triphosphatase activity related to the sodium–potassium pumps.

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Lecithin-splitting Enzyme of *Vibrio* El Tor

It was known that *Vibrio cholerae* and *Vibrio* El Tor excrete a lecithin-splitting enzyme in their culture medium[1-3], but the nature of this enzyme was not properly ascertained. The present communication is concerned with the characterization of the enzyme excreted by *Vibrio* El Tor with special reference to some of its important properties. The enzyme was isolated from the culture filtrate of the organism, which was grown aerobically in surface cultures using peptone–water medium for 48 hr. The culture filtrate was fractionated by ammonium sulphate and the precipitate obtained between 50–75 per cent saturation contained all the enzyme activity. This was dissolved in 0·01 *M* phosphate buffer and after dialysis against the same buffer was kept frozen at − 15° C. for about four weeks, up to which time no loss of enzyme activity has been noticed. Addition of glycerol is not necessary for its storage.

The enzyme has been found to attack only lysolecithin molecules liberating acid-soluble phosphorus and acid-labile choline compounds without any liberation of free choline and is inactive towards purified lecithin. The incubations were performed in 10-ml. stoppered centrifuge tubes into which the requisite amount of lipid substrate in chloroform solution (usually 0·75 μmole) was added. The solvent was removed *in vacuo* at about 40° C. and the lipid was then mixed up in buffer solution (50 μmoles barbital buffer of *p*H 8·0, which has been found to be its optimum *p*H). The enzyme equivalent to approximately 0·15 mgm. protein was then added and the total volume of the incubation mixture was kept 1 ml. The incubations were made at 37° C. for 1 hr., after which the reaction was stopped by the addition of trichloracetic acid solution to a final concentration of 5 per cent in the presence of bovine serum albumin (5 mgm. per flask). This was centrifuged, the centrifugate was extracted once with three volumes of a mixture of chloroform and *iso*butanol (2 : 1, v/v) and was again centrifuged. The aqueous portion which was separated was analysed for the reaction products. It has been noted that there is a decrease in the fatty acyl ester group and simultaneous increase in acid-soluble phosphorus, and these are in quantitative proportion with the release of acid-labile choline compound. This choline compound has been identified as glycerophosphoryl–choline with the help of paper chromatography according to the method described by Dawson and Rowlands[4]. The enzyme is therefore characterized as 'phospholipase *B*'.

Unlike any other phospholipase *B* from *Penicillium notatum*[5], *Aspergillus niger*[6] or from any animal tissue such as pancreas[7] and liver[8], this enzyme has a *p*H optimum of 8·0 and is fairly stable towards heat denaturation. It is destroyed only on heating at 100° C. for 15 min., and heating for 5 min. at that temperature causes about only 50 per cent inactivation. It is fairly insensitive towards metal ions, and when used at a final 1 m*M* concentration only zinc causes about 40 per cent inhibition, whereas copper, manganese, calcium and magnesium have practically no effect. Of the inhibitors studied at 10^{-3} *M* final concentration, cyanide, azaguanine and chloramphenicol have no effect, azide and dinitrophenol inhibit the enzyme to the extent of about 15–20 per cent, whereas sodium fluoride, versene and *p*-chloromercuribenzoate individually cause about 45 per cent inhibition. The inhibition by versene and by *p*-chloromercuribenzoate can be fully reversed by the addition of calcium and glutathione respectively. It has further been observed that glutathione and also cysteine bring about some stimulation of the enzyme. Work on the properties and also on the mechanism of excretion of this enzyme by both *Vibrio cholerae* and *Vibrio* El Tor is in progress.

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Immunity of Bee Keepers to Some Constituents of Bee Venom : Phospholipase-*A* Antibodies

For many years bee venom has been used in the treatment of arthritis and rheumatism, but with little evidence of its value[1]. Bee venom contains three important constituents : the main toxic fraction I, and the enzyme fraction II, containing phospholipase and hyaluronidase. Each enzyme has been isolated in pure form[2,3]. The hæmolytic effect of the venom on blood corpuscles is caused directly by the toxic fraction I, and indirectly by the phospholipase-*A* activity[4]. Among other effects of phospholipase-*A* is retardation of egg yolk coagulation[5].

Antibodies against the toxic fraction have not been found ; those against the enzymatic part of the venom have been demonstrated in the sera of mammals injected with bee venom for a long time[6,7]. We have therefore examined the sera of bee keepers to ascertain whether they contain antiphospholipase bodies.

Samples of blood from bee keepers were taken as test samples, and from ordinary men as control samples. The blood was left to clot at room tem-

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