tion that the ionic-covalent resonance energy in this case should be small. Hence, we would expect  $D_1 > D_2$ , and E(OCI) < E(O). Vier and Mayer' have recently measured E(O) as 71 kcal.; we have pointed out that the value  $D_1$  is greater than the normal Cl-O bond-energy by not more than 20 kcal. Thus, we would conclude that E(OCI) must lie between the limits 51 and 71 kcal.

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<sup>1</sup> Weiss, Trans. Farad. Soc., 43, 173 (1947).

<sup>3</sup> Goodeve and Wallace, *Trans. Farad. Soc.*, 26, 255 (1930).
<sup>3</sup> Goodeve and Marsh, *J. Chem. Soc.*, 1332 (1939).
<sup>4</sup> See, for example, Skinner, *Trans. Farad. Soc.*, 41, 645 (1945).

<sup>5</sup> See, for example, Skinler, Trane. Farad. Soc., 41, 646 (1945).
<sup>5</sup> See, for example, Weiss, Trans. Farad. Soc., 31, 966 (1935). Dwyer and Oldenberg, J. Chem. Phys., 12, 351 (1944).
<sup>6</sup> Bernal and Fowler, J. Chem. Phys., 1, 515 (1933).

7 Vier and Mayer, J. Chem. Phys., 12 28 (1944).

DR. SKINNER was good enough to send me a copy of his communication. More recently we have also been led to assume considerably lower values for the sum of electron affinity and heat of hydration of the ClO radical from a study of the photochemistry of hypochlorites in solution. This seems to suggest a value of  $\{E(\text{ClO}) + S(\text{ClO}^{-})\} \sim 105 \text{ kcal.}$ 

I was, however, unaware of the ClO bond-energy value as given by Goodeve and Marsh to which Dr. Skinner has directed my attention.

Introducing this value into my previous calculations, the corresponding former figures should be diminished by the difference between the values of Goodeve and Marsh, and Goodeve and Wallace (~ 37 kcal.).

Thus one obtains  $\{E(ClO) + S(ClO^{-})\} \sim 128$  kcal. which, with the previously assumed value  $S(CIO^{-}) \sim 60$  kcal., leads to  $E(CIO) \sim 68$  kcal.

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## Physiological Heterogeneity of Metaphosphate in Yeast

IT has been shown that when yeast is fractionated by repeated extractions with cold trichloracetic acid a species of phosphate, found in both the acidsoluble and the residue fractions, is identifiable as metaphosphate<sup>1,2</sup>. The co-existence in a yeast cell of metaphosphate in both acid-soluble and acidinsoluble forms suggests the possibility that these two may differ in their metabolic and physiological function. It is the purpose of the present note to report some recent observations which indicate that this is the case.

Resting cells (S. cerevisiæ, baking type, 48-hour culture) were incubated under anaerobic conditions while suspended in a medium containing 6 per cent glucose, phosphate buffer (pH 4.5) and P<sup>32</sup> labelled inorganic phosphate. After 2 hours incubation at 30° C., these cells were extracted with 5 per cent trichloracetic acid for one and a half hours at 3° C. The resulting supernate  $(S_1)$  was treated according to the flow sheet, Fig. 1. In the precipitation with the magnesia mixture the test solutions were allowed to stand overnight in the cold. The precipitate



10%] Fig. 1. Fractionation of the supernate from a cold trichloracetic acid extraction of yeast. The first step accomplishes a partial separation of metaphosphate by the use of a magnesia mixture which precipitates a mixture of imeta- and orthophosphates. The metaphosphate can then be removed in a highly purified state by a precipitation with Ba<sup>++</sup> at acid pH. As is indicated, the procedure does not remove all the metaphosphate from the original trichloracetic acid extract. Data in parentheses indicate type of phosphate compound found. Numbers in brackets give extent of equilibration with external phosphate in medium.

denoted as  $P_3$  contained a portion of the acid-soluble metaphosphate and exhibited no activity, showing that no exchange had taken place. The ortho phosphate contained in the supernate  $(S_3)$  showed that exchange had occurred to the extent of 24 percent (based on initial P<sup>32</sup> content of the phosphate in the medium).

If the Schmidt<sup>3</sup> method of fractionation is carried out following the cold trichloracetic acid extraction there is obtained a residue  $(R_5)$  which can be shown to contain a large amount of 7-min. hydrolysable phosphate (completely hydrolysed to orthophosphate by treatment for seven minutes with 1 N hydrochloric acid at 100° C.). If this is treated according to the flow sheet in Fig. 2, another metaphosphate fraction  $(P_{\bullet} \text{ of Fig. 2})$  can be isolated. This metaphosphate is a highly active fraction and has, as indicated, exchanged to the extent of 24 per cent. In fact, this is the only fraction thus far examined which approaches in activity that of the internal orthophosphate of the cell.

It appears, therefore, that there are at least two different kinds of metaphosphate in yeast, differing radically in metabolic function. One, which is easily extractable by trichloracetic acid in the cold, presumably does not exchange with any other phosphate fraction in the cell. The other, which is apparently associated with a proteinaceous material, is one of the most highly active organic phosphates in the cell. This result is reminiscent of work reported many years ago by MacFarlane<sup>4</sup>.

Aside from possible implication for the physiology of the yeast cell, the results reported here concern the validity of conclusions derivable from isotope



Fig. 2. Isolation of an active metaphosphate from the residue of a potassium hydroxide extraction of yeast. The procedure followed is essentially similar to that of Fig. 1. A trichloracetic acid extract is made, and an acid barium precipitation employed to separate the metaphosphate from other components. Symbols employed have the same meanings as in Fig. 1.