Osmotic Properties and Permeability of Cancer Cells

I. Relative Permeability of Ehrlich Mouse Ascites Tumor Cells and of Mouse Erythrocytes to Polyhydric Alcohols and to Sodium Chloride*

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Investigations of the osmotic properties of cancer cells have been hindered by the lack of suitable material. However, the availability of several kinds of cancer cells from so-called ascites tumors now makes such studies possible. These cells meet the requirements for quantitative determinations of osmotic phenomena and of the related property of permeability: they are obtainable as isolated uninjured cells of a single type; they are free to undergo volume changes; their size and shape is such as to permit accurate measurements. Such characteristics are shared by only a few other types of animal cells, which therefore have been widely used for osmotic studies—notably, erythrocytes and egg cells of certain marine invertebrates (1). Like them, ascites tumor cells can be obtained in large numbers and kept outside the body for hours without undergoing changes. This has several decided advantages: (a) It permits the use of accurate optical methods for measuring cell size, such as a diffraction method (6, 12) or, as in the present experiments, a photoelectric method (9, 10). By these means precise measurements can be made rapidly and at short intervals of time, and the course of swelling or shrinking of cells can be followed from beginning to completion. (b) Each measurement gives a statistical average of cell size, thereby canceling out individual variability. (c) The cells can be measured while in suspension and can be continuously stirred. (d) In the photoelectric method the measurements are objectively recorded as continuous photographic tracings that can be analyzed at leisure.

A series of penetrating nonelectrolytes were chosen for evaluation of relative permeability. The compounds are related polyhydric alcohols, namely, ethylene glycol, diethylene glycol, and triethylene glycol, glycerol, and erythritol. These are known to penetrate at different rates into erythrocytes (4) and into marine egg cells (8), and in the amounts used are practically harmless.

For the determination of osmotic equilibrium and for the detection of possible cell injury, sodium chloride served as an electrolyte to which the cells are relatively impermeable.

For comparison between the behavior of these various substances toward tumor cells and normal cells, mouse erythrocytes were chosen. Although erythrocytes are not related to cancer cells, they are the only readily available normal cells from the same species—a point of importance in view of the characteristic differences in permeability of the erythrocytes of different species (4, 5).

MATERIALS AND METHODS

Tumor cells.—The Ehrlich mouse ascites tumor was selected for the present experiments. Details of the method for propagating and obtaining the cells are given elsewhere.1 Briefly stated, adult albino mice received each 0.2 cc. of tumor cells from a 7-9-day-old growth. The animals were sacrificed about 1 week afterward, and the tumor suspension was withdrawn by a needle and syringe that had been rinsed with heparin solution. Only milky white suspensions were used; they were strained through gauze to remove possible clumps of fibrin, and kept at a temperature of about 5° C. In each lot the tumor cells were enumerated with a standard hemocytometer, and their total volume, relative to the suspending fluid, was determined by high speed centrifugation, 25,000 X g, in capillary hematocrit tubes (11). Smears and differential counts were made. Lots containing more than an average of 5 per cent leukocytes or 1 per cent erythrocytes were rejected.

Erythrocytes.—Blood was withdrawn by puncture of the exposed heart, the animal having first been sacrificed by frac-
ture of the neck. Heparin was used in an amount just sufficient to prevent clotting. The blood was strained through gauze, and kept at about 3° C.

Measurement of cell volume.—As stated above, the photoelectric method was used (10, 13). For each determination, 0.1 cc. of tumor cell suspension (averaging 10 million cells) or 0.02 cc. of erythrocytes (averaging 100 million cells) was added to 7.5 cc. of isosmotic buffered sodium chloride solution of pH 7.4. The glass chamber in which the cells were placed has a capacity of 10 cc. and was encased in a water bath. Uniform suspension of cells was maintained by gentle stirring with a motor-driven glass rod. A beam of light of constant intensity was passed through the chamber. This light im-

at first shrink. As the solute penetrates, a minimum volume is attained, and then the water lost during shrinkage is regained. The cells gradually return to their original size as the solute becomes equally distributed between cells and medium. From the initial size, the minimum volume attained, and the time required to attain this volume, it is possible to compute the permeability to the solute as well as to the solvent in absolute units. The details of the calculations are given by Jacobs and need not here be repeated. However, since we are dealing with cells of a kind different from those used by Jacobs, it is necessary to compute a series of conversion factors, such as shown in Table 1 of Jacobs' paper (8) and to construct a graph similar to the one shown in Figure 2 of his article. But, while these computations are not laborious, for the present purposes it is not necessary to express permeability values in absolute units. Rather, it seems best to await further information on this and other kinds of cancer cells and, meanwhile, to present our data as relative permeability. By this term is meant the relative rates of passage of penetrating solutes into a particular kind of cell under standard conditions.

As a measure of relative permeability we have used: (a) the time required by the cells to attain minimum volume during the course of shrinkage and (b) the time subsequently required to return in the course of swelling half way to their original size. While either of these points is a measure of relative permeability, we have evaluated both, since each serves as a check on the other.

The temperature for all determinations was 22° C. ± 1° C.

CHART 1.—Course of shrinkage and subsequent swelling of tumor cells in 0.3 M ethylene glycol, diethylene glycol, and triethylene glycol dissolved in isosmotic buffered saline. These initially hypertonic solutions cause shrinkage; but, as the compounds penetrate, the cells swell. The times required to attain minimum volume and subsequently to swell back to one-half original size is somewhat longer for diethylene glycol than for ethylene glycol, indicating less rapid penetration of the former compound. Penetration of triethylene glycol is still slower. Values are given in Table 1, Experiment A.

pinged upon a photronic cell, and the current from the latter was picked up by a Kipp torsion string galvanometer with a period of one-hundredth of a second. The movement of the galvanometer string was recorded photographically on 18-cm. bromide paper. Time, at intervals of 1 second, was impressed on the galvanometer record.

With tumor cells, as with erythrocytes, the amount of light passing through the suspension decreases as the cells shrink, the galvanometer current decreases, and downward deflection is recorded. The reverse occurs when the cells swell. In any one experiment the recording camera is kept at a constant distance from the galvanometer. Measurement of the photographic records gives the degree of galvanometer deflection, which is inversely proportional to cell volume. Typical records obtained by this method are shown in Plate 1.

Determination of permeability.—Jacobs (2, 8) developed an ingenious quantitative method by which cell permeability to a solute as well as to the solvent can be measured. In the present experiments sufficient pure solute (for example, ethylene glycol) was added to the suspension of cells to make the environment 0.5 M with respect to the solute. Since the cell suspension is kept stirred, the medium instantaneously becomes homogeneous. In this initially hypertonic solution the cells

2 Alternately, the solute is first dissolved in isosmotic saline and then the cells are added to the medium. This technique is found best when using erythritol.

RESULTS

Relative permeability of tumor cells to polyhydric alcohols.—The results of a typical experiment are shown graphically in Charts 1 and 2. Here galvanometer deflections are plotted against time in seconds (points beyond 100 seconds are not graphed). The results with the glycols are illustrated separately from those obtained with glycerol and erythritol. It is seen that the times required

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to attain minimum volume and subsequently to regain one-half original size are shortest for ethylene glycol, slightly longer for the diethylene compound, and still longer for triethylene glycol (Chart 1). The times are considerably increased when glycerol or erythritol are the penetrating compounds, as is obvious when Chart 2 is compared to Chart 1.

Comparison of relative values for tumor cells and erythrocytes.—The numerical values of the two experiments graphically represented are collected in Table 1. These two experiments were chosen to illustrate the greatest range of permeability values. The table shows, first, that the order of permeability values is the same for both tumor cells and erythrocytes; second, that there are differences in values for cells from different animals, these differences being more pronounced in the case of tumor cells. Third, the rate of penetration of the test substances is much greater for erythrocytes than for tumor cells. Thus, for the more slowly penetrating compounds, glycerol and erythritol, the difference is over one hundred-fold.

Relative impermeability of tumor cells and erythrocytes to sodium chloride.—Sodium chloride is the chief electrolyte in the natural medium of both tumor cells and erythrocytes. The latter are known to be relatively impermeable to this salt; that is, possible entry occurs so slowly that it cannot be detected under the conditions of the present experiments. It is to be expected that tumor cells behave similarly, and this proved to be the case. When tumor cells or erythrocytes were caused to shrink in hypertonic solutions of sodium chloride, they did not subsequently swell back to original size, as occurs in initially hypertonic solutions of glycerol and erythritol, minimum values were reached in about 1 second or less; hence, the downward curves of shrinkage are omitted from the graph. However, the times for the erythrocytes to return half way to original size differ considerably. In this as well as in other experiments the order of penetration is the same as that given for tumor cells.

TABLE 1

<table>
<thead>
<tr>
<th>Time to</th>
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<tr>
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<td>VOLUME</td>
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<td>Tumor cells</td>
</tr>
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<tr>
<td>Diethylene glycol</td>
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<td>18</td>
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<td>210</td>
</tr>
<tr>
<td>Erythritol</td>
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<td>780</td>
</tr>
<tr>
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<td>Erythritol</td>
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penetrating polyhydric alcohols. On the contrary, after the cells reached minimum volume, they remained at an almost complete level. Hence, under these conditions the minimum volume represents an equilibrium volume.

Typical experiments are graphically represented in Chart 4. In these experiments sodium chloride was added to suspensions of tumor cells or of erythrocytes, so as to make the medium hypertonic with respect to the cells. Both types of cells promptly shrank to a steady state, the erythrocytes shrinking much faster than the tumor cells. The time for attaining this equilibrium volume furnishes an approximation of the relative permeability to water. In the two examples given, tumor cells required 7 or 6 seconds, respectively, to shrink one-half way to a steady state, whereas erythrocytes attained this point in less than 1 second.

Reproducibility of results.—It is well known that erythrocytes may be kept, under proper conditions, outside the body for many hours. That this is equally true of tumor cells is demonstrated by experiments such as are illustrated in Chart 5. Here the cells, removed from the mouse 2 hours before the beginning of the tests, were first shrunken in hypertonic solutions of sodium chloride. After a lapse of 5 more hours another sample of cells was again placed in the more concentrated solution and the course of volume changes recorded. The curves of shrinkage are identical. The persistence of the same state of permeability proves the absence of cell injury.

Quantitative relation between concentration of sodium chloride solution and volume of tumor cells.—When tumor cells are placed into different concentrations of hypertonic sodium chloride solutions, and the course of volume changes is recorded, a series of curves is obtained. In the experiment shown in Chart 6 the cells were caused to shrink in 1.25, 1.50, 1.75, and 2.0 buffered sodium chloride solutions (isosmotic = 1.0). The galvanometer deflections are seen to increase with increasing tonicity. The relation between deflection and volume attained is linear, as illustrated by Chart 7. In these experiments equilibrium values were measured, respectively, in three or four concentrations. In each case the best fit for the points is a straight line. Since galvanometer deflections are brought about by changes in cell volume, it follows that such deflections are inversely proportional to the volume of cells.

The linearity of the relation between galvanometer deflection and concentration of the fluid with which the cells are in equilibrium makes it possible to determine the average volume of the tumor cell, or of the erythrocyte, in the customary units of cubic micra. It is merely necessary to place the cells from any one experimental group into solutions having different concentrations of a nonpenetrating solute and measure first the deflections of the galvanometer and then obtain, by means of hematocrit, the total cell volume per c.cm. in each of the concentrations. Dividing these values by the total number of cells (which is the same in each
concentration) gives the average cell volume. The volume of cells corresponding to any deflection of the galvanometer in other experiments of a given group can now be computed. Since only the initial and minimal volume are required for calculations of permeability in terms of absolute magnitudes, these determinations are not time-consuming.

**DISCUSSION**

The experiments reported were frankly exploratory. They were designed to find out whether ascites tumor cells are suitable for studies on permeability and, if so, what methods to use in such studies. When these preliminary questions were answered, the relative permeability of the cells to a series of related penetrating substances was compared to the behavior of erythrocytes to the same compounds. As a nonpenetrating substance, sodium chloride was selected in order to gain information on osmotic equilibrium states. For reasons stated, we preferred for the present to express permeability values in relative rather than in absolute terms, although one may readily be translated into the other.

It remains to make plain why no attempt was made to refer to the literature, scanty though it is, on permeability determinations hitherto made for cancer. As far as we have been able to learn, all such studies have been made with slices of tissue or with tissue cultures, rather than with cells that naturally occur in a free uninjured state. The osmotic properties of tissues cannot justifiably be assumed to be identical with those of their component cells. Tissues contain not only cells of more than one type, but also blood vessels, lymphatics, tissue spaces, and intercellular substances—all having different properties. Moreover, in tissues free changes of cell volume are interfered with by mutual pressure of component parts and by mutual adhesiveness; the same objections apply to tissue cultures.

Furthermore, it has not been thought profitable, for the present, to compare permeability values of the tumor cells to those of other cells, except erythrocytes of the same species. Such comparisons should await the accumulation of much more information than is now available.

The experiments here reported will serve as a point of departure for further studies.

**SUMMARY**

1. The cell of the Ehrlich mouse ascites tumor has been found to be favorable material for studies on cell permeability.

2. With a photoelectric method, relative permeability of the tumor cell has been evaluated for a series of polyhydric alcohols. The order in which these compounds penetrate the cell is: ethylene glycol > diethylene glycol > triethylene glycol > glycerol > erythritol.
3. The same order holds for mouse erythrocytes, but the relative rates of penetration are much slower for tumor cells.

4. The tumor cells, like erythrocytes, are relatively impermeable to sodium chloride; hence, solutions of this salt are suitable for the detection of cell injury and for studies on osmotic equilibrium.

REFERENCES


PLATE 1.—Photographic records showing that both tumor cells and erythrocytes are relatively impermeable to sodium chloride. To the cells suspended in isoosmotic buffered saline, sodium chloride was added so as to increase the osmotic pressure 1.5 times (Fig. 1, tumor cells) or 2.0 times (Fig. 2, tumor cells; Fig. 3, erythrocytes). It is seen that the cells shrink to a minimum volume and remain shrunken. Note that erythrocytes shrink more rapidly than tumor cells. Time at intervals of one second is represented by the vertical block-outs in the continuous part of the curves; the wider spaced blocks indicate intervals of 15 seconds.
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