Unless it is assumed that the modes of action of gramicidin *S* and the decapeptide differ, despite their great similarity in structure, one is forced to the conclusion that the cyclic structure is not directly or intimately related to activity. The lack of activity of the pentapeptide rules out the specific influence of D-phenylalanine or L-ornithine, although these amino-acids may be essential to an overall structure possessing activity.

The greater activity of gramicidin *S* may be due to a lesser susceptibility to destruction by bacterial enzymes. A cyclic structure can be attacked only by endopeptidases, and the presence of D-phenylalanine and L-ornithine would materially decrease the number of enzymes capable of hydrolysing this compound. On the other hand, the straight-chain compound is also susceptible to amino- and carboxypeptidases.

Work is continuing with the aim of correlating antibacterial activity more closely with molecular structure.

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Intracellular Sites of Metabolism of 3 : 4 Benzpyrene in Mouse Liver

Previous work[1] has shown that after intravenous injection of colloidal 3 : 4 benzpyrene the bulk of the carcinogen is held in the liver. Excretion takes place over approximately twenty-four hours, the hydrocarbon having undergone metabolic oxidation to one or other of two compounds labelled BpX_1 or BpX_2. These are oxidation products and have been characterized by physical methods and analogy with known derivatives of other polycyclic hydrocarbons as being : 8(OR_1) — 9(OH) — 8,9 dihydro 3 : 4 benzpyrene and 8(OR_1) — 9(OR_2) — 8,9 dihydro benzpyrene respectively. The groups R_1 and R_2 are, at present, unidentified radicals. It has also been found[1,2] that during metabolic oxidation the products derived from 3 : 4 benzpyrene are firmly bound within the tissues where they are formed.

Working upon these facts, we have endeavoured to trace the sites at which oxidation occurs. Groups of 6–8 mice have been injected intravenously with 0·05 mgm. of benzpyrene as a colloid in water. At intervals up to 24 hr. they have been killed, the livers removed, homogenized in Tyrode solution and separated centrifugally into nuclei, mitochondria, microsomes and supernatant. Each fraction has been extracted for benzpyrene metabolites, using the method devised by Weigert[3]. Final purification has been by column chromatography on silica. Estimation of the metabolites was by absorption spectra over the range 340–430 mμ and confirmed by the fluorescence spectra.

Under these conditions the metabolite BpX_2 has been found in all four fractions at time intervals of $2\frac{1}{2}$, 4, 8, 12, 17 and 24 hr. after injection of the hydrocarbon. Over the same time intervals BpX_1 was only found in the supernatant fraction. Unchanged benzpyrene was regularly found in the nuclear, mitochondria and microsome fractions, but could only be detected in trace amounts in the supernatant fractions.

Since it is possible to argue that the metabolites were adsorbed to the different fractions during the homogenization or centrifuging, a further series of experiments was undertaken. Batches of untreated liver were fractionated as previously and each fraction was then suspended in Tyrode solution containing colloidal benzpyrene. They were placed in the incubator at 37° C. in total darkness, this latter condition being imposed by the necessity for avoiding any photosensitizing action by the hydrocarbon. After periods of up to $2\frac{3}{4}$ hr. incubation, they were extracted as previously and examined for metabolites. BpX_2 was obtained from all four fractions and BpX_1 from the supernatant only. These results show that metabolism must occur within the fractions themselves.

The penetration of benzpyrene into all parts of the cell and its engagement in chemical activity at various sites closely parallels the findings of Danielli[4] and his co-workers with regard to nitrogen mustard. These workers concluded that the action of this compound, which is also carcinogenic, is 'diffuse', and it now appears that benzpyrene behaves in similar fashion.

A detailed account of this work will be published elsewhere.

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Nitrogen Fixation by Soil Yeasts

Since the discovery by Starkey and De[1] of the soil bacterium *Azotobacter indicum*, which is capable of fixing atmospheric nitrogen under acid conditions, many unsuccessful attempts have been made to isolate this species from acid soils in western Europe. In one such attempt, preliminary experiments had suggested that the soil of a *Betula – Calluna* heath in Kent contained organisms able to fix atmospheric nitrogen, but attempts to isolate these organisms by the 'still culture' enrichment method failed. This method is not suited to the isolation of organisms present in acid soil in very small numbers : for such organisms continuous perfusion of a large volume of soil with selective media is a far superior method.

Columns of heath soil (*p*H 4·5 approx.) taken from the *A*1 horizon of the podsol beneath *Calluna vulgaris* were continuously perfused with media containing a carbon source but no source of fixed nitrogen. On different occasions mannitol, benzoate, various esters and alcohols were used. The perfusate and the soil were sampled at intervals for micro-organisms by