value as authentic 2-deoxy-2-sulphoamino-D-glucose. The electrophoretic mobility of this ^{35}S -labelled product also coincided with that of 2-deoxy-2-sulphoamino-D-glucose.

The enzymically prepared heparan [^{35}S]sulphate was degraded with nitrous acid (Lagunoff & Warren, 1962) and the products were fractionated on a Sephadex G-15 column. Approx. 75% of the radioactivity was eluted with inorganic sulphate. These results suggest that predominantly N-sulphate groups are synthesized in the presence of the ox lung enzyme.

Brown, D. H. (1957). *Proc. natn. Acad. Sci. U.S.A.* **43**, 783.

Lagunoff, D. & Warren, G. (1962). *Archs Biochem. Biophys.* **99**, 396.

Suzuki, S. & Strominger, J. (1960). *J. biol. Chem.* **235**, 257.

Suzuki, S., Trenn, R. H. & Strominger, J. (1961). *Biochim. biophys. Acta*, **50**, 169.

Investigation of the Metabolic Oxidation of Acenaphthen-1-ol

By C. J. SIMMONS, R. P. HOPKINS and P. CALLAGHAN. (*Department of Biochemistry, St Thomas's Hospital Medical School, London S.E.1, U.K.*)

After the administration of acenaphthene, acenaphthylene and *cis*- and *trans*-acenaphthene-1,2-diol to rats, 1,8-naphthalic acid was isolated, as its anhydride, from the acidified urine of the dosed animals (Chang & Young, 1943; Hopkins, Brooks & Young, 1962; Hopkins & Young, 1966). Following the suggestion of Hopkins (1968) that oxidation of the acenaphthene-1,2-diols to 1,8-naphthalic acid might involve an initial dehydrogenation step, acenaphthenequinone was isolated from a microsomal incubation of the acenaphthene-1,2-diols in the presence of NAD^+ (Drummond & Hopkins, 1969). Drummond, Callaghan & Hopkins (1971) have demonstrated that there are at least two systems in rat liver that dehydrogenate acenaphthene-1,2-diol, one in the microsomal fraction and another in the soluble fraction. These findings and the work of Sims (1966), on the structurally related 3-methylcholanthrene, led us to investigate the metabolism of acenaphthen-1-ol in order to determine whether it proceeds by a pathway involving an initial dehydrogenation reaction.

Rats were injected subcutaneously with acenaphthen-1-ol and the urine was acidified to pH 2 and extracted with ether. Examination of the extract by t.l.c. indicated the presence of acenaphthen-1-one and 1,8-naphthalic anhydride, which were isolated and characterized. 1,8-Naphthalic anhydride was also isolated and characterized from urine obtained from rats that had been dosed with

acenaphthen-1-one. Hydrolysis of the extracted urines with 6M-hydrochloric acid or β -glucuronidase gave rise to further quantities of acenaphthen-1-one and 1,8-naphthalic anhydride.

The oxidation of acenaphthen-1-ol was also investigated by incubation at 37°C with a rat liver microsomal preparation and NAD^+ at pH 7.4. Ether extracts of the incubation system, after acidification to pH 2, were examined by t.l.c. and found to contain acenaphthen-1-one, which was isolated and characterized. This reaction was followed in a similar incubation system by measuring the increase in E_{340} due to accumulation of acenaphthen-1-one. The microsomal dehydrogenase system was found to use either NAD^+ or NADP^+ as cofactor.

It was also found that a rat liver microsomal fraction incubated at 37°C with an NADPH-generating system at pH 7.4 converted acenaphthen-1-one into acenaphthenequinone, which was identified by t.l.c. and g.l.c. of ether extracts of the incubation medium after acidification to pH 2. Acenaphthenequinone was isolated and characterized.

These observations will be discussed and a metabolic sequence suggested for the conversion of acenaphthen-1-ol into 1,8-naphthalic acid.

Chang, L. H. & Young, L. (1943). *J. biol. Chem.* **151**, 87.

Drummond, E. C., Callaghan, P. & Hopkins, R. P. (1971).

Biochem. J. **121**, 4P.

Drummond, E. C. & Hopkins, R. P. (1969). *Biochem. J.* **113**, 13P.

Hopkins, R. P. (1968). *Biochem. J.* **108**, 577.

Hopkins, R. P., Brooks, C. J. W. & Young, L. (1962). *Biochem. J.* **82**, 457.

Hopkins, R. P. & Young, L. (1966). *Biochem. J.* **98**, 19.

Sims, P. (1966). *Biochem. J.* **98**, 215.

Investigation on the Effects of Cigarette Smoke on Rabbit Alveolar Macrophages

By G. M. POWELL* and G. M. GREEN. (*Department of Medicine, University of Vermont, Burlington, Vt. 05401, U.S.A.*)

Alveolar macrophages occupy a functional position of primary importance in pulmonary clearance, since they are capable of the uptake, transport and elimination of particles, micro-organisms and fluids through the processes of phagocytosis, pinocytosis and digestion. Green & Carolin (1967) have shown that the presence of the filtered gas phase of cigarette smoke has a very marked inhibitory effect with respect to the phagocytic function of rabbit alveolar macrophages. Green (1968) has also shown that the cells could be protected from the effects of

* Present address: Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, U.K.

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 μ mol/8ml 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.

- Cohn, Z. A. & Morse, S. I. (1960). *J. exp. Med.* **111**, 667.
Green, G. M. (1968). *Science, N.Y.*, **162**, 810.
Green, G. M. & Carolin, D. (1967). *New Engl. J. Med.* **276**, 421.
Sbarra, A. J. & Karnovsky, M. L. (1959). *J. biol. Chem.* **234**, 1355.

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

By P. M. DEY, A. KHALEQUE and J. B. PRIDHAM.
(Department of Biochemistry, Royal Holloway College, University of London, Englefield Green, Surrey, U.K.)

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.

We are indebted to the Science Research Council for financial support.

- Barham, D., Dey, P. M., Griffiths, D. & Pridham, J. B. (1971). *Phytochemistry* (in the Press).
Dey, P. M. & Pridham, J. B. (1969a). *Biochem. J.* **113**, 49.
Dey, P. M. & Pridham, J. B. (1969b). *Biochem. J.* **115**, 47.