

sodium bicarbonate solution. The extract was filtered free from unchanged benzpyrene and from insoluble products of the irradiation.

0.15 ml. of the filtrate was brushed into an area 2 cm. in diameter on the backs of ten mice from a stock in which no spontaneous tumours have occurred over a period of several years. 260 paintings have been given in fifty-four weeks. Five mice have developed tumours on the painted site, and in four cases so far examined histologically, these consisted of multiple keratinizing squamous cell carcinomas with small papillomas.

In order to exclude the possibility that the effect might be caused by the passage of colloidal benzpyrene through the filter, ten control mice have been painted simultaneously with a solution prepared in the same way but without the irradiation. The control has also been weighted against the experiment by adding acetone to the solution to ensure its rapid penetration into the skin. Five of the control animals have been examined (up to fifty-four weeks of painting): none of them has shown any cancerous or pre-cancerous condition. It may therefore be concluded that the aqueous extract from 3:4-benzpyrene contains a carcinogen which is not the hydrocarbon itself.

This conclusion is supported by evidence obtained in experiments in which mice previously sensitized by applying a solution of benzpyrene in benzene have been painted on the same site either with the aqueous extract or, for control, with bicarbonate solution. Twenty out of twenty-eight experimental mice have developed tumours in periods varying from thirty-eight to fifty-four weeks of painting as compared with four of the twenty-eight control animals.

It is impossible at present to identify the active compound. The aqueous extract contains two main substances characterized, at pH 8, by absorption bands at 3600 Å. and 2760 Å. respectively, but all attempts to isolate them have led to decomposition. Similar—possibly identical—substances have been detected in the products of irradiation, under the same conditions as were described in the earlier note¹, of cholanthrene, 1:2:5:6-dibenzanthracene, phenanthrene, anthracene and of naphthalene. The absorption spectra exclude the possibility that they are either photo-oxides⁴, mono- or dihydroxy derivatives⁵, or of the stilbene type⁶.

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² Schulman, J., and Rideal, E. K., *NATURE*, **144**, 100 (1939).

³ Melville, H. W., *Trans. Faraday Soc.*, **32**, 1525 (1936).

⁴ Cook, J. W., *et al.*, *NATURE*, **143**, 1020 (1939); *J. Chem. Soc.*, 1125 (1940).

⁵ Cf. Chalmers, J. G., *Biochem. J.*, **34**, 678 (1940). Boyland, E., *et al.*, *Biochem. J.*, **35**, 184 (1941); *etc.*

⁶ Cf. Dodds, E. C., *et al.*, *NATURE*, **148**, 142 (1941).

Lichenin

THE introduction of cross-linkages into soluble synthetic linear high polymers is followed by a decrease in solubility. In Nature, an interesting parallel appears to be afforded on one hand by the water-soluble polysaccharide lichenin, which consists of an unbranched β -glucopyranose chain $(C_6H_{10}O_5)_n$ where $n = 80-160$ ^{1, 2} and by cellulose on the other, if for the latter the structure of β -glucopyranose chains (or multi-membered loops) linked at intervals by cross-linkages³ be accepted. This communication contributes to the question of the structural difference between lichenin and cellulose.

It has been shown previously⁴ that treatment of potassium hydroxide-cellobiose with dry methyl sulphate followed by hydrolysis yields 2-methyl- and 2:6-dimethyl glucose whereas potassium hydroxide-cellulose when treated in this way yields 2-methyl glucose, but no 6-methyl or 2:6-dimethyl glucose⁵. This seems to indicate that in cellulose the primary alcohol residues are not vulnerable to attack under these mild conditions, and is evidence that the primary alcohol residues are the most likely to be involved in some form of cross-linking between the chains.

We have now shown that lichenin can combine with one molecular proportion of potassium hydroxide for each anhydroglucose unit to form an unstable addition compound, but that both 2- and 6-methyl glucose are present in the products of hydrolysis after methylation under anhydrous conditions. This result may be interpreted as showing that the primary alcohol groups in lichenin are not shielded as in cellulose and is in harmony with the results of Carter and Record¹ who, by their osmometric measurements on acetylated and methylated lichenins, show that the 'physical' molecular weight is of the same order as that found by end-group assay; that is, there is no aggregation of primary chains by cross-linkages, and thus agrees with the suggestion of W. N. Haworth³.

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⁴ Percival and Ritchie, *J. Chem. Soc.*, 1160 (1934).

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Artificially Induced Loss of Theta-Toxin Production by *Clostridium welchii* Types A and C

Macfarlane and Knight¹ having shown fairly conclusively that the alpha toxin of *Cl. welchii* Type A is a lecithinase, we considered it possible that repeated passage through media containing lecithin might enhance the capacity to produce alpha toxin. However, we found that after a dozen rapid passages through Hall's cooked-brain medium, strains showed no loss of capacity to produce alpha toxin when grown in 'test medium' (V.F. broth plus 0.25 per cent glucose), as compared with the same strains after similar passaging through test medium, but that they unexpectedly lost the capacity to produce theta toxin. It would thus appear possible to produce large