

cigarette smoke by the thiols cysteine and GSH. This latter observation, together with the finding that glycolysis is implicated as the energy source for phagocytosis in polymorphonuclear leucocytes (Cohn & Morse, 1960; Sbarra & Karnovsky, 1959), suggested a thiol-blocking role for filtered gas phase on one or more enzymes in the glycolytic pathway. Preliminary experiments were therefore made in which the activity of crystalline rabbit muscle glyceraldehyde 3-phosphate dehydrogenase was measured after exposure to filtered gas phase. It was shown that both filtered gas phase and aqueous extracts thereof were potent inhibitors of enzyme activity, that the degree of inhibition was dependent on the preincubation period and on the relative concentration of filtered gas phase and that in the presence of cysteine (2.5 $\mu\text{mol}/8\text{ml}$ of filtered gas phase) no significant inhibition of activity was recorded.

Further experiments were made to determine whether glyceraldehyde 3-phosphate dehydrogenase activity is inhibited in rabbit alveolar macrophages treated with filtered gas phase. Macrophages were collected and prepared by lung wash-out (Green & Carolin, 1967) and incubated for 2h in the presence of aqueous extracts of filtered gas phase. Cells were disrupted by sonication and cell supernatants were assayed for glyceraldehyde phosphate dehydrogenase activity. Glucose 6-phosphate dehydrogenase and lactate dehydrogenase were also measured in control and filtered-gas-phase-treated cells. Although there was considerable variation in the extent of inhibition of glyceraldehyde phosphate dehydrogenase activity from one experiment to another, the results were consistent with the postulate that filtered gas phase acts as an inhibitor of this enzyme and that the extent of inhibition is dependent on the concentration of filtered gas phase. No decrease in enzyme activity in the presence of filtered gas phase was recorded when cysteine was added to the incubation mixtures. The activities of glucose 6-phosphate dehydrogenase and lactate dehydrogenase in cells exposed to filtered gas phase were not significantly different from those of control cells.

Collectively, these findings are consistent with a thiol-blocking role for filtered gas phase, with particular reference to the activity of glyceraldehyde phosphate dehydrogenase, suggesting a relationship between macrophage phagocytic competence and loss of enzyme activity. It is therefore proposed that one of the biochemical defects resulting from exposure to cigarette smoke is the inhibition of glyceraldehyde phosphate dehydrogenase, a key enzyme in the glycolytic pathway. It is further suggested that the resulting glycolytic block may account for decreased macrophage phagocytic ability.

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Further Observations on the α -Galactosidase Activity of *Vicia faba* Seeds

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Vicia faba seeds contain two molecular forms of α -galactosidase (Dey & Pridham, 1969a) and the properties of these proteins have been examined (Dey & Pridham, 1969b). Multiple forms of the enzyme also occur in seeds from a number of other plant species. In general, the higher-molecular-weight forms predominate in dormant seed tissues, but on germination these are rapidly replaced by α -galactosidases of smaller molecular size (Barham, Dey, Griffiths & Pridham, 1971).

Recent studies have shown that α -galactosidase preparations from *V. faba* seeds are markedly activated by K^+ and to a smaller extent by NH_4^+ ; Na^+ has little effect on the enzyme activity.

The specific activity of the low-molecular-weight form, α -galactosidase II, of α -galactosidase from bean seeds, partially purified by pH precipitation and acetone and ammonium sulphate fractionations, increases when the preparation at pH 5.5 is stored at 4°C. Sephadex G-100 chromatography has shown that this is probably due to the formation of the high-molecular-weight form (α -galactosidase I). Further purification of enzyme II by passage through Sephadex G-100 inhibits the conversion of enzyme II into enzyme I.

Enzyme II appears to consist of two active fractions of similar molecular size, which can be resolved in CM-cellulose.

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