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The adsorption of iodine from solution by micro-organisms and by serum

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The uptake of iodine by baker's yeast, serum, *E. coli* and *Staph. aureus* from aqueous dilutions of an iodine non-ionic surface-active agent complex was compared with that from aqueous dilutions of an ethanol and potassium iodide solution. The form of adsorption isotherms depended upon the iodine system and the substrate. A characteristic of all isotherms was the high affinity at low iodine concentrations. Except where high ethanol concentrations remained, uptake was greater from the ethanol: potassium iodide dilutions. The pH of the iodine system also influenced the uptake, the effect varying with the iodine system and the substrate.

EVANS & Fishburn (1943) have suggested that the first stage of disinfection by water soluble bactericides was an adsorption to the surface of the bacteria, followed by a chemical reaction between the adsorbed bactericide and the active proteins of the bacteria. Knaysi & Gordon (1930) were able to show that iodine was adsorbed by yeast from an aqueous iodine and iodide solution according to the Freundlich isotherm, whilst Habs (1932) undertook an investigation of the binding of iodine by bacterial cultures, from which he postulated the existence of an adsorption process involving an irreversible and a loose binding by the bacteria. Aqueous iodine and cetomacrogol systems do not produce the characteristic blue colour with starch, itself an adsorption phenomenon, and do not usually stain fabrics, in contrast to solutions of iodine and potassium iodide in ethanol. It would appear, therefore, that there is a fundamental difference between the release of iodine from the two systems. Accordingly the uptake of iodine from these two systems by yeast, serum, *Escherichia coli* and *Staphylococcus aureus* was investigated.

Experimental

MATERIALS

An iodine cetomacrogol complex and solution of iodine and potassium iodide in ethanol (Hugo & Newton, 1963); fresh baker's yeast; sterile horse serum containing no chemical preservative (Burroughs Wellcome & Co.); *Escherichia coli* Type I, formerly NCTC 5934, and *Staphylococcus aureus* NCTC 6571; the chemicals were analytical reagent grade.

METHODS

The yeast and serum were suspended in distilled water so that the dry weight of the suspensions were approximately equal to those of the bacterial suspensions, the dry weight being determined by drying to constant weight at 105°. The bacterial suspensions were prepared by washing 24 hr cultures from the surface of agar slopes, centrifuging at 3500 revs/min for 2 min to remove agar and large clumps, shaken

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for 5 min with glass beads to break up clumps, followed by further centrifuging for 5 min to remove the remaining clumps.

Since the effect of temperature on adsorption occurring from solution has been found to be small (Freundlich, 1926), the reactions were carried out at room temperature with the two iodine preparations simultaneously. The suspensions were mixed with an equal volume of the required concentration of iodine in stoppered containers and agitated gently. At the required time interval a sample was removed and the solid matter separated by centrifuging at 4,000 revs/min for 15 min in closed centrifuge tubes. The clear supernantant liquid was sampled and the iodine content estimated by titration with sodium thiosulphate and an amperometric endpoint. For the uptake by serum, separation could not be achieved by the above process. It was found, however, that by adding an equal volume of a 10% solution of trichloroacetic acid, the serum could be precipitated without affecting the iodine content of either system. This precipitate was removed by centrifuging as before. Buffer solutions were prepared from sodium acetate: hydrochloric acid and disodium hydrogen phosphate: citric acid systems (Vogel, 1951), the pH being measured by a Cambridge pH meter with glass and calomel electrodes.

Results and discussion

The uptake of iodine from the two systems by yeast, serum, and bacteria had an initial rapid stage, followed by a slower stage extending over a period of 4 to 6 hr (Fig. 1). To cover both these stages, the uptake

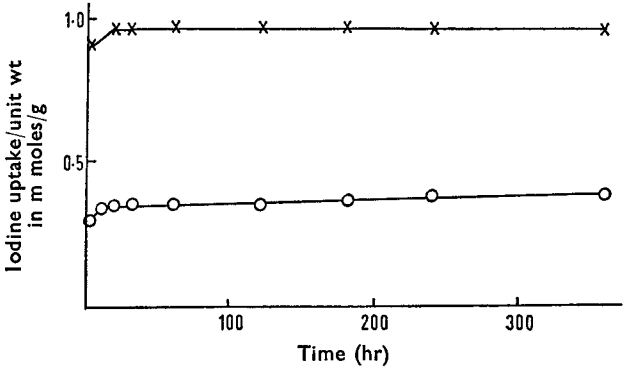

FIG. 1. The rate of uptake of iodine by *Staph. aureus* from iodine formulations. The behaviour of *E. coli*, yeast and serum was similar and over the same range. Original iodine concentration, 1,000 μg/ml. Dry weight of suspension, 1,740 μg/ml. × Iodine solution. O Iodine: cetomacrogol complex.

after 2 min and 5 hr was measured. Whether the mechanism by which iodine is removed from solution involves chemical, or physical mechanisms or a combination of both, cannot be stated from the work carried out. If, however, the uptake of iodine from the two systems is plotted in the form of adsorption isotherms, they show curves the forms of which

can be considered in the light of the classification of isotherms presented by Giles, MacEwan, Wakhwa & Smith (1960) (Figs 2 and 3). In this classification, the isotherms for the adsorption of organic solutes are

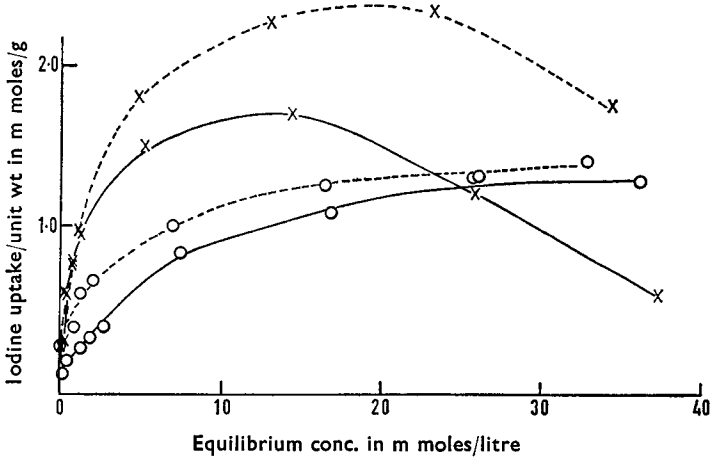

FIG. 2. Adsorption isotherms for the uptake of iodine by yeast (—) and serum - - -) iodine formulations, after 5 hr. Yeast, dry weight of suspension, 2,910 μg/ml for iodine solution and 2,890 μg/ml for the iodine: cetomacrogol complex. Serum, dry weight of suspension, 2,656 μg/ml. × Iodine solution. ○ Iodine: cetomacrogol complex.

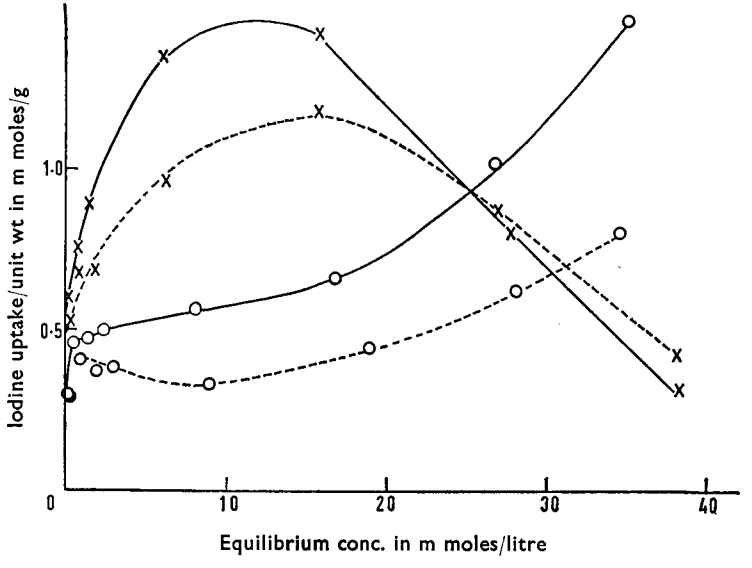

FIG. 3. Adsorption isotherms for the uptake of iodine by *E. coli* (—) and *Staph. aureus* (- - -) from iodine formulations, after 5 hr. *E. coli*, dry weight 2,690 μg/ml for iodine solution and 3,190 μg/ml for the iodine: cetomacrogol complex. *Staph. aureus*, dry weight 3,430 μg/ml for iodine solution and 3,060 μg/ml for the iodine cetomacrogol complex. × Iodine solution. ○ Iodine: cetomacrogol complex.

divided into four main classes, S, L, H and C according to the nature of the initial portion of the curve. Thus for the isotherms of the S type, adsorption is facilitated as the solute is adsorbed, the curve being convex to the x axis. The L type isotherm is of the familiar Langmuir pattern, where adsorption is hindered as the solute is adsorbed, the curve being concave to the x axis. The H type isotherm is similar to the L type, but the affinity of the solute for the adsorbent is so high that the curve commences at a positive value on the y axis. The C type isotherm (constant partition) shows a linear relation between the amount adsorbed and the equilibrium concentration. Each of these four classes are then further divided into five sub-groups, 1, 2, 3, 4, and mx, according to the subsequent shape of the isotherm. The significance of these different isotherms in the diagnosis of adsorption mechanisms is discussed by Giles & others (1960).

In each experiment (Figs 2 and 3) the initial stages of the isotherms follow the H type curve which suggests a high affinity of the iodine for the substrates, such that in dilute solution the amount of iodine remaining in solution could not be measured. After the initial stage, however, the shape of the isotherms varied, depending upon the iodine system and the substrate. There was no difference in the shape of the 2 min and the 5 hr curves, only a displacement to show greater uptake after the longer time.

The whole range of the isotherms investigated for iodine solution takes the same general form for all substrates. From the classification of Giles & others (1960), this shape would be included in the sub-group mx, which is characterised by the occurrence of a maximum, considered to be due to association of the solute. Thus with an increase in concentration the solute:solute attraction begins to increase more rapidly than the solute: substrate attraction. The maximum could also be due to

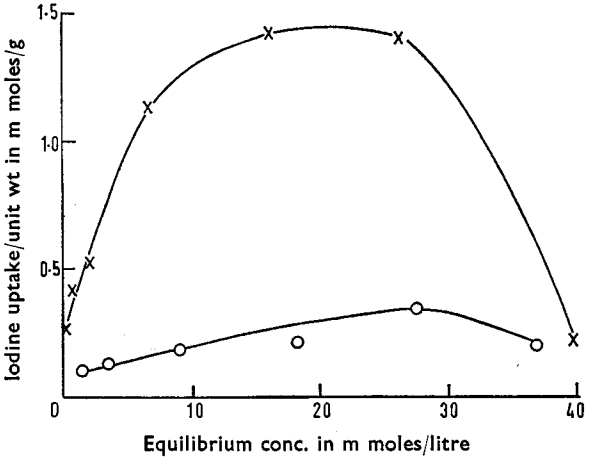

FIG. 4. Adsorption isotherms for the uptake of iodine by yeast from iodine solution, (×) diluted with water and iodine solution (○) diluted with 78% ethanol and 2% potassium iodide, after 2 min. Dry weight of suspension, 3,100 μg/ml.

the change in the solvent which occurs when the iodine solution is diluted with water. To investigate this point, the iodine solution was diluted with the original solvent, that is ethanol: potassium iodide. Fig 4 compares the 2 min uptake by yeast and illustrates the effect of changing the solvent on dilution. Considering the isotherms up to the maxima, they may be classified as H2. This represents the state where as more sites on the substrate become filled, it becomes increasingly difficult for the iodine to find vacant sites, until eventually all the sites are filled with either iodine or solvent and there is a high energy barrier to further adsorption. As these curves are modified Langmuir isotherms, they follow the Freundlich isotherm over a range of iodine concentrations (Fig. 5).

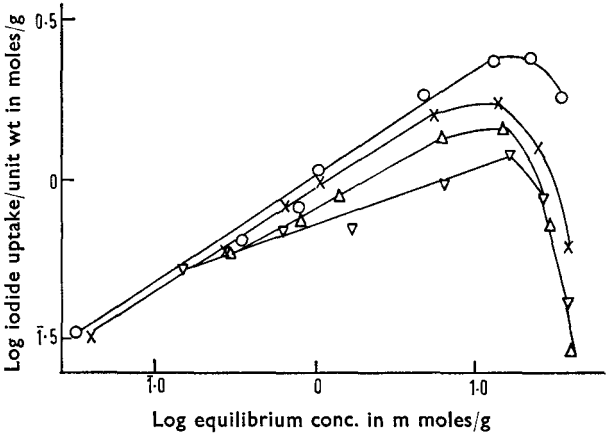

FIG. 5. Freundlich adsorption isotherms for the uptake of iodine by serum (○); yeast (×); *E. coli* (△) and *Staph. aureus* (▽) from iodine solution, after 5 hr. Dry weight of suspension, serum, 2,652 μg/ml; yeast, 2,910 μg/ml; *E. coli*, 2,690 μg/ml and *Staph. aureus*, 3,430 μg/ml.

The type H sub-group 2 isotherm was obtained for the uptake of iodine by yeast and serum from the iodine:cetamacrogol complex (Fig. 2) and thus the same kinds of mechanism are presumably involved. The uptake by the bacteria, however, takes a different form (Fig. 3). Initially there was the same high affinity, but soon a maximum was reached, with a rise as the iodine concentration increased. Thus again there appears to be evidence of the sub-group mx isotherms, with adsorption related to the changes in attraction with concentration. Salton (1951) found that the adsorption of cetyltrimethylammonium bromide by six species of bacteria (including *E. coli* and *Staph. aureus*) was of the H2 type, but irregular isotherms have been noted for the adsorption of cationic surface-active agents by cellulose (Sexsmith & White, 1959) and anionic surface-active agents by cotton (Meader & Fries, 1952). Thus the irregular isotherms for the adsorption of iodine from the iodine: cetomacrogol complex may be related to the surface-active properties of the complex.

In all cases it will be noted that, as anticipated, there was a greater uptake of iodine from the iodine solution, except where the solvent effect dominated the uptake. This suggests that the iodine may be adsorbed from the cetomacrogol system in the form of a complex, or that there is a greater affinity of the iodine for the cetomacrogol than the ethanol: potassium iodide solution. There is also the factor of interfacial tension, Freundlich (1926) considering that adsorption is greatest where the interfacial tension between solvent and substrate is high. The cetomacrogol

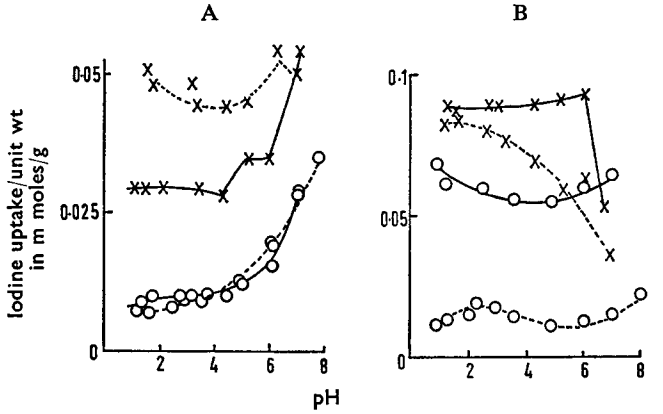

FIG. 6. The effect of pH on the uptake of iodine by (A) *E. coli* (- - -) and *Staph. aureus* (—) and (B) yeast (- - -) and serum (——) from iodine formulations, after 2 min. Original iodine concentration, 1,000 μg/ml.

		Dry weight of suspension μg/ml			
		E. coli	*Staph. aureus*	Yeast	Serum
×	Iodine solution	3,180	3,260	3,100	2,652
O	Iodine: cetomacrogol complex	2,985	3,040	3,200	2·652

can be expected to lower interfacial tension and this may be involved in the smaller uptake. All these factors plus others, however, may be involved in the process. Beckett, Patki & Robinson (1959a,b) found that cetomacrogol reduced the uptake of hexylresorcinol by *E. coli*, and not only prevented the changes in turbidity which took place in aqueous solutions, but considerably reduced the bactericidal activity. The effect of cetomacrogol on the bactericidal activity of iodine will be described by Hugo & Newton (1964).

The influence of pH on the uptake of iodine was dependent on the iodine system and the substrate (Fig 6). The uptake of iodine by yeast from the iodine: cetomacrogol complex changed little with increase in pH whereas a decrease occurred from iodine solution. In connection with this, it was observed that in unbuffered systems there was a fall in the pH after contact with yeast, whilst the converse occurred after contact with bacteria, which show an increased uptake with increase in pH. The difference in the uptake of iodine by yeast is probably related to the difference in the two iodine systems, whilst that between the uptake by yeast and bacteria, to the differences in the nature of the surface of the

adsorbing particles. The increased iodine uptake by bacteria which occurs with increase in the pH is in contrast to the reported decrease in the bactericidal and sporicidal activity which occurs as the pH rises (Gershenfeld & Fox, 1948; Gershenfeld & Witlin, 1949; Chambers, Kalber, Malaney & Bryant, 1952; Hugo & Newton, 1964 and Wyss & Strandskov, 1946).

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