data given for 0 and 15°. The effect of tissue constituents on the inhibition under physiological conditions will likewise have to be assessed by extrapolation. The measurements listed in Table 2 were carried out in a medium containing about one quarter of the amount of tissue material present in the intact organ. Moreover, the protein was denatured by heat treatment.

Choice of inhibitor for physiological experiments. Of the substances tested *p*-sulphonamidobenzoic acid may have advantages over other substances in inhibitor experiments. Its powerful action is not much affected by temperature and by the presence of tissue constituents. The neutral sodium salt is very soluble in water (about 20 % w/v) and can therefore be added in concentrated solution. It is worthy of note that *p*-sulphonamidobenzoic acid has been found in urine after administration of marfanil (Hartles & Williams, 1946) and of *p*-toluenesulphonamide (Flaschen-träger, Bernhard, Löwenberg & Schläpfer, 1934). In some experiments it may be disadvantageous that

the inhibiting principle is contained in an anion, as in this substance; in such cases benzene-, *p*-toluene-, naphthalene- or thiophene-2-sulphonamide may be preferable.

SUMMARY

1. The inhibitory action of twenty-five sulphonamides on carbonic anhydrase was examined under varying conditions. Data are given for the concentration causing an inhibition of 50 % at 0 and at 15°, in dilute enzyme solution, and also in the presence of tissue constituents.

2. Relations between chemical constitution and inhibitory power and the suitability of various substances for inhibitor experiments are discussed.

The author is indebted to Dr James Walker, National Institute for Medical Research, London, N.W. 3, for samples of marfanil, *p*-sulphonamidobenzamidine, *p*-sulphonamidobenzamidoxime, 2-chloropyridine-5-sulphonamide and β -cyanoethanesulphonamide.

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Studies in the Biochemistry of Micro-organisms

79. FUSCIN, A METABOLIC PRODUCT OF *OIDIODENDRON FUSCUM* ROBAK PART 1. PREPARATION, PROPERTIES AND ANTIBACTERIAL ACTIVITY

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Smith (1946), in a note on the occurrence of species of the micro-fungus *Oidiodendron* Robak in Britain, states that 'the genus *Oidiodendron* was erected by Robak (1932) on three species which were all found growing on ground wood pulp in Norwegian mills. The original three species are *O. nigrum*, *O. fuscum* and *O. rhodogenum*. Later, Robak (cited by Melin & Nannfeldt, 1934, p. 440) described a fourth species, *O. griseum*, and a further addition to the genus was made by von Szilvinyi (1941) with his *O. flavum*.'

The purpose of the present communication is to describe the preparation, properties and antibacterial activity of a hitherto undescribed mould metabolic

product, isolated from two different strains of *O. fuscum*, for which the name *fuscine* is proposed. Both the strains were identified by Mr G. Smith and were isolated originally from British sources, one—strain Ag. 112—by the late J. H. V. Charles from gun cotton at Ardeer, Scotland, in 1926, the other—strain Camb. 111—by Dr Alan Burges in Cambridge, England, in 1945, from the surface humus in a pine plantation.

It has been found that, when either of these strains is grown on Czapek-Dox 5 % glucose solution, the yellow-orange culture filtrate after 28–30 days' incubation at 24° shows a marked antibacterial activity against *Staphylococcus aureus*. Aeration of

the clear culture filtrate (pH 5.0) from strain Ag. 112 leads to the deposition of an orange-red microcrystalline precipitate of crude fuscine, $C_{15}H_{18}O_5$, and the disappearance from the culture filtrate of most of its antibacterial activity. It has been proved that in this case the freshly prepared culture filtrate contains a mixture of fuscine with considerably larger amounts of its reduction product, dihydrofuscine, $C_{15}H_{18}O_5$, which on aeration is oxidized by an enzyme system to fuscine. Strain Camb. 111 behaved rather differently, since aeration of its culture filtrate (pH 6.2) led to no deposition of fuscine. However, on acidification and ether extraction of this culture filtrate, fuscine was readily isolated and purified.

The isolation of fuscine and its reduction product dihydrofuscine from cultures of the same strain of *Oidiodendron fuscum* lends further support to the growing belief that metabolic products of this type play some part in the oxidation-reduction mechanism of the moulds which produce them. Thus, from culture filtrates of *Aspergillus fumigatus* Fresenius, Anslow & Raistrick (1938) isolated fumigatin, 3-hydroxy-4-methoxy-2:5-toluquinone and the corresponding quinol, 3-hydroxy-4-methoxytoluquinol; Posternak (1939) obtained the substituted ditoluquinone, phoenicin, and its leuco derivative tetrahydrophoenicin from *Penicillium rubrum* Grasberger-Stoll; Ashley, Raistrick & Richards (1939) found that certain species in the *Aspergillus glaucus* series produce, along with physcion, 4:5-dihydroxy-7-methoxy-2-methylanthraquinone, two reduction products of physcion, i.e. the anthranols 4:5-dihydroxy-7-methoxy-2-methyl-9-anthranol and 4:5-dihydroxy-7-methoxy-2-methyl-10-anthranol; and Ashley & Raistrick (1938) separated from the mycelium of *Helminthosporium leersii* Atkinson yellow luteoleersin, $C_{26}H_{38}O_7$, and its colourless reduction product alboleersin, $C_{26}H_{40}O_7$. All these quinonoid metabolic products and their corresponding leuco derivatives have been shown to be interconvertible.

Fuscine crystallizes in brilliant orange diamond-shaped plates, m.p. 230°. Its chemistry and its derivatives and breakdown products, a number of which have been prepared, will form the subject of a future communication. Hence it will suffice here to state that it has many properties suggestive of a quinonoid structure. Thus it readily sublimes unchanged in a high vacuum; it dissolves in alkalis with the formation of intensely purple-coloured salts; it is easily reduced to colourless dihydrofuscine by cold aqueous sodium hyposulphite or catalytically by hydrogen in the presence of a palladium catalyst; conversely, dihydrofuscine is readily reoxidized to fuscine by aeration of its solution in pH 9.2 buffer.

Fuscine has marked antibacterial activity, particularly against all the Gram-positive bacteria tested, the growth of which it completely inhibits at con-

centrations of 1:80,000 to 1:1,280,000. It is much less active against Gram-negative organisms. *Vibrio cholerae* was inhibited at 1:80,000, six other species at 1:5000 to 1:10,000, but four species, including two of *Salmonella* and two of *Shigella*, were not completely inhibited even at concentrations of 1:5000. Two species of mycobacteria were inhibited at 1:10,000 and 1:20,000, while it was inactive against a third species.

Several antibiotics, notably penicillin, streptomycin and patulin, are inhibited in the presence of thiol compounds, but the nature of this inhibition is not yet clearly understood. When a slightly alkaline solution of fuscine is treated with thioglycolic acid the initial intensely purple colour of the solution quickly disappears. Subsequent acidification of the almost colourless solution leads to the deposition of a microcrystalline precipitate which, on crystallization from hot water, gives colourless needles, m.p. 200°, the analysis of which corresponds to a complex of 1 mol. fuscine + 1 mol. thioglycolic acid + 1 mol. water. It is perhaps somewhat surprising that the conversion of fuscine into this complex causes little change in its antibacterial spectrum.

EXPERIMENTAL

Cultures

Fuscine was prepared from two different strains of *Oidiodendron fuscum* Robak. They bear the London School of Hygiene and Tropical Medicine (L.S.H.T.M.) catalogue numbers Ag. 112 and Camb. 111. Strain Ag. 112 was used exclusively for the bulk production of fuscine.

Strain Ag. 112 was isolated by the late J. H. V. Charles in April 1928 from guncotton in the research laboratories of Nobel's Explosives Co. Ltd., Ardeer, Scotland.

Strain Camb. 111 was isolated by Dr Alan Burges, School of Botany, Cambridge in October 1945 from the surface humus in a pine plantation at Wangford Warren, near Brandon.

Both strains were identified by my colleague, Mr George Smith, who reports that 'growth is very slow, grey to fuscous, powdery, with some dirty brown pigment in the medium (Smith, 1946).'

Cultural conditions

A volume of 350 ml. Czapek-Dox solution (glucose, 50 g.; $NaNO_3$, 2.0 g.; KH_2PO_4 , 1.0 g.; KCl, 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; $FeSO_4 \cdot 7H_2O$, 0.01 g.; distilled water, 1 l.) was measured into each of a number of 1 l. conical flasks, and sterilized. The medium was inoculated with a heavy spore suspension prepared from 3-4-week cultures of *O. fuscum* strain Ag. 112 grown on Czapek-Dox agar slopes. Batches of 100 such flasks were incubated in the dark at 24° and were harvested after 28-30 days, when the surface of the mould mycelium was grey in colour, the reverse almost black, and the culture fluid orange to reddish brown. Crystals of fuscine were sometimes observed in or on the mycelium of older cultures (5-6 weeks).

Isolation of fuscine from the culture fluid

The culture fluid was separated by filtration from the mycelium. The culture filtrate (33–34 l.) still contained from 2.8 to 3.0% of glucose, had almost invariably a pH of 5.0 and completely inhibited the growth of *Staphylococcus aureus* at a dilution of 1:80 in 2% glucose broth after 24 hr. incubation. The filtrate, initially clear and orange in colour, although it was quite stable in the absence of air, gradually changed on exposure to air either at room temperature or even after chilling, to a dark brown and in the course of a few hours an orange-red precipitate of crude fuscine began to separate. It will be shown later that the culture filtrate, before exposure to air, contained a mixture of fuscine with a much larger amount of its reduction product, dihydro-fuscine. Since dihydrofuscine is relatively unstable because of the readiness with which it is oxidized to fuscine, the stable quinonoid form, the following procedure was adopted for isolation. The culture filtrate was distributed equally between four 10 l. bottles. A vigorous stream of air was passed through the bottles for 10 hr. at room temperature, and after standing overnight the resulting precipitate was separated by filtration through a layer of kieselguhr, washed well with water and dried. The crude material consisted of lens-shaped, microscopic, orange-brown crystals. The filtrate, now very dark, contained very little fuscine and was discarded.

The crude fuscine was purified by extraction of the dried fuscine-kieselguhr powder with boiling ethanol (1300–1400 ml./100-flask batch). On cooling the ethanol solution after filtration from the kieselguhr, almost pure fuscine separated in shining, orange-red, diamond-shaped plates which were filtered off, washed with ice-cold ethanol and dried. Evaporation of the ethanolic mother liquors and washings to about 75 ml. yielded a second crop, usually about 1 g./batch, of crystalline fuscine, of almost equally good quality.

In all, 25 batches of 100 flasks each were prepared and yielded a total of 242 g. of crystalline fuscine (first and second fractions combined) which corresponds to an average yield of 9.68 g./batch or 0.315 g./l. of culture filtrate. The maximum yield in any one batch was 12.4 g. and the minimum 7.86 g.

Isolation of fuscine from Oidiodendron fuscum, strain Camb. 111. Ten 1 l. flasks, each containing 350 ml. of Czapek-Dox solution, were inoculated with *O. fuscum*, strain Camb. 111, and incubated at 24°. When the flasks were harvested after 21 days they appeared very similar to those of strain Ag. 112. The mycelium was grey with a dark reverse. The yellowish brown culture filtrate had the following characteristics: residual glucose, 3.2%; pH, 6.2; completely inhibited the growth of *Staph. aureus* at a dilution of 1:320.

The culture filtrate was vigorously aerated, but, unlike that from strain Ag. 112, no precipitate was formed. The filtrate was therefore made acid to congo red with HCl and extracted with two portions of ether each of 750 ml. On removal of the solvent from the orange-brown extract there remained 0.55 g. of a brown powder which was crystallized from hot ethanol. The resulting orange crystals had all the properties of fuscine from strain Ag. 112; e.g. m.p. and mixed m.p. 230°, identical colour reactions with ethanolic FeCl_3 and with aqueous alkalis, and similar antibacterial activity against *Staph. aureus*.

Isolation of dihydrofuscine from culture filtrates of Oidiodendron fuscum, strain Ag. 112. The contents (about 2.3 l.) of seven 1 l. flask cultures of *O. fuscum*, strain Ag. 112, 28 days old, were filtered quickly on a Buchner funnel directly into conc. HCl (100 ml.), and all subsequent operations were carried out, as far as possible, in an atmosphere of nitrogen. The acid filtrate was extracted three times with 500 ml. portions of ether. The combined ether extracts were concentrated to 10 ml. and chilled overnight. The resulting crystals were collected and recrystallized from ether yielding 0.13 g. of buff-coloured prisms, m.p. 204°, not depressed on admixture with dihydrofuscine, m.p. 206°, prepared by the reduction of fuscine (see p. 531). The combined mother liquors yielded on evaporation a further crop of crystals consisting of a mixture of colourless dihydrofuscine and orange fuscine.

The oxidation of dihydrofuscine to fuscine by aeration of culture filtrates of *O. fuscum*, strain Ag. 112, appears to be effected by an enzyme system. Such a filtrate, 100 ml. of which gave a good precipitate of fuscine on direct aeration, afforded no fuscine when treated as follows: (a) 100 ml. were brought to the boil in an atmosphere of N_2 , cooled and aerated for 16 hr.; (b) 200 ml. were acidified with conc. HCl (10 ml.), kept overnight and then carefully readjusted to pH 5.0 with conc. aqueous KOH, and vigorously aerated.

Similarly, aeration of suspensions of dihydrofuscine (20 mg.) in acetate buffer solutions (100 ml.) of either pH 5.0 or 6.0 failed to yield fuscine, both the buffer solutions and the undissolved crystals remaining colourless.

Analysis and general properties of fuscine

Fuscine was prepared for analysis by repeated recrystallization from ethanol and was finally obtained in brilliant orange diamond-shaped plates, m.p. 230°. It sublimes in a high vacuum at 160–180° without decomposition. (Found: C, 65.0, 64.9; H, 5.9, 5.7; N, S, Cl, OCH_3 , nil; active hydrogen in pyridine, 0.38; in anisole, nil; $\text{C}-\text{CH}_3$, 10.2. $\text{C}_{15}\text{H}_{16}\text{O}_5$ requires C, 65.2; H, 5.8; 1 active hydrogen atom, 0.36; $2\text{C}-\text{CH}_3$, 10.9%.)

A molecular weight determination by the depression of the melting point of camphor failed owing to decomposition. Dr W. H. Taylor, Department of Physics, University of Manchester, kindly carried out an X-ray analysis. He reported as follows: 'The unit cell of the crystal was found to be 1102, and the mol. wt. was calculated from this as $1102/2 = 551$ or $1102/4 = 275$. The symmetry of the crystal is such that it was not possible to decide between the two alternatives.' $\text{C}_{15}\text{H}_{16}\text{O}_5$ requires a mol. wt. of 276 and this value was confirmed by molecular weight determinations on derivatives of fuscine.

Fuscine is soluble in chloroform, acetone, hot ethanol, ethyl acetate, and acetic acid, moderately soluble in ether and benzene, slightly soluble in cold ethanol, and almost insoluble in light petroleum and water. It is readily soluble in aqueous alkalis (NH_3 , NaHCO_3 , Na_2CO_3 and NaOH) with the formation of an intense purple colour, and in aqueous pyridine with a purple-blue colour. A 1% solution in chloroform showed no rotation in a 1 dm. tube. An ethanolic solution of fuscine gives a purplish red colour with ethanolic FeCl_3 . It is soluble in cold conc. H_2SO_4 with the formation of a greenish yellow, not very intense, colour. An ethanolic solution does not give a precipitate with Brady's reagent, nor does an acetone solution restore the colour to Schiff's reagent. With ethyl cyanoacetate in ethanolic NH_3 no blue

colour is developed, but only the purple colour which fuscine gives with any alkali. The purple colour changes to orange-brown in the course of half an hour.

Preparation of dihydrofusicin from fuscine

(a) *By reduction with sodium hyposulphite.* A solution of fuscine (20 g.) in chloroform (1600 ml.) was shaken vigorously with a solution of $\text{Na}_2\text{S}_2\text{O}_4$ (200 g.) in water (1 l.). When the original orange colour had disappeared completely the chloroform solution was washed once with a little freshly boiled-out water and evaporated to dryness in an atmosphere of N_2 . The dry residue was crystallized from ethanol (120 ml.) yielding *dihydrofusicin* (15.4 g.) as colourless rhombic crystals, m.p. 206° . (Found: C, 64.3, 64.2; H, 6.6, 6.7. $\text{C}_{15}\text{H}_{18}\text{O}_5$ requires C, 64.7; H, 6.5%.)

(b) *By catalytic reduction.* A solution of fuscine (0.5 g.) in glacial acetic acid (100 ml.) was reduced by shaking in an atmosphere of H_2 with a catalyst prepared from PdCl_2 (0.2 g.) and norite charcoal (0.5 g.). Reduction was complete in 5 min. when 45.4 ml. (corr.) of H_2 were absorbed. Theory for uptake of 1 mol. H_2 = 40.6 ml. The colourless solution was separated from the catalyst by filtration and evaporated to dryness *in vacuo*. The residue, crystallized from dry ether, gave colourless, thick, irregular tablets, wt. 0.30 g., m.p. 206° alone or in admixture with *dihydrofusicin* prepared by method (a) above. Adams & Shriner's (1923) platinum oxide catalyst similarly gave *dihydrofusicin*, no further reduction being effected.

Dihydrofusicin is moderately soluble in ethanol or ether and is almost insoluble in light petroleum or water. Its ethanolic solution gives a red colour with FeCl_3 .

Preparation of fuscine from dihydrofusicin

When suspensions of *dihydrofusicin* in buffer solutions of pH 8 or below are aerated, there is little evidence of oxidation. At pH 9 or above oxidation is rapid and appears to be accelerated by concentrations of FeSO_4 equal to those present in Czapek-Dox solution. The optimum conditions are as follows. *Dihydrofusicin* (0.1 g.) was suspended in 0.05M sodium borate buffer solution, pH 9.2 (10 ml.), containing 0.1 ml. of a 0.1% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution. The *dihydrofusicin* quickly dissolved with the formation of a dark purple solution which was vigorously aerated for 3 hr., and acidified with 2N-HCl. The resulting orange flocculent precipitate (70 mg.), on crystallization from ethanol, gave typical orange plates, m.p. 230° alone or in admixture with authentic fuscine.

Compound of fuscine with thioglycollic acid

Thioglycollic acid (0.5 ml. of 90%) was added to a solution of fuscine (0.24 g.) in water (20 ml.) and 0.1N-NaOH

(50 ml.). The initial purple colour disappeared at once and a pale yellow solution, pH 5.0, resulted. Four drops of 2N-HCl were now added and colourless needles quickly began to crystallize. After chilling overnight they were collected, washed and dried; wt. 0.29 g., m.p. 193° after shrinking at 120° . Recrystallization of 0.2 g. from hot water (60 ml.) yielded 0.13 g. of colourless needles, m.p. 200° . (Found: (a) on different samples of crystals, m.p. 193° ; C, 52.8, 52.8; H, 5.8, 5.2; S, 8.2, 9.2; (b) on crystals, m.p. 200° ; C, 52.6; H, 5.2; S, 8.7. $\text{C}_{17}\text{H}_{22}\text{O}_8\text{S}$, i.e. $\text{C}_{15}\text{H}_{18}\text{O}_5 + \text{C}_2\text{H}_4\text{O}_2\text{S} + \text{H}_2\text{O}$, requires C, 52.8; H, 5.8; S, 8.3%.) The compound is slightly soluble in cold and readily soluble in hot water. It also dissolves readily in 0.05M-phosphate buffer, pH 8.0, to a colourless solution. When dissolved in an excess of 0.1N-NaOH under conditions ensuring rigid exclusion of O_2 , a strong purple colour developed at once, but attempts to isolate fuscine, after acidification, failed. Much of the substance was recovered unchanged. It is remarkably stable towards acids. Thus 0.2 g. suspended in 0.5N-HCl (20 ml.) was boiled under reflux for 6 hr. in an atmosphere of N_2 . The solution remained colourless and 0.16 g. of unchanged material, m.p. 200° , was recovered from the cooled solution.

SUMMARY

1. The preparation, from culture filtrates of two different strains of the microfungus *Oidiiodendron fuscum* Robak, of a hitherto unreported mould metabolic product *fuscine*, $\text{C}_{15}\text{H}_{18}\text{O}_5$, orange plates, m.p. 230° , is described.

2. It is accompanied in the culture filtrates by its reduction product *dihydrofusicin*, $\text{C}_{15}\text{H}_{18}\text{O}_5$, colourless rhombic crystals, m.p. 206° . These two compounds, which are readily interconvertible, account for the greater part of the antibacterial activity of culture filtrates of *O. fuscum*.

3. The general properties of fuscine and *dihydrofusicin* are described. Fuscine has many properties suggestive of a quinonoid structure, and *dihydrofusicin* those of the corresponding quinol.

4. Fuscine forms a stable crystalline compound with thioglycollic acid, having the molecular formula $\text{C}_{17}\text{H}_{22}\text{O}_8\text{S}$, i.e. $\text{C}_{15}\text{H}_{18}\text{O}_5 + \text{C}_2\text{H}_4\text{O}_2\text{S} + \text{H}_2\text{O}$.

The author offers his best thanks to The Therapeutic Research Corporation of Great Britain Ltd. for a grant enabling him to carry out this work, and to Dr W. H. Taylor, Department of Physics, University of Manchester, for the determination of the molecular weight of fuscine by X-ray analysis.

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Appendix. Antibacterial Activity of Fuscine

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METHODS

A sterile 1:2500 solution of fuscine was prepared by dissolving fuscine (12 mg.) in 0.1N-NaOH (1 ml.) and diluting with sterile phosphate buffer (4 ml. of pH 7.0) and sterile water (25 ml.). The clear, intensely purple solution was mixed with an equal volume of sterile 2% glucose broth, the pH of the resulting solution being 7.6. Successive two-fold dilutions were made in 2% glucose broth giving a range of concentrations of fuscine of 1:5000, 1:10,000, 1:20,000 up to 1:1,280,000. All the bacteria listed in Table 1 were tested on this medium except *Vibrio cholerae*, in which case the 2% glucose was omitted from the medium. The serial dilutions were quickly inoculated with 1 loopful of an undiluted 24 hr. culture of the desired micro-organism. Growth readings were taken after 24 hr. incubation at 37° in all cases except *Corynebacterium diphtheriae* (48 hr.) and the mycobacteria (72 hr.).

A sterile solution of fuscine-thioglycollic acid was prepared by dissolving 33 mg. of the pure crystalline compound (see p. 531) in sterile water (50 ml.) and sterile 0.05M phos-

phate buffer (10 ml. of pH 7.0). The resulting almost colourless solution, corresponding to a concentration of 1 in 2500 of uncombined fuscine, was used for making serial dilutions in sterile 2% glucose broth. These were inoculated with ten species or strains of micro-organisms, incubated at 37° and growth readings were taken after 24 hr.

RESULTS

Fuscine. The growth of all the eighteen species or strains of Gram-positive bacteria tested was completely inhibited by fuscine, at concentrations varying from 1:80,000 to 1:1,280,000. Of the Gram-negative bacteria it completely inhibited the growth of *Vibrio cholerae* at a concentration of 1:80,000, but six other species or strains were only inhibited at concentrations of 1:5000 to 1:10,000, and it was without appreciable effect on *Shigella flexneri* Type Z, *S. sonnei*, *Salmonella paratyphi* B and *Salm. enteritidis* Gärtner. It was completely active against

Table 1. Bacteriostatic power of fuscine against two species or strains of *Mycobacterium*, eleven of Gram-negative and eighteen of Gram-positive micro-organisms

Micro-organism	Catalogue no. (National Collection of Type Cultures or of London School of Hygiene and Tropical Medicine)	Limiting dilution for inhibition of growth	
		Complete	Partial
<i>Salmonella enteritidis</i> (Gärtner)	N.C.T.C. 125	—	5,000
<i>Salm. paratyphi</i> B	N.C.T.C. 14	—	5,000
<i>Shigella flexneri</i> Type Z	L.S.H.	—	5,000
<i>S. sonnei</i>	L.S.H.	—	5,000
<i>Escherichia coli</i>	L.S.H.	5,000	10,000
<i>E. coli</i>	N.C.T.C. 86	5,000	—
<i>Salmonella typhi</i>	N.C.T.C. 2128	5,000	—
<i>Proteus vulgaris</i>	N.C.T.C. 5887	5,000	10,000
<i>Shigella shigae</i>	N.C.T.C. 4837	10,000	—
<i>Salmonella typhi-murium</i>	N.C.T.C. 74	10,000	20,000
<i>Mycobacterium phlei</i>	N.C.T.C. 525	10,000	20,000
<i>Myc. smegmatis</i>	N.C.T.C. 523	20,000	—
<i>Vibrio cholerae</i>	N.C.T.C. 1633	80,000	160,000
<i>Bacillus subtilis</i>	L.S.H.	80,000	160,000
<i>Staphylococcus aureus</i>	N.C.T.C. 4736	80,000	320,000
<i>Staph. aureus</i>	N.C.T.C. 3093	80,000	320,000
<i>Staph. albus</i>	N.C.T.C. 3256	80,000	320,000
<i>Corynebacterium diphtheriae gravis</i>	L.S.H.	160,000	640,000
<i>Staphylococcus aureus</i>	N.C.T.C. 3750	320,000	—
<i>Staph. aureus</i>	N.C.T.C. 4163	320,000	640,000
<i>Staph. aureus</i>	L.S.H. 2	320,000	640,000
<i>Bacillus anthracis</i>	N.C.T.C. 5444	320,000	640,000
<i>Corynebacterium diphtheriae intermedius</i>	L.S.H.	320,000	640,000
<i>C. diphtheriae mitis</i>	L.S.H.	320,000	1,280,000
<i>Staphylococcus aureus</i>	L.S.H. 1	640,000	1,280,000
<i>Staph. aureus</i>	N.C.T.C. 3095	640,000	1,280,000
<i>Streptococcus viridans</i>	N.C.T.C. 3166	640,000	1,280,000
<i>Strep. pyogenes</i> Griffith Type 1	N.C.T.C. 2432	640,000	1,280,000
<i>Bacillus anthracis</i>	L.S.H.	1,280,000	—
<i>Staphylococcus aureus</i>	N.C.T.C. 3761	1,280,000	—
<i>Staph. citreus</i>	N.C.T.C. 2301	1,280,000	—

Mycobacterium smegmatis at a concentration of 1:20,000 and *Myco. phlei* at 1:10,000 but was completely inactive against an unidentified species of *Mycobacterium* not listed in Table 1.

Fuscin-thioglycollic acid. The results obtained are summarized in Table 2. They indicate that, in general, the conversion of fuscin into its thioglycollic acid derivative caused a remarkably small change in its antibacterial spectrum, cf. Table 1.

SUMMARY

1. Fuscin is a somewhat powerful antibacterial substance, being particularly active against Gram-positive bacteria; but much less active or substantially inactive against mycobacteria and Gram-negative bacteria.

2. The conversion of fuscin into its thioglycollic acid derivative causes a remarkably small change in its antibacterial spectrum.

Table 2. Antibacterial activity of fuscin-thioglycollic acid

Micro-organism	Catalogue no.	Limiting dilution for inhibition of growth	
		Complete	Partial
<i>Escherichia coli</i>	N.C.T.C. 86	—	5,000
<i>Proteus vulgaris</i>	N.C.T.C. 5887	5,000	—
<i>Vibrio cholerae</i>	N.C.T.C. 1633	20,000	—
<i>Bacillus subtilis</i>	L.S.H.	40,000	80,000
<i>Staphylococcus aureus</i>	N.C.T.C. 3093	40,000	160,000
<i>Staph. aureus</i>	L.S.H. 2	40,000	80,000
<i>Staph. aureus</i>	Oxford H	160,000	320,000
<i>Staph. aureus</i>	L.S.H. 1	160,000	320,000
<i>Bacillus anthracis</i>	N.C.T.C. 5444	640,000	1,280,000
<i>Corynebacterium diphtheriae mitis</i>	L.S.H.	640,000	1,280,000

Metabolism of Fluorene in the Rabbit

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The metabolism of the hydrocarbon fluorene does not appear to have been examined previously, although the compound has been investigated for carcinogenic activity in skin-painting experiments with mice (Bloch, 1922, quoted by Kennaway, 1930; Twort & Fulton, 1930) and more recently in feeding experiments with rats (Wilson, De Eds & Cox, 1947). The results of these experiments led to the conclusion that fluorene is not carcinogenic. The high carcinogenic potency of 2-acetamidofluorene is now well established, as may be seen from the review by Bielschowsky (1947), but the only publication which has appeared to date concerning the metabolism of this carcinogen is that of Bielschowsky (1945) who showed that the metabolite, 7-hydroxy-2-acetamidofluorene, may be isolated from the urine of rats fed 2-acetamidofluorene.

In the course of further studies of the metabolism of 2-acetamidofluorene in rabbits an investigation was made concerning the fate of fluorene in these animals with the primary object of determining whether the reactive 9-methylene group of this compound could undergo any change during the passage

of the hydrocarbon through the animal body. This work might lead to a better insight into the metabolism of the related amine carcinogen. It was also of interest to see if fluorene is metabolized by rabbits according to the hydroxylation mechanism which has been found to play a part in the detoxication of a number of carcinogenic and non-carcinogenic polycyclic hydrocarbons. Adequate reviews of this detoxication mechanism have been given by Boyland & Weigert (1947) and by Williams (1947); only one example will be quoted here. Young (1947) found that the urine from rats ingesting naphthalene contained the diol, 1:2-dihydroxy-1:2-dihydronaphthalene, which yielded α -naphthol on mild acid hydrolysis, and Bourne & Young (1933) showed that naphthalene in man and dog gave rise to α -naphthol and the glucuronide of this compound. These results are in accord with the views of Fieser (1941) that the detoxication reaction involves primarily the addition of the elements of hydrogen peroxide to the hydrocarbon molecule in a perhydroxylation reaction giving rise to diols which are usually unstable and which pass over into phenolic compounds by