

alterations occurred with increasing dose of radiations. Therefore, I conclude that the depolymerization of hyaluronic acid observed is the result of both indirect and direct effects of X-rays.

Hence X-rays are able to depolymerize an acid mucopolysaccharide such as hyaluronic acid in the same way as the specific enzyme does; it seems that ionizing radiations, like hyaluronidase, act on hyaluronic acid by opening the N-acetylglucosaminic bond.

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Occurrence of Glutaminase in *Endamoeba histolytica*

THE existence of glutaminase in bacteria has been demonstrated¹⁻³. However, to our knowledge no work has been done on other micro-organisms such as Protozoa. Little is known on the enzymatic make-up of *Endamoeba histolytica*⁴. Recently, proteolytic enzymes were demonstrated in the amoebae⁵. Since a method became available for obtaining large quantities of bacteria-free amoebae^{6,7} an investigation was undertaken to study the glutamine hydrolysing enzyme in this organism.

The amoebae for the glutaminase assay were prepared by growing the organisms in a medium which consisted of egg slants overlaid with horse serum-Ringer solution, glucose, adenosine triphosphate, ribose-5-phosphate, rice powder, sodium thioglycollate, penicillin and streptomycin. The amoebae were harvested after 3-4 days of incubation at 37°C. and washed 2-3 times in saline solution before performing the enzyme assay. These cultures of *E. histolytica* contained no viable bacteria. Three strains of amoebae (NRS, UC, HUS-100) were employed; the results with the three strains were essentially identical.

The incubation mixture consisted of 0.3 ml. amoeba suspension (approximately 1,000,000 amoebae per ml.), 0.5 ml. 0.1 M veronal-hydrochloric acid buffer, 0.1 ml. 0.1 M l-glutamine, 0.1 ml. 0.9 per cent sodium chloride solution. The mixture was incubated in 25 ml. penicillin bottles for 30 min. at 37.5°C., after which the bottles were stoppered, injected with 1 ml. of 20 per cent sodium carbonate solution, and assayed for ammonia⁸. Glutamine activity was calculated by subtracting ammonia formed both in the absence of substrate and in the absence of amoeba from ammonia liberated in the presence of both substrate and amoeba.

The optimal pH of the glutaminase of *E. histolytica* was found to be between 7.0 and 7.5. This is markedly different from that of *Clostridium perfringens*, which was reported to be around 5.0; it is interesting to note that these organisms are found in the soil whereas the intestinal tract of man is the normal habitat for *E. histolytica*. Differences in pH and other environmental factors may account for the optimum pH of the glutaminase of *E. histolytica*. With regard to activators of glutaminase, the amoebic enzyme

is more like mammalian glutaminase than the bacterial enzyme. The former is activated by phosphate and the latter by monovalent anions. Only phosphate activated the amoebic glutaminase; sodium chloride did not activate this enzyme in the amoebae.

Thus the occurrence of a phosphate-activated glutaminase in *E. histolytica* has been established.

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Occurrence of Flavokinase Activity in Plants

RIBOFLAVIN functions biologically as either flavin mononucleotide or flavin-adenine-dinucleotide, which constitute the prosthetic group of a variety of enzymes¹. A study of pathways through which these nucleotides themselves are synthesized from the free vitamin would be valuable. The only reports in the literature regarding the enzymatic synthesis of flavin mononucleotide pertain to the study of the enzyme from baker's yeast² and a riboflavin-excreting mutant yeast *BY2*³. On the basis of the specificity and stoichiometry of the reaction, the enzyme was designated 'flavokinase'. In this communication, the occurrence of the enzyme in plants is reported for the first time.

For the preparation of enzyme extracts, 5 gm. of the acetone-dried powders were ground with equal weights of 'Pyrex' glass powder and 10-15 ml. water, and extracted in the cold (0-5°) for 16-18 hr. The supernatant of this extract was dialysed at 0-5° for 4 hr. The precipitate resulting after dialysis was centrifuged off and the supernatant was used as the enzyme. The identity of flavin mononucleotide was established as follows. A 40 ml. reaction mixture consisting of 2 ml. adenosine triphosphate solution (obtained by converting 40 mgm. of barium-adenosine triphosphate⁴ to the sodium salt), 2 ml. sodium fluoride solution ($1 \times 10^{-1} M$), 2 ml. of manganese sulphate solution ($3.0 \times 10^{-4} M$), 4 ml. of riboflavin solution ($1.0 \times 10^{-4} M$), 14 ml. of veronal-hydrochloric acid buffer (0.1 M) pH 8.4 and 16 ml. of enzyme preparation (from *Phaseolus radiatus*) was incubated at 37° for 18 hr. The reaction was stopped by adding 16 ml. of 17.5 per cent trichloroacetic acid and heating at 80-85° for 5 min. The supernatant was subjected to preparative circular paper chromatography⁵. The eluate of the band corresponding to flavin mononucleotide showed absorption maxima in the region 264-266 m μ . It was chromatographically identical with an authentic sample of flavin mononucleotide. On chemical, as well as enzymatic, hydrolysis of the product, liberation of phosphate occurred, and riboflavin was the only flavin formed after such