the effect of cadmium on testicular tissue is not comparable to the effect of a lack of zinc, the former being rapid and sudden. In our experiments it was possible to protect testicular tissue of adult rats completely from the destructive effect of cadmium (0·04 m.mole cadmium chloride/kgm. body-weight subcutaneously) by the simultaneous administration of 80, 100 and 200 times the amount of zinc, injected in the form of acetate subcutaneously in divided doses before, simultaneously with, and after, application of cadmium.

Of all soft tissues, spermatozoa (and the prostate) contain the largest amount of zinc. Turnover of zinc is greatest in the testes[2]. Cadmium and zinc are physico-chemically very close to each other. It is therefore attractive to speculate that cadmium displaces zinc from those molecular structures into which zinc is probably incorporated during the maturation of spermatozoa. In view of the fact that it has been possible to suppress the destructive effect of cadmium on the testis by using many times the dose of zinc, a mechanism of competitive inhibition between these two metals may be suggested.

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Action of an Autolytic Enzyme of *Bacillus subtilis* on the Cell Wall

Many biologically produced substances are known to lyse living micro-organisms. Among them, lysozyme has been studied most extensively for the mechanism of its action, and it has been shown that this action is the dissolution of cell wall, probably the hydrolysis of mucopolysaccharide contained in it[1]. Studies on the action of autolytic enzymes, on the other hand, are few, and their site of action has not hitherto been known. Recently, one of us observed very rapid autolysis of amylase-producing strains of *Bacillus subtilis*[2] and isolated an autolytic substance(s) from the lysate[3]. Studies on this autolytic substance(s) have shown that it lyses the cell wall.

An autolytic enzyme preparation was obtained from the lysate of *B. subtilis H* by precipitation with ammonium sulphate. Cell-wall preparation was obtained from the same bacterium. Washed cells were disrupted by boiling at 100° C. for 10 min. and, further, by exposure to supersonic vibration. Disrupted cells were digested successively with crystalline trypsin, crystalline *B. subtilis* protease and crystalline chymotrypsin. The residue seemed to consist mainly of cell wall when examined in the electron microscope. As shown in Fig. 1, this cell-wall preparation was dissolved by the autolytic enzyme solution (crystalline lysozyme also dissolved it, but crystalline ribonuclease had no effect). Electron microscope examination also confirmed this result.

Further investigation of the mechanism of lytic action was undertaken against living bacteria. Recently, Weibull[4] succeeded in dissolving the cell wall only, by the controlled treatment with lysozyme in sucrose, and obtained protoplast. The protoplast of our strain, *B. subtilis H*, was also formed by treatment with lysozyme in the presence of 10–15 per cent sucrose or polyethylene glycol. Therefore, the effect of high concentrations of these substances on the lytic action of the autolytic enzyme solution was examined. Complete protection was observed during the first 1–2 hr., but thereafter lysis occurred and action seemed to take place in two steps: the dissolution of the cell wall, and disruption and lysis of the resulting protoplast. When the enzyme solution was heated at 100° C. for 10 min., the autolytic activity was completely abolished. This boiled solution was added to the cell suspension in sucrose (15 per cent) together with the lysozyme solution. Complete lysis occurred, whereas only protoplast formation was observed without boiled enzyme solution. Therefore it was concluded that this enzyme solution contains a heat-stable substance(s) which causes the disruption of the protoplast in addition to the heat-labile enzyme which dissolves the cell wall.

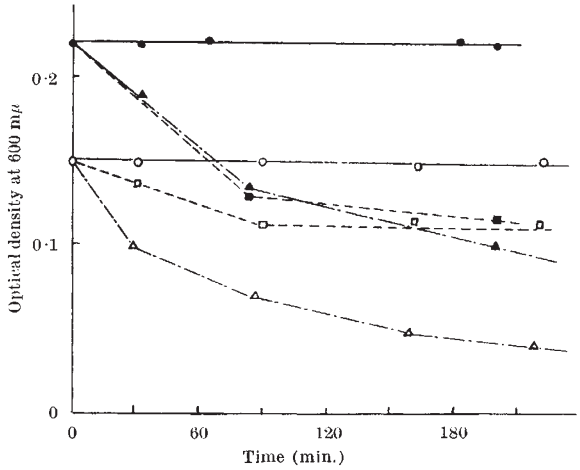

Fig. 1. Action of the autolytic enzyme solution and lysozyme on the cell-wall preparation. The cell-wall preparation was suspended in *M*/30 phosphate buffer, mixed with the autolytic enzyme solution or lysozyme solution as indicated. Total volume, 6 ml., *p*H 7·2. Rate of dissolution at 30° C. was followed by measuring the changes of optical density at 600 mμ

Set 1: ●—●, control; ■—■, plus 1 ml. autolytic enzyme solution; ▲·—·▲, plus 3 ml. autolytic enzyme solution. Set 2: ○—○, control; □–□, plus lysozyme 0·67 γ/ml.; △·—·△, plus lysozyme 6·7 γ/ml.

In view of these and other results, it seems reasonable to conclude that the primary site of action of this autolytic enzyme solution is the cell wall, probably the polysaccharide contained in it (polysaccharide was isolated from the cell and its depolymerization by this enzyme solution was demonstrated (unpublished results)). A more detailed account of this work will be published elsewhere.

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