As a result of the work of Levvy *et al.* (1948), it is no longer necessary to postulate a synthetic role for β -glucuronidase in the body (Fishman, 1940) in order to explain the changes in the activity of the enzyme which can be produced in various organs. The view that β -glucuronidase is not involved in glucuronide synthesis (Levy, 1948) receives support from the failure of saccharic acid to influence formation of *o*-aminophenylglucuronide by mouse-liver slices, and of β -glucuronidase preparations to effect condensation of glucuronic acid with *o*-aminophenol.

Certain sex hormones are known to be excreted as glucuronides. The effect of administering saccharic acid on the metabolism of these compounds might repay investigation.

SUMMARY

1. Hydrolysis of phenylglucuronide by β -glucuronidase was strongly inhibited by saccharic acid. Closely related compounds were much less effective.

Inhibition by saccharic acid was competitive, and a value of $6 \times 10^{-5} \text{ M}$ was obtained for K_i , the dissociation constant of the enzyme-inhibitor complex.

2. Saccharic acid had no marked effect on synthesis of *o*-aminophenylglucuronide by mouse-liver slices.

3. Administration of large doses of saccharic acid to mice did not influence liver regeneration after damage or growth in infant animals.

4. No conjugation of *o*-aminophenol with glucuronic acid was observed after incubation in the presence of β -glucuronidase.

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The Metabolism of Chrysene: the Isolation of 3-Methoxychrysene by Methylation of the Phenolic Metabolite of Chrysene from Rat Faeces*

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Attention has been drawn to the fact that in mice and rats there is a remarkable similarity in the position of metabolic oxidation in the molecule for the three hydrocarbons 1:2-benzanthracene, 1:2:5:6-dibenzanthracene and 3:4-benzpyrene (Berenblum & Schoental, 1943). It was pointed out that these positions are not the ones which are chemically the most reactive. A preliminary report by Dickens (1945), that 9:10-dimethyl-1:2-benzanthracene is metabolized into its 4'-hydroxy derivative, brings

this hydrocarbon into line with those mentioned above, and provides additional support for the similarity in metabolic pattern.

It was thought that useful information might be derived from a study of the metabolism of chrysene, in which the anthracenoid ring structure is lacking. The following investigation was, therefore, undertaken.

EXPERIMENTAL

The procedure adopted was essentially the same as that used by the authors in their previous metabolic studies. Thirty rats were injected intraperitoneally with 2 ml. of a warm,

* A preliminary report on this work was communicated to the Biochemical Society (*Biochem. J.* 1945, **39**, lxiv).

supersaturated solution of purified chrysene (m.p. 251–252°) in arachis oil, representing about 25 mg./rat. The animals were kept in metabolism cages permitting the separate collection of urine and faeces. Their diet consisted mainly of rat cakes (North-Eastern Agricultural Co-operative Society Ltd., Aberdeen), supplemented occasionally with wholemeal bread and cod-liver oil, and water *ad lib*.

Isolation. The faeces were collected daily, dried in air, ground to a fine powder, and extracted with benzene by percolation at room temperature. The benzene extracts were shaken with dry 'precipitated pure silica' (British Drug Houses Ltd.) in order to remove a fluorescent, strongly adsorbable material (? a conjugated metabolite), and the solution, after filtration, was passed through columns of alumina for chromatographic separation. On developing the columns with benzene, three main zones could be distinguished:

A (at the top of the column). A strongly adsorbed zone, greyish in ordinary light, with a weak violet fluorescence in ultraviolet light. (This contained some of the fluorescent material mentioned above, most of which had been adsorbed on the silica.)

B (appearing below A). A narrow brownish zone, with a bluish fluorescence in ultraviolet light. (This contained the phenolic metabolite.)

C (appearing below B). A wide zone exhibiting a weak greenish fluorescence in ultraviolet light. (This zone contained the bulk of the sterols present in the extract.)

Zone B was cut and extracted with methanol. The extract was concentrated under reduced pressure, methylated by treatment with dimethyl sulphate and sodium methoxide in the cold, and extracted with benzene in the usual manner. The methylated product was purified by repeated chromatographic adsorption from light-petroleum solution, the fluorescent zone being extracted by benzene after cutting up the column each time. On concentration, the final eluate deposited a crystalline substance. This was recrystallized from light petroleum, and yielded colourless, fluorescent leaflets, m.p. 185–186°. It formed a complex with 2:7-dinitro-anthraquinone in xylene, which crystallized in fine crimson needles, m.p. 282–284°.

Identification. For reference, the six isomeric monomethoxychrysenes were synthesized, and their 2:7-dinitro-anthraquinone complexes prepared, by one of us (R. S.) in collaboration with Prof. J. W. Cook, F.R.S. (Cook & Schoental, 1945).

The properties of the methylated metabolite were found to correspond closely to those of 3-methoxychrysene as regards the colour and crystal form of the compounds and their respective complexes; the melting points of the complexes agreed closely, but the value for the methylated metabolite itself (185–186°) was higher than that quoted previously (Cook & Schoental, 1945) for 3-methoxychrysene (167–168°). Therefore, a synthetic specimen of 3-methoxychrysene was further purified (at the suggestion of Prof. Cook) by regeneration from the complex with 2:7-dinitro-anthraquinone by chromatography, and it had a melting point of 185–186°. The identity of the methylated metabolite with 3-methoxychrysene was confirmed by lack of depression of the melting points of mixtures of the two compounds, as well as of their dinitroanthraquinone complexes.

In our earlier metabolic studies on 3:4-benzpyrene (Berenblum, Crowfoot, Holiday & Schoental, 1943) and 1:2-benzanthracene (Berenblum & Schoental, 1943), the amounts of metabolite were often so small as to preclude

the possibility of obtaining them in pure enough form for melting-point determinations, and reliance had to be placed on fluorescence and ultraviolet absorption spectrography, and on other micromethods of identification. In the present investigation these methods also proved useful, especially in the early stages, before the methylated metabolite was available in crystalline form, when comparison of its fluorescence spectrum with those of the synthetic methoxychrysenes gave an early indication of the position of substitution.

Table 1. *Positions of fluorescence spectral bands of chrysene, methylated metabolite of chrysene, and the six isomeric monomethoxychrysenes*

	Wavelengths of main bands (mμ.)
Chrysene	(366), 382, 404
Methylated metabolite	368, 386, 408
3-Methoxychrysene	368, 386, 408
4-Methoxychrysene	369, 387, 410
2-Methoxychrysene	369, 389, 412
6-Methoxychrysene	371, 389, 412
5-Methoxychrysene	373, 391, 415
1-Methoxychrysene	374, 393, 418

For greater accuracy of measurement of the fluorescence bands, a method was employed for obtaining 'peak' photographs (in place of the semi-quantitative representations used in our previous publications), by incorporating a 'moving wedge' behind the slit of the spectrograph. Details of the method, and reproductions of the fluorescence spectra of the six isomeric methoxychrysenes are given elsewhere (Berenblum & Schoental, 1946a): in Table 1 are given the positions of the main bands. The distinct differences between the spectra of the different isomers give an indication of the relative specificity of fluorescence spectrography for this class of compounds. The fact that the spectrum of the methylated metabolite was indistinguishable from that of 3-methoxychrysene confirmed their identity.

A study of the ultraviolet absorption spectra of this series of compounds was undertaken by Holiday & Jope (unpublished results). They found very distinct differences in the absorption spectra of the six isomeric methoxychrysenes, and reported that the spectrum of the methylated metabolite was identical with that of 3-methoxychrysene, thus providing further confirmation of their identity.

DISCUSSION

Since the methylated metabolite of chrysene has now been identified as 3-methoxychrysene, and since the metabolite itself possesses the properties of a phenol, it may be concluded that the chrysene metabolite present in the rat faeces is 3-chrysenol.

This result is in keeping with the principle, previously noted in connexion with other polycyclic hydrocarbons (Boyland & Levi, 1935; Berenblum & Schoental, 1943) that metabolic oxidation does not occur in the positions of the molecule which are chemically the most reactive, i.e. the 2-position in the case of chrysene (Newman & Cathcart, 1940).

In comparing the structural formula of the rat metabolite of chrysene (I or II) with those of 1:2-benzanthracene (III), 1:2:5:6-dibenzanthracene

(IV), and 3:4-benzpyrene (V and VI), a similarity in pattern of metabolic oxidation is clearly discernible. It would seem, at first sight, that this

similarity is dependent on the manner of presentation, i.e. that formula I corresponds to 8-benzopyrenol, and formula II to 10-benzopyrenol. In fact, the feature common to them all is that the sites of metabolic oxidation are all in the alpha position to the phenanthrenoid 9:10 double bonds, and, since benzpyrene has two such double bonds (1:2 and

6:7), there are two corresponding alpha positions of metabolic oxidation (Berenblum & Schoental, 1946b).

An indication of the chemical mechanism of metabolic oxidation is given by the finding by Boyland & Levi (1935) that animals fed on anthracene excrete 1:2-dihydroxydihydroanthracene, and that this product readily undergoes conversion into 1-anthranol by mild acid treatment. Similar conversions of hydrocarbons to diols in the animal body have been observed in the case of naphthalene and phenanthrene (Young, 1947; Booth & Boyland, 1947; Boyland & Woolf, 1948). It thus seems a plausible hypothesis (Fieser, 1941) that a dihydroxydihydro-derivative might constitute an intermediate stage in the metabolic conversion of other hydrocarbons into their respective phenols.

The results with chrysene, described in the present communication, are not inconsistent with this view. However, this would not explain why the supposed 'perhydroxylation' does not take place in the reactive, adjacent, 1- and 2-positions (which so readily yield to oxidation; see Bamberger & Burgdorf, 1890), unless these positions are already blocked by some group, possibly enzymes, in the process of metabolism.

SUMMARY

After intraperitoneal injection of chrysene into rats, a phenolic fraction was isolated from their faeces. The isolated product yielded on methylation a compound identified as 3-methoxychrysene, indicating that the phenolic metabolite was 3-chrysenol.

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