Functional Implications of Interactions of Extracellular Ions with Ligands of the Cell Membrane

By Aser Rothstein, Ph.D.

ULTIMATELY IT IS the goal of most "membranologists" to understand the function and structure of the plasma membrane in molecular terms. Inasmuch as the primary function of the membrane is the regulation of traffic moving into or out of the cell, membranology has been seriously concerned with the nature of traffic-regulating systems. Unfortunately the road to this particular paradise is hardly visible, being well hidden by thorny problems. With some effort and ingenuity it has been possible to estimate the flow of substances into and out of the cell, and from such kinetic measurements deductions have been made concerning the underlying regulating systems. The result has been a multiplicity of sophisticated, interesting, but often untestable hypotheses and considerable confusion among biologists in general.

The problem of identifying membrane structure and function in molecular terms is a biochemical one. However, the standard biochemical procedure of separating cellular structures and studying isolated systems has met with limited success in the case of the plasma membrane. Firstly, few pure membrane preparations have been prepared, and secondly, none of them, with the possible exception of the red-cell ghosts, behaves as an intact, functional membrane. Some of the membrane fractions do, however, display biochemical activities which seem to be related to membrane function. One such activity is the Na-K activated adenosinetransphosphatase (ATPase)\( ^1,2 \) which has been demonstrated in many types of cells; another is the Na-sensitive phosphatidic acid system of epithelial tissues.\(^3 \) In each case the conclusions must rest on the strikingly parallel behavior of the membrane transport systems in the intact cells and the biochemical events in the cellular fractions.

Because of the inherent problems associated with isolated membrane fractions, some attempts have been made to describe the chemical events in the membrane by studies with intact cells. Basically the technique involves the use of chemical probes, that is, of chemical agents which react with ligands of the membrane and which disturb membrane function. Implicit in such studies is the requirement for two kinds of information. Firstly, it is necessary that the chemical probe interferes with a membrane function, and secondly, that the interaction of the chemical probe with the membrane can be measured in a chemical sense and, perhaps, can be attributed to combination with a known ligand. While it is true that many chemical substances are known to alter membrane function, in most cases very little or nothing is known concerning interaction in a chemical sense. In fact, some

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substances may act on membrane functions without interacting with the membrane at all, by penetrating into the interior of the cell and interfering with internal metabolic functions which support membrane activities. In the present discussion three examples will be presented of the use of chemical probes in the intact yeast cells.

**Uranyl Ion on Sugar Uptake**

Although the studies with UO$_2^{++}$ have already been reported in detail, a brief summary will be presented here because it can serve as an excellent, uncomplicated example of the use of chemical probes. From a chemical point of view, the interactions of uranyl ion with molecules present in biological systems is fairly simple. In somewhat acid pH (below 4.5) used in the experiments, uranyl ion is unhydrolyzed (in the form UO$_2^{++}$). It forms complexes with carboxyl groups and phosphoryl groups of biological molecules but not with sulfhydryl groups. When uranyl ion in low concentrations is presented to the cells, most of the eation is bound in the reversible complex which can be described in terms of a simple mass law equation, assuming a one-to-one complex:$^5$

$$ U + Y \rightleftharpoons UY $$

(1)

$$ K = \frac{(U)(Y)}{(UY)} \cdot \frac{fu \cdot fy}{fu_y} $n

(2)

$$ K_1 = \frac{(U)(Y)}{fu} \cdot \frac{fu}{fu_y} $$n

(3)

where $U$, $Y$, and $UY$ are free uranyl, free yeast groups, and bound uranyl respectively, and the $fu$, $fy$, and $fu_y$ are activity coefficients. The validity of equation 3 is justified by the data of table 1, in which the calculated $K_1$ is relatively constant despite a twenty-fold change in uranyl ion concentration.

The maximal binding of uranyl ion by intact cells is of the order of 1 mM/Kg. (wet weight). On the other hand, the total binding capacity of the cellular constituents for uranyl ion, determined by breaking the cells, is over fifty times as high. It is obvious that only a small fraction of the uranyl binding capacity of the intact cell is accessible to the uranyl ion. That this small fraction is located on the outer surface of the cell is indicated by a number of experiments.$^6$ For example, the distribution of UO$_2^{++}$ is determined by small concentrations of extracellular, inorganic phosphate and not by the much larger concentrations of intracellular phosphate.

Other extracellular cations will compete with uranyl ion for the binding sites on the surfaces of the cell. The array of affinities of the various ions is shown in table 2. It can be noted that uranyl ion is bound more firmly by a considerable degree than any of the other cations tested. With manganese (as manganous ion) as a probing ion, two different kinds of binding sites could be demonstrated on the cell membrane.$^7$ The data are plotted according to equation 4, which is a simple rearrangement of equation 3:

$$ \frac{(UY)}{(U)} = \frac{(UY)}{fu} \cdot \frac{fu}{fu_y} $$

(4)

where $Y_i$ is the total number of yeast groups.

<table>
<thead>
<tr>
<th>Total UO$_2^{++}$</th>
<th>K$_1 \times 10^{-7}$ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>4.4</td>
</tr>
<tr>
<td>1.6</td>
<td>3.0</td>
</tr>
<tr>
<td>2.4</td>
<td>3.7</td>
</tr>
<tr>
<td>3.2</td>
<td>4.1</td>
</tr>
<tr>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td>4.8</td>
<td>3.5</td>
</tr>
<tr>
<td>6.0</td>
<td>3.5</td>
</tr>
<tr>
<td>6.8</td>
<td>3.4</td>
</tr>
<tr>
<td>8.0</td>
<td>4.6</td>
</tr>
<tr>
<td>16.0</td>
<td>5.3</td>
</tr>
</tbody>
</table>

**Table 2**

**The Relative Binding Affinities of Cations for Groups on the Cell Surface**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Number of Groups (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UO$_2^{++}$</td>
<td>330</td>
</tr>
<tr>
<td>Cu$^{++}$, Hg$^{++}$</td>
<td>1.0</td>
</tr>
<tr>
<td>Ba$^{++}$</td>
<td>10</td>
</tr>
<tr>
<td>Zn$^{++}$</td>
<td>3</td>
</tr>
<tr>
<td>Co$^{++}$, Mg$^{++}$</td>
<td>1.5</td>
</tr>
<tr>
<td>[K$^+$, Rb$^+$, Cs$^+$]</td>
<td>$.003 to .005</td>
</tr>
<tr>
<td>Ca$^{++}$, Sr$^{++}$</td>
<td>1.0</td>
</tr>
<tr>
<td>Mn$^{++}$</td>
<td>TRIS$^+$</td>
</tr>
<tr>
<td>Mn$^{++}$</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>
In figure 1 the ratio of bound-to-free manganese is plotted against the concentration of bound manganese. Instead of the expected straight line of the negative slope, the line breaks to give a second more shallow slope. The steep slope extrapolates to give a value for $Y_1$ of the same as found for the uranyl binding. The shallow slope can be accounted for by a second species of binding site with a lower affinity for manganese. By competition studies, it was demonstrated that uranyl ion also binds to both types of membrane ligands.

The two species of binding sites have been tentatively identified as phosphoryl and carboxyl groups, respectively.8 However, the exact chemical nature of the substances involved is difficult to determine. In the case of the phosphoryl sites, the phosphate groups must be arranged sufficiently close to give a chelating structure, such as is found in polyphosphate, inorganic polyphosphates, ATP, or nucleic acids.8 An unconfirmed Japanese study with the enzyme ribonuclease implicates ribonucleic acid as the binding substance.9

Two distinct physiological consequences are associated with the binding of uranyl ion to the two types of ligands (fig. 2). At low concentrations of uranyl ion, sugar uptake of the cell is blocked, whereas at higher concentrations invertase activity is blocked.10 By parallel studies of binding, it has been demonstrated that the inhibition of sugar uptake is associated only with the binding of uranyl by the phosphoryl groups and that the inhibition of invertase activity is associated only
with the binding of uranyl ion by the carboxyl groups.

First, consider the inhibition of invertase which represents a relatively simple situation. This particular enzyme is located on the outer surface of the yeast cell. Because of its location, it is directly influenced by the extracellular rather than the intercellular environment. The properties of the enzyme can therefore be studied in vivo, that is, in the intact cell. The action of heavy-metal inhibitors, such as mercury, silver, and uranyl, are little different on the enzyme of the living cell than on the purified enzyme. In the case of uranyl ion, the inhibitory action is due to formation of a complex with carboxyl groups of the enzyme. In the presence of an inhibiting concentration of uranyl ion, the enzyme cannot carry out its normal function, which is apparently a digestive one. That is, the enzyme splits substrates, such as sucrose, which are only poorly used by the cell into products—glucose and fructose—which can be rapidly metabolized.

The situation in the case of sugar uptake is considerably more involved. The yeast cell and many other cells possess a specific system by which the sugars pass through the membrane to reach the metabolic sites. Uranyl ion blocks the transferring system but does not interfere with the metabolic machinery. Thus a uranyl-blocked cell can utilize stored glycogen, can form glycogen, can absorb potassium, can utilize 2- and 3-carbon substrates, but cannot use sugars.

The mechanism by which uranyl ion bound to membrane-phosphoryl groups can block the transfer of sugar is not at all clear. The total number of uranyl binding sites is about 10⁵ per cell, but it is not known whether each of these sites is involved in the block of sugar uptake. It can be concluded that the sugar sites must have the same affinity for uranyl ion as do other phosphoryl sites, also that a particular site is either blocked or not blocked. The block is effective against sugar leaving as well as entering the cell. It is not clear whether uranyl ion acts directly upon the carrier system for sugar or whether uranyl ion by forming a chelate with adjacent groups, in some way sterically prevents the access of sugar to the carrier site.

Agents that Affect the Integrity of the Membrane

In the previous section (table 2), it was pointed out that cations in general can combine with the same sites as uranyl ion. Among these ions are some that interfere with the membrane in an entirely different manner. These are the cationic dyes (such as methylene blue) and the cationic detergents.

The binding of methylene blue to the cell has not been studied directly. However, the binding can be characterized in terms of competition with uranyl ion or with manganous ion. Although monovalent, the cationic dyes have a much higher affinity for the binding sites than do other monovalent cations, such as sodium or potassium. They do not, however, bind as firmly as the bivalent cations.

Unlike uranyl ion, the binding of the cationic dyes does not lead to a block in sugar uptake, but it does lead to an increase in the rate of potassium efflux. With relatively high concentrations of a dye, such as methylene blue, the rate of potassium efflux may be 50 or even 100 times that of the control, but the high rate of leakage ceases after 15 to 20 minutes (fig. 3), even though a great deal of potassium is still left within the cells of the population. Consequently, both the rate of efflux and the maximal loss are related to the dye concentration. If the maximal losses are plotted against the log of the dye concentration, the resulting S-shaped curve can be fitted exactly by a normal probability curve. An explanation for such behavior is all-or-none response for individual cells. Those cells in which the threshold is exceeded become leaky and lose all of their potassium within 15 to 20 minutes, whereas the remaining cells are normal. The explanation was confirmed by examining the cells under the microscope. In low concentrations of dye, almost no stained cells are found, but with increasing concentrations the percentage of stained cells increases. Figure 4 shows the relationship...
The leakage of $K^+$ from yeast cells exposed to different concentrations of methylene blue.

The relationship between staining of cells with methylene blue and $K^+$ loss.

between the percentage of stained cells and percentage of potassium lost from the population. The one-to-one correspondence suggests that those cells which have lost their potassium are permeable to the dye and can be stained. The same kind of relationship can be demonstrated for ability of cells to form colonies.

Once initiated, the insult to a cell resulting from the action of the dyes is irreversible. The membrane is damaged to the extent that it is no longer a permeability barrier but is highly permeable not only to cellular potassium and dye molecules but to cellular phosphate compounds and to other substances which do not normally penetrate.

From a chemical point of view, the interaction of the cationic dyes with the cells must be considered as a two-step reaction. The first step involves the reversible binding of the dyes to the phosphoryl sites on the cell membrane, discussed previously in connection with $UO_2^{++}$ binding. The second step presumably involves a redox reaction, because only the oxidized form of the dye is effective, even though the reduced dye can be bound. Without the first step the second cannot occur. For
cerns the protective action of cations (such as $\text{UO}_2^{2+}$, $\text{Ca}^{++}$, or $\text{Mg}^{++}$). Cations protect against cationic dyes and detergents but do not protect against mercury, copper, x-irradiation, or UV-irradiation.

Using radioactive mercury, an attempt was made to titrate the cellular ligands responsible for the membrane defect. Unfortunately, however, relationship between binding and response was obscured by the large amounts of mercury bound by cellular ligands not concerned with membrane integrity. The curve for mercury tends to reach a maximum binding of something over 20 mM/Kg. cells (fig. 5), but the physiological response occurs only in the bottom segment of the curve. Approximately 4 mM mercury is bound per Kg. of cells with almost no response (potassium leakage); approximately 12 mM/Kg. is bound at maximum response (100 per cent leakage), but no inflection points are seen on the binding curve at either of these levels. Such a diversion of metals to nonresponsive sites often obscures the primary dose-response relationship of heavy metals and other chemical agents.21

No single common denominator obviously connects the cationic redox dyes, the cationic detergents, mercuric chloride, cupric chloride, x-irradiation, and UV-irradiation. The cationic dyes and cationic detergents possess the common property that they must bind to phosphoryl groups of the cell membrane before they can produce damage, but the same is not true for mercury or for irradiation. In the case of redox dyes, mercury, and x-irradiation, a common denominator is the potential reaction with sulfhydryl groups in the cell. However, the action of detergents and probably UV could not be readily explained on the basis of sulfhydryl interactions. Furthermore, each of these agents can, at lower concentrations than those that damage the membrane, produce highly specific and highly individual graded responses. The cationic detergents produce specific metabolic effects$^{16,17}$ related to the metabolism of 2-carbon substrates,22 the redox dyes influence the active transport of sodium and potas-

This reason other cations which compete for the binding sites (such as $\text{UO}_2^{2+}$ or $\text{Ca}^{++}$) can protect against concentrations of dyes which normally render the whole population permeable. Furthermore, anionic dyes which cannot be bound, do not induce K loss even if the redox potential is the same as that of the cationic dye.

Parallel observations have been made with detergents.$^{16,17}$ The cationic but not anionic detergents are effective, producing an all-or-none response in terms of leakage of potassium or other constituents. As in the case of the cationic redox dyes, binding to anionic sites is a prerequisite for the physiological effects. Thus other cations, by competing for the binding sites, protect the cells.

A number of other agents including mercuric chloride,18 cupric chloride,19 x-irradiation, and UV-irradiation20 produce an all-or-none response in terms of K leakage. Phosphate leakage, stainability, and viability, which is similar to that produced by cationic dyes and detergents. In fact, in the case of mercuric chloride, the effects are quantitatively additive with those of methylene blue.18

The one major difference between the cationic dyes and detergents and the other agents concerns the protective action of cations (such as $\text{UO}_2^{2+}$, $\text{Ca}^{++}$, or $\text{Mg}^{++}$). Cations protect against cationic dyes and detergents but do not protect against mercury, copper, x-irradiation, or UV-irradiation.
sium; mercury bound to the membrane gives a specific block of sugar uptake; x-irradiation and UV-irradiation produce mutational effects.

The only common denominator for all of the agents is the physiological response, the all-or-none release of potassium and other cellular constituents. It is, therefore, in the nature of the response that clues must be sought concerning the basic chemistry of the response. The properties of the response, although already mentioned can be listed:

1. All-or-none for individual cells;
2. Nonspecific in the sense that the permeability is markedly increased to all small molecules and ions that have been tested;
3. Nonspecific inasmuch as diverse agents can produce the response;
4. Irreversible, leading to cell death (failure to form colonies).

The response represents a disturbance in the basic membrane structure rather than interference with the specific active site of an enzyme or of a transport system. Chemically the reaction probably involves cross-linking of sulfhydryl groups in the case of redox dyes, mercury, copper, and x-irradiation, and perhaps disturbances of lipid structures in the case of detergents (lipid solvents produce a similar effect). The structure of the cell membrane can tolerate a certain number of reactions with the agents without loss of stability, although the specific membrane functions listed previously are disturbed. Each interaction, however, can be considered as a stress on the membrane. When the stress reaches a limiting value for the cell, the membrane structure breaks down, leading to an irreversible loss of cellular constituents equivalent to lysis in an unwalled cell.

From a chemical point of view, the effectiveness of sulfhydryl agents points to a role for sulfhydryl groups in the maintenance of membrane integrity. In fact, specific agents such as parachloromercuribenzoate can act in the same way as mercuric chloride. At the same time, it must be recognized that the integrity also depends on other ligands. The phosphoryl groups of the membrane apparently play only an indirect role in the presence of the cationic dyes and detergents. The cationic dyes and detergents can only reach the “sensitive sites” if they first bind to the phosphoryl groups of the cell surface. Either the binding is a prerequisite to the penetration of the dyes to the “sensitive sites” or is prerequisite for physiological action in the sense that only in the bound form can the active group on the dye or detergent be in appropriate or steric position to react with the “sensitive site.” In the latter case, it would follow that the phosphoryl groups and “sensitive sites” are in close proximity, only a few bond lengths apart.

**Action of Arsenate on Phosphate Transport**

The yeast cell possesses a transport system for absorbing phosphate which has been characterized in some detail and which will be very briefly reviewed. The uptake of phosphate requires the presence of specific substrates, the sugars, and proceeds at about the same rate under aerobic and anaerobic conditions. Thus the metabolic support for the transport must come from the glycolytic systems. The transport is a one-way flux, that is, during the uptake process no phosphate leaves the cell. For this reason as phosphate is taken up, the specific gravity of the $\text{P}^{32}$ in the medium is unchanged, with no dilution by cold phosphate from the cell. The uptake follows a typical saturation curve which can be fitted by the Michaelis-Menton equation. The transported ion is the monovalent anion $\text{H}_2\text{PO}_4^-$ with ionic balance maintained by an equivalent loss of $\text{OH}^-$, the cell becoming more acid and the medium more alkaline.

The phosphate uptake is markedly increased by potassium, but the effect is an indirect one. In fact, the cell can be allowed to take up potassium prior to the exposure to phosphate, and the stimulating effect will still be observed. The potassium effect can be explained on a basis of the acid-base balance of the cell. When cells are allowed to absorb potassium, they do so by an exchange of cellular hydrogen ion for the potassium ion, the potassium in the cell being balanced by or-
The uptake of phosphate and of arsenate by fermenting yeast.

The inhibition of phosphate uptake by arsenate and of arsenate uptake by phosphate in fermenting yeast.

Organic anions, such as succinate and bicarbonate. A cell, rich in potassium, can absorb a considerably larger quantity of phosphate without upsetting the acid-base balance, the compensating effect being the disappearance of the organic anion in the metabolic cycle. In the experiments to be reported here the uptake of phosphate was maximized and an initial lag period in uptake was eliminated by pre-exposing the cells to potassium chloride and glucose for 10 minutes prior to the addition of the phosphate. This time is sufficient to absorb a very large amount of potassium and to reach a maximal rate of metabolism.

In figure 6, data are presented for the uptake of phosphate with different initial concentrations added to the suspension. With a low concentration, $3 \times 10^{-4}$ M, all of the phosphate is absorbed in 20 minutes. With the highest concentration, $5 \times 10^{-3}$ M, 60 per cent is absorbed in 60 minutes, but if the experiment is allowed to proceed for 90 minutes, all of the phosphate will be absorbed. The actual rates of uptake, as mentioned previously, follow asymptotic relationship with the maximal rate at $1 \times 2 \times 10^{-3}$ M.

Arsenate can also be transported into the yeast cell, with properties of the system in many ways similar to those of phosphate transport. For example, the arsenate is transported only if glucose is present, and the arsenate once taken up is not exchangeable with external arsenate. Furthermore, arsenate and phosphate mutually interfere with each
other so that they apparently are taken up by a common mechanism. On the other hand, the kinetics of transport of arsenate are not the same as those for phosphate, and the mutual inhibition is not a simple competitive inhibition. In contrast to the curves for phosphate, the arsenate uptake proceeds for 20 to 30 minutes and then ceases (fig. 6). Only at concentrations of arsenate as low as $1 \times 10^{-5}$ M is uptake complete. At higher concentrations of arsenate, the maximal uptake approaches a limiting value which is of the order of 5 mM/Kg. of cells, regardless of how high the external arsenate concentration is. The arsenate uptake behaves like a self-inhibiting system. However, complete blockage does not require that a given amount of arsenate be absorbed. For example, at $1 \times 10^{-4}$ M arsenate the uptake ceased completely when the total amount taken up was 0.76 mM/Kg. In contrast, at $5 \times 10^{-4}$ M arsenate, the uptake ceased at 1.4 mM/Kg.

In figure 7, the influence of increasing arsenate concentrations on phosphate uptake is demonstrated. Arsenate not only inhibits the uptake of phosphate in terms of a reduced rate, but the maximum uptake becomes progressively less than 100 per cent. In other words, the self-blocking effect of arsenate is also reflected in a parallel manner in terms of phosphate uptake. Reciprocally, increasing concentrations of phosphate reduce both the rate of uptake of arsenate and the maximum. For example, at $1 \times 10^{-2}$ M phosphate the arsenate uptake is blocked when the total amount absorbed is 0.4 mM/Kg., whereas in the control the transport ceases when 2.5 mM/Kg. are absorbed.

The rate of development of the self-blocking effect of arsenate on its own uptake or on phosphate uptake is demonstrated in figure 8. In this experiment the data are plotted in terms of the rates of phosphate uptake as a function of the time of exposure to varying concentrations of arsenate. As the arsenate concentration is increased, the block develops more rapidly. Once established the block is not easily reversed. Washing with water or

---

**Figure 8**

The rate of development of the arsenate block in fermenting yeast.
with phosphate has little effect. However, exposure to high phosphate plus glucose induces some reversal in an hour.

Because the rate at which the inhibitory process develops is dependent on both the arsenate and the phosphate concentrations, the kinetics of inhibition are more difficult to measure. In order to deal with this situation, a series of experiments were performed in which arsenate and phosphate uptake were measured simultaneously, with the two substrates being present in various concentration ratios. In each experiment, it was evident that phosphate or arsenate are competing for a common transport system. For example, if the ratio of phosphate to arsenate was constant but the absolute concentrations were varied over a hundredfold range, the relative rates of phosphate and arsenate uptake were always the same. Another type of experiment is shown in figure 9. In this case, the arsenate and phosphate were present in the same concentration. The rates of uptake are different, with phosphate being absorbed about twice as fast as the arsenate. Both rates fall continuously but in a parallel manner, so that the ratio of the uptake remains constant. It is apparent that the two ions compete for a common transport reaction, but superimposed on the kinetics of competition is the irreversible component which develops continuously and which finally leads to the cessation of uptake of either ion. In other words, arsenate inhibits phosphate in two ways, firstly by competing in a reversible manner for a common transport system, and secondly, by irreversibly blocking the transport system.

The mechanism by which arsenate blocks the phosphate transport is not clear. The competition for a common transport reaction could be expected on the basis of chemical similarities, but the noncompetitive irreversible block that develops with arsenate could not. In attempting chemical explanations, the limiting factor is the virtual absence of chemical knowledge concerning phosphate transport. Once within the cell, the transported phosphate appears largely in the form of granules containing inorganic polyphosphates of high molecular weight. The one reaction in glycolysis starting from glucose, in which inorganic phosphate is esterified, is the reaction of phosphate with 3-phosphoglyceraldehyde in the presence of phosphoglyceraldehyde dehydrogenase to form 1,3-diphosphoglyceric acid. Phosphate esterified in this manner then is transferred to ATP from which polyphosphate can be formed. This sequence of reactions does not necessarily offer an explanation for active transport or for the action of arsenate. For example, the chemical reactions are all reversible, allowing exchange of P\(^{32}\) labeled phosphate, whereas the transport does not.

Some tentative general conclusions can be drawn without specifying the nature of the chemical reactions involved. In figure 10, the transport reaction by which either phosphate or arsenate is moved into the interior of the cell is labeled X. Actually, a sequence of reactions or a cycle may be involved. The irreversible arsenate block is represented by the formation of AsY, which represents an inert substance formed at the expense of a transport component. The scheme is drawn as a branched system to explain the following observations: Firstly, the blockage of transport does not seem to be due to a feedback from the arsenate that is transported into the interior of the cell, for example, inhibition of glycolytic reactions. By controlling the time

![Figure 9](https://circ.ahajournals.org/)

**Figure 9**
The simultaneous uptake of phosphate and arsenate by fermenting yeast.
of exposure to arsenic, the temperature, the arsenate, and the phosphate concentrations, complete blockage of transport can be attained with or without significant inhibitions of metabolism and, in terms of arsenate uptake, with as much as 5 mM/Kg. net weight or as little as 0.05 mM. Thus the arsenate content of the cell and events within the cell do not seem to be responsible. Secondly, if the arsenate were to block directly one of a sequence of transport steps in the membrane, the arsenate would then inhibit phosphate transport but would not itself be transported.

**Figure 10**

A scheme to explain the action of arsenate on phosphate uptake.

Furthermore, the amount of arsenate that can be transported is over 100 times that necessary to produce a complete block. Therefore, it has been assumed that one of the transport intermediates, when in the arsenate form, is susceptible to irreversible conversion to the inactive form AsY, at a rate that is not controlled by the rate of transport.

Potentially, arsenate may provide a tool for exploring the chemistry of the phosphate transport system. As yet, only a few preliminary observations have been made. For example, when arsenate uptake is large before the blockage of transport is complete, much of the arsenate is found in the trichloroacetic acid extractable fraction. When, however, the uptake is minimal, the arsenate is nearly all nonextractable and nonexchangeable. It is the identity of the arsenic compounds in the latter fraction that may provide a chemical clue to phosphate transport.

**Discussion**

Three examples of the use of chemical "probes" have been discussed. In the case of uranyl ion, the interactions in a chemical sense are relatively nonspecific, involving the general ligands—phosphoryl and carboxyl groups. The physiological effects, on the other hand, are quite specific—inhibition of sugar uptake and of invertase activity. The specificity of the responses is not related to the chemistry of uranium interactions but to the geography of the cell. Uranyl ion cannot penetrate into the cell. The only phosphoryl or carboxyl groups that are accessible are those on the cell membrane, some of which are concerned in sugar uptake and invertase activity. In the case of the lytic agents, the interactions are also relatively nonspecific in a chemical sense. The resulting physiological damage may be specific at low concentrations of the agents but is a nonspecific breakdown of membrane structure at higher concentrations. Again the accessibility of reactive ligands in the membrane is a primary factor in the response. In the case of arsenate, the interactions and the response are highly specific, being limited to the pathways of phosphate transport. Potentially, arsenate may yield more information concerning the chemistry of membrane systems than any of the nonspecific agents.

It will be obvious that attempts to probe the chemistry of the membrane in the living cell have met with only limited success. Small bits and pieces of information have yielded themselves to rather strenuous effort. Yet progress has been made, and who can tell when a breakthrough will be achieved. A rather pertinent analogy is based on the premise that the primary function of the membrane is the regulation of traffic into and out of the cell. In the analogy, we are asked from observations of overall traffic flow in New York City how many policemen are working, whether they are fat or thin, right-handed or left-handed; but we are never allowed to watch them work. We can trap or catch segments of the general population, but the policemen are never caught in their uniforms.
What we would need is an identifying characteristic for policemen, such as flat feet. And who knows—perhaps with patience, we will catch a flat-footed membrane component.

References

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