SURFACE ACTIVITY AS THE CAUSE OF THE ANOMALOUS BEHAVIOR OF URETHANE AS AN INHIBITOR OF YEAST RESPIRATION

CARL LAMANNA AND JACK J. R. CAMPBELL

Department of Microbiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland, and Department of Dairying, University of British Columbia, Vancouver, British Columbia

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The oxidation of glucose by yeast is inhibited by urethane, and at sufficiently high concentrations of the inhibitor oxygen uptake may be suppressed completely. However, no direct proportionality exists between the degree of inhibition and urethane concentration. This discontinuity in the relationship between degree of inhibition and concentration of urethane has been adduced by Fisher and Stern (1942) as evidence for the existence of two parallel and independent systems of respiration differing in their sensitivity to urethane. The more sensitive system has been labeled by these investigators as an "activity" respiration specifically providing the energy utilized in assimilation for growth and multiplication.

It does not seem to be realized generally that urethane is a nonionic surface active agent. The surface activity of urethane is an expression of its composition, \( \text{O} \parallel \text{NH}_3\text{C}-\text{OCH}_2\text{CH}_3, \) which includes polar structures at one end of the molecule and the nonpolar ethyl group at the other end. Within the range of concentrations generally employed in metabolic studies the surface tensions of aqueous solutions of urethane at 28°C fall from a value of 66.5 for a 0.1 M solution to 51 dynes per cm\(^2\) at 1.0 M. At the same temperature the surface tension of distilled water is approximately 71 dynes per cm\(^2\).

In evaluating the influence of concentration on the toxicity of a metabolic inhibitor it must be recalled that the suspension of organisms in a solution of the inhibitor acts as a system of discontinuous phases. The effective concentration of the inhibitor is not the concentration added to the system but is the concentration of the drug accumulating in the interface at the bacterial surface. Characteristically the concentration of a surface active agent will tend to be greater at the interface between the solution and the bacterium than it will be in the bulk of the solvent phase. It is an important fact, too, that under a fixed set of conditions a change in concentration of a surface active agent is not accompanied by a proportionate change in the concentration of the surface active agent at an interface nor, therefore, of interfacial tension. The Freundlich and Langmuir adsorption isotherms are mathematical expressions of this finding. Since micelle formation tends to occur only within certain ranges of concentration of the surface active agent, discontinuities observed in interfacial tension-surface active agent concentration curves also may be due to micelle formation or the aggregation of solute molecules.

That this latter factor is an important cause of abrupt change in the slope of surface tension-solute concentration curves has been shown to be true for even so simple a surface active substance as butyric acid (Grindley and Bury, 1929). Similarly other physical characteristics of butyric acid, such as density, freezing point, electric conductivities, and vapor pressure, show a continuous relationship when the values are plotted against concentration. Biologically this phenomenon is important since organisms may not respond in an identical manner to individual molecules and aggregates of the same substance. For one thing the permeability of biological membranes to aggregates would probably be less than to individual molecules.

In the present report data will be presented which indicate that the discontinuous curves obtained when oxygen consumption of yeast is plotted against urethane concentration are similar to the curves obtained by plotting the surface tension of the solutions against urethane concentration. In the light of the principles outlined above this finding suggests that the discontinuities in the relation of urethane concentration to oxygen consumption are a reflection of the surface active nature of urethane and not of the existence of independent mechanisms of respiration of different degrees of sensitivity to urethane. Such a conclusion is fortified by the demonstration in
the past that disinfection and surface tension curves obtained with surface active agents are similar (Cowles, 1940).

METHODS

The methods of cultivating the organism, preparing the suspensions of cells, and determining the degrees of inhibition were those employed by

Figure 1. The effect of change in the concentrations of urethane upon the oxygen consumption of yeast and upon surface tension. In imitation of the work of Fisher and Stern (1942) the solutions of urethane were prepared in 0.025 M potassium acid phosphate with 2.5 per cent glucose added. Oxygen uptake readings are for 60 minutes.

Since the method of determining surface tension is very sensitive, we have preferred to connect the individual points rather than to draw best fitting curves. Slight errors in measuring out reagents and other manipulative procedures would result in differences from the intended concentrations of urethane detectable by the surface tension measurement.

Fisher and Stern (1942). Resting cellular suspensions were prepared from 48 hour cultures of the organism grown at room temperature on tomato juice agar (Miller et al., 1933). Suspensions used in the experiments on endogenous respiration were washed twice but were not aerated. All cellular suspensions were standardized using a Klett colorimeter. Warburg vessels contained a final volume of 2.15 ml.

Surface tension measurements were made with a Du Nöy tensiometer, Cenco model 70520. The concentrations of urethane to be studied were made up as double strength solutions. One-half of a solution was diluted with an equal volume of yeast suspension and served for study in the Warburg manometer flask. The other half which was used for surface tension determination was diluted in the same manner except that yeast cells were not included in the diluent.

The organism studied was an isolate from a pressed yeast cake of Fleischmann's baker's yeast (Saccharomyces cerevisiae).

EXPERIMENTAL RESULTS AND DISCUSSION

In figure 1 the effect of urethane in varying concentration on the oxygen consumption of yeast suspended in 0.025 M potassium acid phosphate with 2.5 per cent glucose at 22 C and 30 C has been recorded. Surface tension-urethane concentration curves for the same solutions are included also in the figures. It will be noted that the shapes of the oxygen consumption-urethane concentration curves conform to those of the surface tension-urethane concentration curves. Both sets of curves exhibit their most linear and most irregular portions within the same ranges of urethane concentration. The correspondence in shape between oxygen consumption-urethane concentration and surface tension-urethane concentration curves is not peculiar to the particular solvent system employed in the experiments recorded in figure 1. Figure 2 shows the same phenomenon with urethane in Locke's solution plus 2.5 per cent glucose at pH 7.2.
The lack of a linear relationship of the inhibition of oxygen consumption to urethane concentration occurs with endogenous respiration as well as with the oxidation of an external source of oxidizable substrate. However, experience with the endogenous system of which figure 3 is an example yielded a much more erratic picture of oxygen consumption with variation of urethane concentration than was the case with glucose oxidation. There is only a very rough correspondence in the endogenous respiration-urethane concentration curves to surface tension-urethane concentration curves. Nevertheless, it could not escape notice that both types of curves tend to plateau within the same range of urethane concentrations. In figure 3 an anomalous rise in oxygen consumption without any break in the surface tension curves should be noticed at 0.75 mM urethane. This was observed constantly in repeated experiments. On the other hand, the discontinuity at 0.35 mM did not appear regularly. But discontinuities when they did occur at this latter concentration and neighboring values seemed to follow upon irregularities appearing in surface tension-urethane concentration curves.

Cetyl pyridinium chloride is a useful and effective cationic surface active disinfectant. It is hardly to be expected that its mechanism of inhibiting oxygen consumption of yeast would be the same as that for urethane. Thus, it was of interest to discover that with this compound, just as with urethane, discontinuities were observed in oxygen consumption-inhibitor concentration curves, and that the oxygen consumption curves correspond in shape to surface tension-inhibitor concentration curves. The dramatic breaks in the oxygen consumption and surface tension curves in the range of 0.1 to 0.25 mM cetyl pyridinium chloride in all probability have their origin in micelle formation. Ordal and Borg (1942) have recorded previously that there is a sharp increase in lactate oxidation by bacteria at the concentration at which cetyl pyridinium chloride largely assumes the form of micelles.

The data which have been recorded cast doubt upon the assumption of Fisher and Stern (1942) that breaks in the slopes of oxygen consumption-urethane concentration curves are indicative of the presence of independent metabolic systems of varying sensitivity to urethane. Before such a conclusion could be accepted it would have to be shown that the effective concentration of urethane available for poisoning the organisms is directly proportional to the amount of urethane added to the biological system. For the kinds of experiments which have been performed to date such a relationship is doubtful in view of the surface active nature of urethane. Because urethane is surface active its concentration at the interface between the inhibitor solution and bacterial surface will not be directly proportional to the amount added. Furthermore, assuming that aggregated urethane molecules are less effective than their unassociated counterparts, any tendency for urethane molecules to aggregate would make it possible for solutions of higher concentration to be less inhibitory than slightly more dilute solutions of the same series.

It is unfortunate that no direct method exists for measuring the interfacial tensions at the bacterial surface. It must be emphasized that surface tension measurements while instructive for our purposes do not necessarily reflect with accuracy the accumulation of the surface active material at the bacterial surface, the primary data which would provide the answer to these problems. It must be emphasized that we do not view the depression of surface tension as the cause of interference with metabolic function by urethane.

Fisher and Henry (1944) have shown that oxygen consumption-urethane concentration curves for fertilized sea urchin (Arbacia punctulata) eggs show discontinuities whereas oxygen consumption curves for unfertilized eggs do not.
They have interpreted these data to mean that fertilized eggs possess in addition to the urethane sensitive respiratory system present in unfertilized eggs an independent and still more sensitive respiratory mechanism supplying energy for growth. This is not the only possible interpretation of their data. The discontinuities with the fertilized egg systems could be due to differences in the chemical potential of urethane at varying concentrations. The lack of breaks in the oxygen consumption curves with unfertilized eggs probably was due to the fact that the investigators necessarily were working with high concentrations of urethane. In figures 1, 2, 3, and 4 it should be noticed that beyond certain concentrations of surface active agents the surface tension curves tend to become smoother, linear, and of minimum slope, a result which would be expected also from the mathematical expressions of adsorption isotherms. This finding is most evident in the insert of figure 4 where data over the widest range of concentrations of surface active agent were obtained in the present instance. Concentration dependent actions therefore will be regular and linear at these higher concentrations of a surface active agent.

**SUMMARY**

Urethane is shown to be a surface active agent. The effect of changes in concentration of a surface active agent upon surface tension and upon oxygen consumption by yeast is similar. Thus, the lack of a linear relationship between the degree of inhibition of oxygen consumption and concentrations of urethane and cetyl pyridinium can be attributed to the surface active nature of these compounds rather than to the existence in yeast of independent pathways of respiration of different degrees of sensitivity to the presence of the inhibitors. Surface activity as a cause must

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**Figure 4.** Relationships of oxygen consumption and surface tension to the concentration of cetyl pyridinium chloride at 30 C. The surface active agent was dissolved in 0.025 m potassium acid phosphate with 2.5 per cent glucose added. Oxygen uptake readings are for 60 minutes.

The insert is a graph of a duplicate experiment performed over a greater range of concentrations of cetyl pyridinium chloride and includes points up to a concentration of 0.025 M.
be excluded before the possible alternative explanation of the existence of alternative pathways of metabolism can be accepted definitively as the cause of the anomalous behavior by a surface active agent as an inhibitor of metabolism.

REFERENCES


