V. Cytoplasmic Compartmentation

The Inhibition of Adenine Nucleotide Translocation by Atractyloside

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Origin and Chemical Structure of Atractyloside

Atractyloside occurs in the rhizomes of *Atractylis gummifera* (in German Mastix-Distel or Leim-Distel), a thistle growing in the southern mediterranean area. The ancient Egyptians already knew this drug as reported by Pedanios Dioskurides in “De Materia Medica”, Vol. III, which was written about 80 A.D. Dioskurides listed the drug as “Chamaileon Leukos”. Later it was known as an abortive agent. The isolation and crystallisation was reported in 1867 by Lefranc, who then named it atractylic acid.

Atractyloside is a glycoside, the glucose moiety being esterified with two molecules of sulfuric acid and one molecule of isovaleric acid (Lefranc, 1868; Angelico, 1910; Wunschendorf et al., 1931). The aglycon, which is called atractyligenin, has been characterized by Piozzi et al. (1966) to be a derivate of norditerpenoic acid, containing one free carboxylic group and two hydroxyl groups, one of the latter being attached to the glucose moiety. The most probable structure of atractyloside is shown in the Appendix. Thus atractyloside contains three anionic groups. The double bond between C-16 and C-17 appears to be essential for its inhibitory effect; hydration renders the compound inactive (Santi et al., 1962).

Pharmacology of Atractyloside

For a long time, the effect of atractyloside was regarded as being similar to that of strychnin. The investigations on the pharmacological effect were largely stimulated after a serious
accident had happened in Sicily: a class of school children was severely poisoned after having eaten the sweetish rhizomes. Three of the children died. Extensive investigations by Santi and co-workers (see Santi, 1958) showed that injection of atractyloside into mammals caused a decrease of oxygen consumption, a mobilisation of liver glycogen with a short period of hyperglycemia, followed by hypoglycemia (Marras, 1935) when the liver glycogen was exhausted. Apparently the resynthesis of glycogen was inhibited. Furthermore, an acidosis, due to a rise of lactic acid in the blood, was observed. Usually the animals died in a convulsive crisis, this being typical for hypoglycemia. The convulsions disappeared after administration of glucose and bicarbonate, but the animals died anyway in a state of deep depression. Thus the metabolic block appeared to be irreversible. These data indicated that atractyloside might exert its inhibitory effect on the respiratory system.

**Effect of Atractyloside on Mitochondrial Phosphorylations**

From subsequent studies with rat liver mitochondria it was shown that atractyloside inhibited oxidative phosphorylation (Bruni, Bistocchi et al., 1958) and uncoupler or Mg\(^{++}\)-stimulated ATP hydrolysis (Bistocchi et al., 1960, Vignais et al., 1961; Bruni and Luciani, 1962), but did not inhibit uncoupler-stimulated respiration (Bruni et al., 1961). From these findings the effect of atractyloside appeared to be rather similar to the effect of oligomycin (see Slater, this symposium). But soon important differences were noted between the effects of these inhibitors. Whereas oligomycin maintains its effect in disrupted mitochondria, the effect of atractyloside disappears on disintegration of the mitochondria. Thus pretreatment of the mitochondria with deoxycholate, hypotonic swelling (Bruni and Luciani, 1962; Bruni et al., 1962), digitonin (Vignais et al., 1962) or sonication (Löw et al., 1963), diminished the inhibition of oxidative phosphorylation and ATPase by atractyloside. Unlike oligomycin, atractyloside was shown to inhibit, in addition to the oxidative phosphorylation, the substrate level phosphorylation linked to ketoglutarate oxidation (Bruni et al., 1964). In contrast to oligomycin, the inhibitory effect of atractyloside on oxidative phosphorylation and uncoupler-stimulated ATPase was competitively reversed by ADP or ATP-