The Binding of Mercury by the Yeast Cell in Relation to Changes in Permeability

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ABSTRACT Yeast cells exposed to mercuric chloride suffer irreversible damage to the membrane, resulting in a loss of potassium and cellular anions to the medium. The maximal loss of K⁺, but not the time course of K⁺ loss is related to the mercury concentration, the relationship following a normal curve on a graph of log-concentration versus effect. It is concluded that the response is all or none for individual cells, and that with increasing concentrations of metal, the threshold is exceeded in an increasing proportion of the cells. Parallel studies of the binding of mercury by the cells indicate two distinct phases, only one of which is associated with the physiological response. The binding process is relatively slow but reaches an equilibrium state. Desorption is markedly dependent on temperature. No simple stoichiometric relationship exists between the binding of mercury and the physiological response (K⁺ loss).

When cells are exposed to heavy metal cations, a sequence of interactions can be expected starting from the outside of the cell and proceeding inward. That is, the first reaction between the metal and the cell should take place with those ligands of the cell membrane for which the metal possesses a chemical affinity. Second, as the metal ion passes through the membrane into the cytoplasm, it will react with internal constituents at a rate determined principally by the rate of entry of the metal, by the rate of mixing with the cytoplasm, and by the kinetics of the chemical reactions involved.

In the case of uranyl ion and the yeast cell, the situation is relatively simple. Combination occurs only with ligands of the membrane, with no penetration into the interior of the cell (1). The membrane ligands are of two chemical types, phosphoryl groups (2) and carboxyl (3). Physiological functions...
associated with these ligands are sugar uptake in the case of phosphoryl groups and invertase activity in the case of the carboxyl groups.

With some of the other metals, the situation is more complicated. In muscle, for example, a rapid interaction of mercury or copper with the membrane is followed by a slow penetration into the interior of the cell (4). A physiological function associated with the action of the metal on the membrane is the uptake of glucose. One associated with the interior of the cell is the respiration. In other cells a similar sequence of events presumably occurs. Some of the reported effects on membrane function include: changes in the permeability of the red cell to glycerol produced by copper (5) and by mercury (6); losses of K⁺ from red cells produced by lead, mercury, gold, and silver (7); inhibition of surface-bound invertase in the yeast cell by silver and mercury (8) and inhibitory effects on absorptive activities of kidney with organic mercurials. Effects of metals attributable to action on internal cellular structures might include the inhibition of metabolic activities (respiration and glycolysis) and the block of cell division.

From the foregoing brief review, it is apparent that studies of the actions of heavy metals on membrane function may lead not only to an understanding of the nature of the toxic action of the metal at the cellular level, but also provides a means, in some cases, of determining certain properties of the membrane itself. In such studies, however, complicating factors must be taken into account. First, the binding of the metal by the cell may represent not only a binding by membrane ligands, but a penetration of the metal into the interior of the cell as well. Second, an interaction of the metal with certain of the membrane ligands may result in a physiological response, while interactions with other ligands may produce no measurable effect. In this case, the relationship between metal binding and physiological response is distorted by the diversion of metal onto physiologically inactive sites. Finally, the physiological response itself may be related to the membrane ligands in a variety of ways. For example, the inhibition of sugar uptake in yeast by uranyl ion is proportional to uranium binding over a range of uranium concentrations (9). On the other hand, the action of lead on the permeability of the red cell membrane to K⁺ involves an all or none response of individual cells so that increasing concentrations of lead are associated with an increasing proportion of leaky cells. The dose response curve, therefore, represents the variation in resistance within the cell population (10).

In the present study an attempt has been made to analyze the actions of sulfhydryl-seeking metals, particularly mercury, on the yeast cell membrane. The response chosen for study is the marked leakage of potassium induced by mercury (11). In this regard, a recent study of leakiness to potassium induced by basic redox dyes (12) is of interest because a common mechanism
may underly both the dye effect and the mercury effect. The action of the
dyes is related to two membrane sites, phosphoryl groups to which the dye
is reversibly bound, and oxidizable sites, perhaps sulfhydryl groups, respons-
ible for the leakiness to potassium. The physiological response is irreversible,
with a threshold for individual cells. That is, at any particular concentration
of dye, a fraction of the cellular population becomes leaky and the rest are
normal. With increasing concentrations of the dye, the fraction of leaky cells
increases in a log-dose probability response.

With mercury, the nature of the membrane defect responsible for K+
leakage has been investigated in some detail, including direct comparisons
with the action of the basic dye, methylene blue. Other metal cations, such
as lead, copper, and zinc were also tested. In a parallel study, the binding
of mercury by the cell was determined under a variety of conditions using
Hg^{2+} as a tracer, in an attempt to correlate the chemical events with the
physiological response.

Methods

Fresh bakers' yeast (Standard Brands, Inc.) was washed thoroughly. After 3 to 4
hours of aeration, to achieve constant endogenous metabolism, the cells were resus-
pended in distilled water adjusted to pH 3.0 with HCl and agitated by bubbling
air. If not otherwise stated, the final concentration of cells was 60 mg. per ml. of
suspension, the temperature 28°C., and the initial pH, 3.0. The substances to be
tested were added and at appropriate times samples were taken, centrifuged, and
the supernatant decanted for analysis. Mercury was determined by scintillation
counting of the gamma-emitting isotope, Hg^{203}. Potassium was analyzed with the
flame photometer (attachment for DU spectrophotometer). Titration data were
obtained with an automatic titrator (International Instrument Company, Canyon,
California). The same device was also used to maintain a constant pH in certain of
the dye uptake experiments.

RESULTS

1. The Nature of the Damage to the Membrane Induced by
Mercury

A. POTASSIUM LOSS The potassium content of bakers' yeast is relatively
high, about 0.2 m per kg. of cells. Nevertheless, the amount of potassium that
normally leaks out of cells suspended in distilled water is very small, due to
the relative impermeability of the cell membrane (11). In the presence of
mercuric chloride, however, the leakage of potassium was increased mark-
edly. The time course of the leakage was always the same regardless of the
mercury concentration, rapid for about 60 minutes and virtually ceasing after 90 to 100 minutes. (Fig. 1 represents a typical experiment.) With mercury concentrations of 1.6 to 2.0 mM or higher, essentially all of the potassium was lost from the cells, but with lower concentrations of metal, only a fraction of the potassium was lost even after 4 hours. It is obvious, then, that the parameter that best relates the physiological response to mercury is not the rate of K⁺ loss, but the maximal K⁺ loss.

B. POPULATION RESPONSE. The relationship between mercury concentration and the maximal K⁺ loss is almost identical with that obtained for K⁺ loss in cells treated with redox dyes (12). The response as a function of the log of mercury or dye concentration is a symmetrical S-shaped curve, which fits a "normal distribution." Presumably the same explanation for the curves holds true for mercury and for the dyes. That is, the response (K⁺ loss) is "all or none" for individual cells. As the concentration of mercury is raised, the threshold is exceeded in a higher proportion of cells. On this basis, the maximal values for K⁺ loss of Fig. 1, represent the proportion of cells in which the threshold has been exceeded and the S-shaped response curves represent the distribution of thresholds in the population. In the case of methylene blue, the all or none interpretation could be tested by demonstrating a 1 to 1 correspondence between the per cent loss of K⁺ and the per cent of cells which are visibly stained by the dye (by microscopic observation of individual cells). An attempt to stain the mercury-damaged cells with basic dyes¹ was only partly successful. At concentrations of mercury below 0.5 mM (producing approximately 50 per cent loss of K⁺ from the

¹ Methylene blue and Nile blue gave the best differentiation. There are technical problems in using the dyes, because they may not only stain cells damaged by mercury, but may themselves damage the membrane and render the cells stainable (12). By careful study a concentration of dye was found which was high enough to visibly stain mercury-treated cells, but which produced no membrane defect (as judged by K⁺ loss). The dye concentrations were 0.3 mM for methylene blue and 0.03 mM for Nile blue. The deleterious effects of the dyes can be prevented by various salts, but these also interfere with staining.
population) a good correlation was found between K\textsuperscript{+} loss and the percentage of stainable cells. At higher concentrations of mercury, however, the cells became granular and poorly stainable, so that differentiation of stained and unstained cells was very difficult. Nevertheless, the staining data over the range in which it could be studied, the normal distribution for the log dose-response curve, and the almost identical response of the cells to mercury and to redox dyes, support the theory that the all or none interpretation is valid for the action of mercury on permeability.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{Figure2.png}
\caption{Increase of the anion content of the medium after mercury poisoning. (Same experiment as in Fig. 1.) Buffer capacity of medium was estimated by back titration of the supernatant to the initial pH value of 3.0. The values at zero time represent the chloride concentrations of the supernatant. These values differ from one curve to another since with increasing HgCl\textsubscript{2} concentration an increasing amount of chloride ions had been introduced into the system. The initial Cl\textsuperscript{-} concentration in the control is due to HCl added to adjust the pH.}
\end{figure}

C. ION MOVEMENTS ACCOMPANYING K\textsuperscript{+} LOSS Normal yeast cells are relatively impermeable to anions. Gains or losses of K\textsuperscript{+} are usually balanced by equivalent losses or gains of H\textsuperscript{+}, as in cation exchange systems (13). In the experiments with mercury, however, cation exchange cannot account for K\textsuperscript{+} loss. At the end of a typical experiment, the K\textsuperscript{+} concentration of the medium was as high as 8 mm/liter. H\textsuperscript{+}, the only cation available for exchange, was present at a concentration of 1 mm/liter (pH 3.0). The pH rose to only 5 to 6. Thus, H\textsuperscript{+} exchange could account for less than 15 per cent of the K\textsuperscript{+} loss. The remainder must be accounted for by the escape of anions from the cell.

The release of cellular anions was measured experimentally by titrating the medium from its final pH of about 6.0 back to its initial value of pH 3.0. Comparison of Figs. 2 and 1 demonstrates the balance between the release of K\textsuperscript{+} and the release of anions. The K\textsuperscript{+} is partly balanced by the Cl\textsuperscript{-} added as HgCl\textsubscript{2} and the rest by the release of titratable anions from the cell.
of the mercury is bound by the cell (see following section). It is apparent, therefore, that mercury causes a generalized increase in permeability to cellular cations and to anions. The nature of the escaping cellular anions was not extensively investigated. A large proportion can be accounted for as inorganic and organic phosphates.

The changes in permeability induced by mercury were investigated with exogenous cations and anions which do not readily permeate the membrane of the normal cell. The first of these was the rather large anionic dye molecule, orange II (molecular weight 370), which has a high affinity for proteins. In normal cells, the orange II was taken up slowly (Fig. 3), but in mercury-treated cells, all of the dye was absorbed within 5 minutes. In the case of cationic dyes, it has already been mentioned that methylene blue (molecular weight 320) and Nile blue (molecular weight 371) can penetrate into mercury-damaged cells, producing a microscopically visible staining. Other ions

![Diagram](https://example.com/diagram.png)

**Figure 3.** β-Naphthol-orange (orange II) uptake in the presence and absence of HgCl₂. Dye concentration of supernatant, 3.0 mM/liter. The amount of mercury added, 26 mM/kg cells, pH had been kept constant at pH 3.0 throughout the experiment by automatically controlled addition of HCl.

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8The studies with orange II must be done at constant pH. At lower pH values, the dye is bound to the surface of the cell. Any shifts in pH resulting from changes in permeability will alter the dye-binding and complicate the data. For this reason, the experiment of Fig. 3 was carried out with the pH maintained constantly at 3 by an automatic titrator.
tested were Mn++ (using the isotope Mn^{69}) and phosphate (using P^{32}). In normal cells neither of these ions can exchange across the membrane, and only under special metabolic conditions are they absorbed (14, 15). In mercury-treated cells, the barrier to the penetration of both phosphate and Mn++ into the cell was broken down.

D. Recovery Once rendered permeable by mercury, the membrane does not recover during a 24 hour period, even if the cells are placed in a medium containing a high concentration of potassium phosphate (to minimize the losses of K+ and phosphate); or if the cells are maintained in a thick suspension (to minimize dilution of escaping constituents), or if they are given substrates or nutrients. Even if the mercury is removed from the cells by treatment with cysteine for 2 hours, the permeability to K+ is just as high as in freshly poisoned cells.

2. Mercury Binding

Mercury is bound rapidly by yeast cells. The half-time is 2 to 4 minutes at pH 3.0 and 26°C. and maximal binding is achieved in 15 to 20 minutes (Fig. 4). The maximal binding depends on the initial Hg concentration. On a percentage basis, the uptake is greater for lower concentrations of the metal, but, on an absolute basis, the maximal binding is related to mercury concentration according to the upper curve of Fig. 5. The curve shows a sharp inflection when the binding is 20 mm/kg of cells (initial mercury concentration = 0.5 mm) suggesting two distinct types of binding sites. Measure-
ments of chloride uptake give confirmation to the concept of two kinds of mercury uptake. In the experiments of Fig. 5, mercury was added as mercuric chloride. In aqueous solution this salt does not dissociate but exists primarily as a complex ion. Nevertheless, almost no chloride was taken up by the cells in the steep portion of the mercury uptake curve (Fig. 5). On the other hand, above the inflection point, the uptake of Hg and of chloride are almost parallel at a 1 to 2 ratio, as though the absorbed entity was HgCl₂. Normal yeast cells do not take up chloride.

Further evidence that the steep part of the uptake curve represents a binding of Hg⁺⁺ rather than HgCl₂, is the reduced rate of uptake in the presence of a surplus of NaCl. The half-time for uptake was 2 minutes in the presence of KNO₃, NaNO₃, MgSO₄ (at ionic strength of 0.2 M) or in distilled water, but this was reduced to 6 minutes in NaCl (at 0.2 M), due presumably to a reduction in the concentration of free Hg⁺⁺ by formation of the chloride complex.

The binding of mercury can be almost completely prevented by the addition of a surplus of cysteine. If, however, the uptake of mercury is allowed to proceed to maximum before the addition of cysteine, then desorption of the metal proceeds slowly, and is very sensitive to temperature. At the lowest temperature tested (1.5°C.) the desorption was only 10 per cent after 2 hours. At 38°C., however, the desorption approached 90 per cent in the same time (Fig. 6).

The binding of mercury is a very complex process. More than one type of binding is involved. Both the binding and desorption are sluggish processes, responding to temperature in a complicated fashion. Taking such observations into account, any analysis of the mercury distribution data in terms of

**Figure 5.** Mercury and chloride distribution between cells and supernatant after an equilibration period of 80 minutes.
mass law equilibria or adsorption isotherms would not be meaningful at the present time.

3. Mercury Binding vs. Mercury Effects

The minimal concentration of mercury which induced a measurable K+ leakage was 0.2 mm/liter. That which induced an almost maximal leakage was 0.6 mm/liter (Fig. 1). The amounts of mercury bound by the cells at these mercury concentrations were 4 mm/kg of cells and 12 mm/kg of cells. Examination of Fig. 5 indicates that the whole range of physiological changes in the membrane falls within the steep section of the binding curve. However, the inflection point occurs at a somewhat higher amount of mercury binding than does the maximal physiological effect, so that it can be concluded that only a portion of the binding sites represented by the part of the curve below the inflection point are involved in the physiological response.

The binding curve represents the average uptake of mercury by all the cells. The physiological response, on the other hand, represents the fraction of cells in which a threshold has been exceeded. It is not possible to determine whether the variation in responses of individual cells is predicated on differences in the amount of mercury bound by each cell, or by differences in effects produced by the same amount of mercury bound by each cell. In other
Figure 7. The action of various heavy metals on the potassium permeability of the yeast cell. Hg concentration, 2.0 mM/liter. All other heavy metal concentrations 4.0 mM/liter.

Figure 8. The combined effects of mercury and methylene blue as compared with the effects of each of these agents alone. The K⁺ loss of an untreated control was subtracted from all values.
words, the relationship between binding and response is not definable in terms of any simple formulation but is determined by the unknown nature of the threshold phenomenon itself. Furthermore, among the binding sites, a proportion may not contribute to the measured physiological response.

4. Effects of Other Agents

Although \( \text{HgCl}_2 \), at a concentration of 2 mM produced complete leakage of potassium, \( \text{CuCl}_2 \) at double this concentration had a much smaller effect, while \( \text{ZnCl}_2 \) and \( \text{PbCl}_2 \) had no measurable effect (Fig. 7).

Redox dyes such as methylene blue produce a response which seems to be identical with that produced by mercury. In Fig. 8, the \( K^+ \) leakage was measured with each agent alone and with both together. The response in the latter case is approximately that which would be expected if the two agents were interchangeable, acting on the same sites in the same manner.\(^3\)

DISCUSSION

In the light of recent studies on the action of lead on the red cell membrane (10) and on the effects of methylene blue on the yeast cell (12), the peculiar time course of the permeability changes caused by mercury has been interpreted in terms of statistically distributed differences in resistance of individual cells. Each cell behaves in an all or none fashion. If its threshold is exceeded its membrane becomes leaky toward ions. The most sensitive cell must bind an appreciable amount of mercury (about 4 mM/kg of yeast) before its threshold is reached. The distribution of thresholds within the population follows a log-dose, probability pattern, with the least sensitive cell requiring the binding of mercury to the extent of about 12 to 16 mM/kg.

With most inhibitory agents, the physiological response is related to the concentration of agent in a manner predictable from mass law considerations. Why does the response of the yeast membrane to mercury behave differently? The answer is implicit in the nature of the responses. When a specific enzyme or receptor functions by combining with a substrate or an effector substance, and the inhibitor inactivates the receptor molecule, then the pattern of inhibition can be described in terms of concentrations of effector, receptor, and inhibitor molecules. But in the case of mercury (and of redox dyes) the response is not due to a specific block of a process involving an effector–receptor–inhibitor combination, but rather a general breakdown of the membrane as a permeability barrier is involved, as a result of the interaction

\(^3\) The expected result is not the simple addition of the observed \( K^+ \) leakages with the two agents, but must be read from the S-shaped curve relating \( K^+ \) loss and the concentration of the agent.
of mercury with structurally important ligands. Each interaction of mercury with a membrane site constitutes a stress. No physiological response is observed, however, until the total stress reaches the breaking point for the membrane. The response is probably equivalent to hemolysis in red blood cells, except that the cellular structure remains largely intact because of the presence of the cell wall.

It has been pointed out that the actions of mercury and methylene blue are similar. Both agents irreversibly damage the membrane, resulting in loss of selective ion permeability. Both produce the all or none response. The ligand with which both substances might react is the sulfhydryl group, the methylene blue by formation of disulfide bridges (only the oxidized form of the dye is effective) and the mercury, by formation of $-S\text{-Hg}\text{-S}$ bridges. One difficulty inherent in this concept is the high degree of specificity with regard to heavy metals. Mercury, copper, lead, and zinc can all react with sulfhydryl groups, yet in the range of concentrations tested, only mercury and copper produced $K^+$ leakage. A somewhat similar situation was found in red blood cells, except that an effect was found with lead, silver, gold, and mercury and not with copper, cobalt, zinc, cadmium, and others. Metal specificity has also been observed in the inactivation of invertase located on the yeast membrane. The inhibition is due to sulfhydryl reaction in the case of mercury, but to other ligands in the case of silver (8), even though silver can readily combine with sulfhydryl groups in the test tube. It has been suggested that the unexplained chemical specificities found in intact cells such as these described above do not contradict the view that the sulfhydryl groups are important in membrane integrity, but rather, may be explained by steric factors relating to the exact location of the sulfhydryl groups in the membrane structure (16).

REFERENCES

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