Thus both phospholipases A_1 and A_2 were present in the cell fractions. In the supernatant, phospholipase A_1 activity (0·4nmole/hr./mg. of protein) was greater than that of phospholipase A_2 (0·15nmole/hr./mg. of protein), but in all others phospholipase A_2 activity exceeded that of phospholipase A_1. The greater phospholipase A_2 activity could arise partly from the fact that the pH used, 7·4, was closer to the optimum pH, 6·5, for phospholipase A_2 compared with the optimum pH, 4·4, for phospholipase A_1 (Smith, 1969). Experiments with compound (II) were consistent with the above findings and showed that phospholipases B and C were also present in all cell fractions, but mainly in the mitochondria and lysosomes. Compound (III) was degraded rapidly by phospholipase B by all fractions (3–10nmoles/hr./mg. of protein), so clearly this 'intermediate' in phospholipid metabolism would not tend to accumulate to an extent where it could damage organelle structure. Hydrolysis of compound (III) was strongest in mitochondria and lysosome fractions. From the experiments with compound (IV) sphingomyelinase activity was observed strongest in lysosomes, supernatant and mitochondria, the values being 0·95, 0·39 and 0·23nmole/hr./mg. of protein respectively.

Since phospholipases A_1, A_2 and sphingomyelinase have been detected in the supernatant fraction of brain tissue cells, it is possible that this could be the source of the phospholipases found in cerebrospinal fluid, because it has been shown by Smith (1969) that the above can be released into the perfusate of the perfused adrenal gland.

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The Proteolytic Action of Arvin on Human Fibrinogen

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Arvin is a purified proteolytic enzyme fraction isolated from the venom of *Agkistrodon rhodostoma*, the Malayan pit viper (Esnouf & Tunnah, 1967). It possesses considerable clinical potential as a means of producing controlled defibrination in patients requiring anti-coagulant therapy, but, unlike the usual anticoagulants, acts only at the fibrinogen–fibrin level. It is capable of decreasing the plasma fibrinogen concentration virtually to zero without apparently affecting other plasma clotting factors (Bell, Pitney & Goodwin, 1968; Sharp, Warren, Paxton & Allington, 1968; Bell, Bolton & Pitney, 1968).

The action *in vitro* of arvin on purified human fibrinogen results in the production of a clot that is much 'softer' and more readily dispersed than that produced by thrombin, indicating a different mechanism of action. Thrombin is known to release four soluble fibrinopeptides, designated A, AP, AY and B (see Blomback, Blomback, Edman & Hessel, 1966). It has now been shown that under identical experimental conditions a more complex pattern of fibrinopeptides results from the action of arvin preparations. This pattern can be resolved by chromatography of desalted clot supernatants on Dowex 50 resin or by high-voltage electrophoresis. Six acidic peptides can be separated in addition to a number of more basic ones. Three of the acidic peptides are identical with the fibrinopeptides A, AP and AY released by thrombin. Two of the remaining acidic peptides have also been characterized and are heptapeptides derived from the *N*-terminal ends of fibrinopeptides A and AP. Preliminary investigations indicate the remaining acidic peptide to have been derived from the *N*-terminal section of the fibrinogen β-chains. The structures of the basic peptides are currently under investigation.

The complexity of the peptide pattern obtained with arvin suggested the existence of more than one proteolytic enzyme in the preparation and it has been possible to demonstrate the presence of at least two such enzymes. The one present in largest amounts is responsible for the clotting phenomenon and liberates only fibrinopeptides A, AP and AY. Fibrinopeptide B is not released by this enzyme. Time–activity studies suggest that this enzyme is responsible for the initial attack on the fibrinogen molecule, leading to clotting.

The significance of these various findings will be discussed.

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