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Proteolytic Enzymes in *Entamoeba histolytica*

THE presence of proteolytic enzymes in *Entamoeba histolytica* has been suspected for many years and the extracellular secretion of these enzymes has been put forward by many authors as an explanation of the mechanism of tissue invasion by this parasite[1]. Experimental evidence of their occurrence in *E. histolytica* has been presented by several authors[2], though a detailed study of them has not been made.

The proteolytic enzymes of *E. histolytica* (strain $P + A^1$) were investigated by making an extract of amoebae grown *in vitro* in association with *Escherichia coli*. The amoebae were collected in suspension from the culture medium (solid egg base covered with dilute horse serum, supplemented with rice starch) and washed with Tyrode solution. The amoebae were re-suspended in a volume of Sørenson's phosphate buffer, *p*H 7·2, to give a calculated concentration of 5 million amoebae/ml. The amoebae were then frozen overnight. After thawing, the rice starch and debris were removed by centrifuging and the supernatant was shaken with a few drops of chloroform to inhibit the growth of bacteria. Excess chloroform was removed by centrifuging. Extracts prepared in this manner from strain $P + A^1$ were sterile.

The extract was incubated at 37° C. with casein solution as substrate and with ammonia – citric acid buffer to give a range of *p*H from 4·0 to 8·5. After incubation, the reaction was stopped by the addition of trichloracetic acid. The degree of hydrolysis of the casein was determined with Folin–Ciocalteau phenol reagent, and expressed as μgm./ml. of tyrosine. The method is similar to that described by Holter and Løvtrup[3].

The extract showed high activity from *p*H 6·5 to 8·5 with a maximum at *p*H 7·9–8·0. When the extract was incubated with the substrate at the optimum *p*H, the amount of hydrolysis increased linearly with time up to the end of 2 hr.; thereafter the rate of hydrolysis decreased. The activity was not potentiated by cysteine or inhibited by iodoacetate.

The proteolytic enzymes in extracts of strain $P + A^1$ also dissolved formalin-denatured gelatine and reduced the viscosity of gelatine solutions.

Extracts of the associated strain of bacteria, *Escherichia coli*, showed no proteolytic activity on the casein substrate, nor did they affect the viscosity of gelatine solutions.

Other strains of *E. histolytica* are being examined by this technique and all show some proteolytic activity. Further details of experiments showing the correlation of virulence and proteolytic activity will be published elsewhere.

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Disomic and Tetrasomic Inheritance in a *Solanum* Hybrid

IN the course of a cytogenetic study of the nature of differentiation of diploid species ($2n = 24$) of the section Tuberarium, genus *Solanum*, the mode of inheritance of three loci could be followed in a cross between *S. macolae* and *S. simplicifolium*, two species of the series Tuberosa occurring in Bolivia and Argentina. The *simplicifolium* parent used in the cross had simple leaves, a prominently winged stem and white corolla, in contrast to the compound leaves, rudimentary stem wing and purple corolla possessed by the *macolae* parent. Except for corolla colour in *macolae*, the two species bred true for their characters. The *macolae* parent was found to be heterozygous for *P*, a dominant factor necessary for the formation of purple colour in the corolla.

Meiosis was regular in the F_1 plants, and the plants set berries with viable seeds. These plants had compound leaves and prominently winged stems. There was segregation for corolla colour; six plants had purple and four plants had white corollas. In F_2 progenies from F_1 plants with compound leaves, prominent stem wing and purple corolla, there was monogenic segregation for all the characters. In backcrosses between the F_1 and recessive and dominant parents, 1 : 1 and ∞ : 0 segregations respectively were obtained. Thus, the genotypes of the *macolae* and *simplicifolium* parents were determined as *LLwwPp* and *llWWpp* (*L*, for compound leaf; *W*, for prominent stem wing and *P*, for purple corolla) respectively. There was no evidence of linkage among factors *L*, *P* and *W* in the F_2 and backcross data.

Seeds from the initial cross were treated with colchicine, and tetraploid hybrid plants were thus obtained. At first metaphase in the doubled hybrid, there was a mean frequency of 1·19 quadrivalents 0·095 trivalents, 21·025 bivalents and 0·905 univalents per cell. The number of quadrivalents per cell ranged from 0 to 4. The tetraploid plants were fertile. When the C_2, C_3 and C_4 plants (corresponding to the F_2, F_3 and F_4 plants of the diploid hybrid) were grown in following years, it was found that all