(\frac{P'_d}{P_d} - \frac{P'_n}{P_n} \right) \quad (5)$$

where $P'_e + P'_d + P'_n = P' = \text{constant}$, and P_e, P_d, P_n are constant. These simultaneous equations have the following solution:³

$$\begin{aligned} P'_e &= A_1 e^{-\alpha t} + B_1 e^{-\beta t} + SP_e \quad (SP_e = P'_e(t=0), \text{ etc.}) \\ P'_d &= A_2 e^{-\alpha t} + B_2 e^{-\beta t} + SP_d \\ P'_n &= A_3 e^{-\alpha t} + B_3 e^{-\beta t} + SP_n, \text{ with} \end{aligned} \quad (6)$$

$$A_1 + A_2 + A_3 = 0, \text{ and } B_1 + B_2 + B_3 = 0, \text{ and (considered at } t = 0)$$

$$A_1 + B_1 = P'_e - SP_e = SP_i, \quad A_2 + B_2 + SP_d = 0, \text{ and } A_3 + B_3 + SP_n = 0$$

Differentiating (6),

$$\frac{dP'_e}{dt} = \frac{dP'_i}{dt} = \alpha A_1 e^{-\alpha t} + \beta B_1 e^{-\beta t},$$

and the initial slope

$$\frac{dP'_i}{dt} \Big|_{(t=0)} = \alpha A_1 + \beta B_1 = (\text{from 5}) k_d S_{e0} \quad (7)$$

The constants in (6) now have the following relationships to those in (5):

$$\alpha + \beta = k_d \left(\frac{1}{P_e} + \frac{1}{P_d} \right) + k_n \left(\frac{1}{P_d} + \frac{1}{P_n} \right) \quad (8)$$

$$\alpha\beta = k_d k_n \frac{P_e + P_d + P_n}{P_e P_d P_n} \quad (9)$$

$$A_1 = \frac{k_d S_{e0} - \beta SP_i}{\alpha - \beta}, \quad A_2 = P_d A_1 \left(\frac{1}{P_e} - \frac{\alpha}{k_d} \right)^*, \quad A_3 = -A_1 - A_2 \quad (10)$$

$$B_1 = SP_i - A_1, \quad B_2 = -SP_d - A_2, \quad B_3 = -B_1 - B_2$$

* This is derived from the first equation in (5).

It is now possible to derive values for k_d and k_n from experimental data showing uptake of P^{32} by a culture by graphical analysis. This is done by plotting $\ln(SP_i - P'_i)$ against time (Fig. 3b); this is equal to $\ln(P'_e - SP_e)$ and (from 6) to $\ln(A_1 e^{-\alpha t} + B_1 e^{-\beta t})$. If $\alpha \gg \beta$, this will become, after a certain time, $\ln(B_1 e^{-\beta t})$. A line is therefore drawn along the latter straight part of this curve; its slope will be equal to $-\beta$ and the extrapolated line will intercept the ordinate at $t = 0$ to give $\ln B_1$. $B_1 e^{-\beta t}$ is then calculated for various time

³ We are indebted to Professor Philip Franklin of the Department of Mathematics, Massachusetts Institute of Technology, for deriving this solution.

intervals, $A_1 e^{-\alpha t}$ is obtained by subtraction, and α and A_1 are calculated in similar manner by plotting $\ln (A_1 e^{-\alpha t})$ against time. k_d is then obtained from the equation $k_d = \frac{\alpha A_1 + \beta B_1}{S_{e0}}$ and k_n from equations (8) and (9). The remaining constants are obtained by substitution into equations (10) and, finally, curves are drawn according to equations (6), which should correspond to actual uptake observations.

In making these calculations, P_d is assumed to correspond to the cell inorganic phosphate obtained from analyses of tissue, and P_n to the organic phosphorus.

An approximation method may also be used, which has the advantage of simplicity. The permeability constant can be approximated by drawing a line tangent to the uptake curve as was done in the case of potassium. This is shown in Fig. 3a. Using the first part of equation (5) above, this slope again is $k_d \cdot S_{e0}$ at $t = 0$, and it is clear that derivation of k_d in this way is valid regardless of the complexity of further reactions involving the isotope. The initial slope is always maximal at this time, since $S_e - S_d$ can only decrease as penetration occurs. A value for k_n can be obtained by assuming an approximate one and calculating the uptake curve (P'_i) from the above equations.

The appropriateness of the calculation has been checked by analyses of cultures sacrificed at intervals to obtain the specific activities of phosphorus in medium and in cell inorganic and organic phases. One series of analyses is shown in Fig. 4 together with theoretical values according to cases *a* and *b* (4). It will be seen that the cell inorganic specific activity S_d never rises to meet the external S_e , as would occur in case *a* (4) if the phosphorus of the medium were exchanging directly with the organic phosphorus of the cell as well as with its inorganic phase. The same tendency of the cell inorganic specific activity to reach an intermediate specific activity has been seen in two parallel experiments of eight cultures each, using chick embryo muscle and mouse sarcoma.

The examples given in Figs. 2 and 3 have been chosen to illustrate the techniques which have been used in deriving constants from data on the uptake of isotopes by tissues. In these examples, k_1 is for potassium 0.14 and for phosphate 0.174, indicating that the turnover times are about 7 and 6 hours, respectively. k_2 for phosphorus is 0.012. Approximately 60 additional cultures of chick embryo muscle cultivated under the same conditions have yielded data showing that these constants are representative of this tissue in culture. In future papers we shall present these data, together with parallel data on other normal and malignant tissues grown under various conditions.

The part played by cell division in the uptake of ions will be considered in a later paper. Preliminary experiments have indicated that the permeability rates (k_1) are little affected by complete inhibition of mitosis. k_2 , on the other

hand, is greatly influenced by growth, as might be expected from our data on nucleic acid phosphorus turnover in resting and growing liver (7).

Permeability Rates.—Although metabolic turnover is customarily expressed by rate constants such as the above (*i.e.*, indicating the rate by which turnover approaches completion) it is usual to express permeability differently. The term permeability implies the passage of material across a surface or membrane; its rate should therefore be proportional to the area of the surface. The fact that ions must cross the cell surface in order to enter the cell does not, of course, mean that this surface is the limiting factor determining the rate of exchange between medium and protoplasm. It is desirable, nevertheless, to express the data in terms of cell surface.

An approximate calculation of total cell surface in a culture can be made on the basis of individual cell measurements. A large series of such measurements were made on spindle-shaped chick embryo cells in a previous study (8). Cells varying between 400 and 2000 μ^3 in volume had ratios of surface to volume ($\mu^2:\mu^3$) of 0.5 to 0.85. Spherical cells of the same volume range, which might occur in denser areas, would have ratios from 0.39 to 0.65. The surface:volume ratio of such a culture might therefore be expected to fall between 0.3 and 1.0 μ^{-1} , if all cell surfaces are intact. Translating the ratios to $\text{cm}^2:\text{mm}^3$, we have taken the approximate surface area of cells to be 6.0 cm^2 per mg. wet weight of the tissue, recognizing a possible error in either direction by a factor of 2. Final wet weights of the cultures have been calculated from the phosphorus content of the tissue, which as has been shown previously is a reasonably constant measure of tissue weight (4).

Permeability can be calculated from k_d (micrograms passing into the cell per hour) by dividing this by the estimated cell surface in cm^2 , this in turn being derived from the cell phosphorus content or by direct weighing of the tissue at the end of the experiment. Converting micrograms to millimoles, permeability then appears in customary terms; *e.g.*, millimoles/ cm^2 /hour.

In the examples given, the permeability to phosphorus of a 31 mg. culture, where k_d was 1.56, is $p = 1.57 \times 10^{-7}$; that to potassium of a 36 mg. culture with $k_d = 19.6$, is $p = 14.0 \times 10^{-7}$. The latter figure may be compared with the permeability of erythrocytes to potassium, which for various species ranges between 0.5 and 5×10^{-7} millimoles/ cm^2 /hour (9). As stated above, these figures are probably representative for this tissue.

It should be emphasized that in both cases, notably that of potassium, measurements of turnover involve two processes, either of which may be of major importance in determining the passage rate of ions across the cell surface, and their exchange with the protoplasm, where they are held in higher concentration than in the external medium. Thus, although permeabilities have been expressed in terms of cell surface the rate of permeation of the radioactive isotopes may rather be limited by the binding of these ions within the

cell than by the passage of ions into the cell. This matter will be discussed further in future papers.

SUMMARY

By using radioactive isotopes in tissue cultures, the rate of permeation of substances into cells can be measured independently of concurrent metabolic reactions of these substances. Techniques of obtaining and analyzing data are described. Examples are given using radioactive potassium and phosphorus.

Using cultures of chick embryo muscle, turnover time for cell potassium is 6 hours, and for cell inorganic phosphate is 7 hours in the examples cited. Permeability rates, based on estimates of the cell surface involved and expressed as millimoles per cm.² per hour, are of the order of magnitude of 10^{-6} for potassium and of 10^{-7} for phosphate.

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