participates in a bridge between adjacent platelets is not answered by the present investigation.

The potential usefulness of the reaction by which ADP-8-^{14}C is incorporated in platelets as a tool for assessment of platelet function is apparent and is at present being pursued in our laboratory.

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PATHOLOGY

A Lecithin-hydrolysing Enzyme which correlates with Haemolytic Activity in El Tor Vibrio Supernates

Recently, while studying the action of culture supernatants of *Vibrio cholerae* and El Tor vibrio on the phospholipids of human serum, the presence of a lysolecithinase previously reported by other workers[1,2] was confirmed. In addition, an enzyme which hydrolysed lecithin was found in culture supernatants of haemolytic El Tor vibrio, but could not be detected in supernatants of either *V. cholerae* or non-haemolytic El Tor strains. (Three such El Tor strains were obtained from the Walter Reed Army Institute of Research through the courtesy of Dr. Felsenfeld).

Supernatants were prepared according to the method of Watanabe and Felsenfeld[3]. The hydrolysis of serum phospholipids was studied in the following manner. Human serum (1·5 ml.) containing 0·02 per cent thiomersal was incubated with culture supernatant (0·5 ml.) for 24 h at 37° C. The serum lipids were extracted three times with 13 ml. of chloroform : methanol 1 : 1 (v/v), the extracts pooled, and evaporated to dryness. The dried residue was re-extracted with chloroform : methanol, 2 : 1 (v/v). This extract was analysed for phosphorus and an aliquot containing 12 μg of phosphorus was examined by silicic acid paper chromatography using di-isobutyl ketone : acetic acid : water, 40 : 25 : 5, as solvent[4]. The separated components were identified by standard staining procedures[4–7]. Loss of lecithin and lysolecithin indicated action on these components.

In contrast to the action on the phospholipids in serum, culture supernatants of haemolytic El Tor vibrio failed to hydrolyse purified egg lecithin although purified lysolecithin was hydrolysed. Addition of purified sphingomyelin and lysolecithin, the two other major phospholipids of serum, to the lecithin emulsion resulted in the hydrolysis of the added lysolecithin but no hydrolysis of lecithin. When, however, an emulsion of serum lipids, extracted as previously described, was used as substrate, hydrolysis of both lecithin and lysolecithin occurred. This behaviour indicates an activation of the lecithinase either by the nonphospholipid portion of serum or by one of the minor phospholipids present.

To identify reaction products, a serum phospholipid extract, in which complete hydrolysis of lecithin and lysolecithin had occurred, was examined by paper electrophoresis[8] together with controls of glyceryl phosphoryl choline and phosphoryl choline. A phosphorus staining zone migrating to the position of the control of glyceryl phosphoryl choline was observed. No other phosphorus staining compound was present. Fatty acid was demonstrated as the other reaction product after correcting for the presence of a lipase in the culture supernatant. This lipase hydrolyses the glycerides in the serum to give free fatty acids as a product. Fatty acids and glycerides were separated from phospholipids by dialysis[9] of both the control and the reaction mixture which had gone to completion. The lipase activity was determined by the loss of ester groups in the glyceride fraction. By subtraction of the fatty acid due to the lipase activity from the total fatty acid determined by titration, it was possible to show that the remaining fatty acid was equimolar to the two phospholipids hydrolysed.

The following mechanisms are suggested:

$$\text{Lecithin} + H_2O \xrightarrow{\text{Lecithinase}} \text{lysolecithin} + \text{fatty acid}$$

$$\text{Lysolecithin} + H_2O \xrightarrow{\text{Lysolecithinase}} \text{glyceryl phosphoryl choline} + \text{fatty acid}$$

The possibility that the lysolecithinase in the El Tor culture supernatants could hydrolyse lecithin as well as lysolecithin if the concentration of the enzyme was sufficiently high was investigated. Several haemolytic supernatants (containing lecithinase and lysolecithinase activity) and non-haemolytic supernatants (containing lysolecithinase activity only) were assayed quantitatively for lysolecithinase activity[10] using purified lysolecithin as substrate. There was no significant difference between the lysolecithinase activity in haemolytic and non-haemolytic supernatants respectively.

As only haemolytic vibrios possess the lecithinase activity, a correlation between the haemolysin and lecithinase seemed likely. The effects of heat treatment, toxoiding with formalin, and Seitz filtration on a culture supernatant were investigated with the view of obtaining evidence on this correlation. Haemolytic and lecithinase activities were lost after each of the following procedures: (*a*) heat treatment at 60° C for 10 min; (*b*) toxoiding with formalin; (*c*) Seitz filtration through a Ford sterile mat. In contrast, the lysolecithinase activity required 30-min heat-treatment at 60° C for complete inactivation, resisted toxoiding, and was not removed by Seitz filtration. In addition, a haemolytic variant from one of the non-haemolytic, lecithinase negative El Tor strains was found to have acquired the lecithinase activity.

Thus it has been shown that haemolytic El Tor vibrio produces a lecithinase in addition to the reported lysolecithinase. The evidence suggests a possible correlation between the haemolytic and lecithinase activities of culture supernatants of this organism. Further work using purified haemolysin[11] is necessary to determine the role of the enzyme in the haemolytic mechanism.

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