2. The enzyme is assayed spectrophotometrically by measuring the rate of reduction of 2,6-dichlorophenol-indophenol (or cytochrome *c*), or manometrically by measuring the rate of oxygen uptake. Both assay systems require phenazine methosulphate as primary hydrogen acceptor.

3. Dialysed enzyme preparations require ammonia or methylamine for activity.

4. The enzyme activity is independent of nicotinamide nucleotide coenzymes.

5. The optimum pH value of the enzyme is 9·0 and K_m for methanol is 20 μM.

6. The enzyme is not specific for methanol as substrate; it will also catalyse the oxidation of other *normal* aliphatic alcohols.

We thank the University of Reading for the award of a Research Scholarship to C. A.

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Biochem. J. (1964), **92**, 621

Metabolism of Polycyclic Compounds

25. THE METABOLISM OF ANTHRACENE AND SOME RELATED COMPOUNDS IN RATS*

BY P. SIMS
Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W. 3

(*Received* 30 *January* 1964)

In the metabolism of benz[*a*]anthracene by animals, a compound was detected that, after the hydrolyses of its sulphuric acid and glucuronic acid conjugates, yielded benz[*a*]anthracene-7,12-quinone (Boyland & Sims, 1964*b*). The structure of this compound was not determined, but it seemed possible that it was either 7,12-dihydro-7,12-dihydroxybenz[*a*]anthracene or the related compound 7,12-dihydroxybenz[*a*]anthracene. The metabolism of anthracene has, therefore, been reinvestigated and the formation of 9,10-dihydro-9,10-dihydroxyanthracene, 9,10-dihydroxyanthra-

* Part 24: Boyland & Sims (1964*b*).

cene and a number of compounds derived from them has been demonstrated.

Previous work on the metabolism of anthracene (Boyland & Levi, 1935, 1936*a*, *b*) showed that the hydrocarbon is converted by animals into *trans*-1,2-dihydro-1,2-dihydroxyanthracene, which is excreted free and in conjugation with glucuronic acid, and into 1-anthrylmercapturic acid. The latter compound probably arises from an acid-labile mercapturic acid (Boyland & Sims, 1958; Knight & Young, 1958), since the urines were acidified during the working-up procedures. Boyland & Levi (1936*b*) also isolated small amounts of 9,10-anthraquinone from the urines of animals dosed with anthracene, but it was thought to be a contaminant of anthracene present in the animals' food. 9,10-Anthraquinone is converted by rats into 2-hydroxy-9,10-anthraquinone and its sulphuric ester (Sato, Fukuyama, Yamada & Suzuki, 1956; Sato, Suzuki & Yoshikawa, 1959).

EXPERIMENTAL

All melting points are uncorrected.

Materials

1-Hydroxyanthracene was prepared by the method of Schmidt (1904). 2-Hydroxyanthracene (Imperial Chemical Industries Ltd.), anthracene and anthrone (British Drug Houses Ltd.) and 9,10-anthraquinone (L. Light and Co. Ltd.) were commercial preparations. The large-scale feeding experiment described below was carried out with commercial anthracene, but in a second experiment the anthracene used had been purified by chromatography in benzene–methanol (9:1, v/v) on silica gel (100–200 mesh) (L. Light and Co. Ltd.) followed by two recrystallizations from benzene. 9,10-Anthraquinone could not be detected in either of these samples of anthracene in the modification of the test described below, as used by Nelson & Senseman (1922) for the determination of small amounts of the quinone in the hydrocarbon.

cis- and *trans*-9,10-Dihydro-9,10-dihydroxyanthracene was prepared by Dr D. Manson by the reduction of 9,10-anthraquinone with lithium tetrahydroaluminate (Boyland & Manson, 1951, and unpublished work). The *cis*-isomer formed needles from benzene, m.p. 162–165°, and the *trans*-isomer plates from benzene, m.p. 140–142°. Both the isomers, when heated on the steam bath with ethanol–conc. HCl (1:1, v/v), deposited almost colourless crystals, m.p. 180–190°, which are presumed to be 9-hydroxyanthracene. In both cases attempted recrystallization of the products from ethanol yielded anthrone in pale-yellow plates, m.p. and mixed m.p. 157–159°. The *cis*-isomer was much more stable than the *trans*-isomer, both in the solid state and in solution. The *cis*-isomer could be kept indefinitely at room temperature exposed to air and in daylight, whereas the *trans*-isomer rapidly turned yellow under these conditions, presumably because of quinone formation. When the isomers were separately incubated at 37° overnight with 0·1 M-acetate buffer, pH 5·0, either with or without the addition of β-glucuronidase, and the products examined on thin-layer chromatograms as described below, it was found that the *cis*-isomer yielded trace amounts of the *trans*-isomer whereas the *trans*-isomer yielded a mixture of unchanged *trans*-isomer, the *cis*-isomer and anthrone. 9,10-Anthraquinone was also detected by means of the test described below.

9-Anthryl acetate was prepared by the acetylation of anthrone with acetic anhydride in pyridine (Barnett & Matthews, 1923), and 9,10-diacetoxyanthracene by the reductive acetylation of 9,10-anthraquinone with zinc dust and acetic anhydride in pyridine (Barnett, Goodway, Higgins & Lawrence, 1934). 2-Hydroxy-9,10-anthraquinone was prepared from 2-amino-9,10-anthraquinone by the method of Perkin & Whattam (1922). 1,2-Anthraquinone was obtained from (±)-*trans*-1,2-dihydro-1,2-dihydroxyanthracene, isolated from the urine of rats treated with anthracene as described below, by oxidation with aq. $FeCl_3$ (cf. Dienel, 1906).

Chromatography

Paper chromatography was carried out on Whatman no. 1 chromatography paper by downward development for 18 hr. with butan-1-ol–propan-1-ol–aq. 2 N-NH_3 (2:1:1, by vol.). The dried chromatograms were examined in u.v. light and (1) sprayed with a freshly diazotized solution of *p*-nitroaniline (0·02% in 0·1 N-HCl) followed by aq. 10% (w/v) Na_2CO_3, or (2) sprayed with a solution of diazotized *p*-nitroaniline (0·2% in 4 N-HCl), after which the papers were heated to 80° until colours appeared and then sprayed with aq. 10% (w/v) Na_2CO_3, or (3) dipped in the platinic iodide reagent of Toennies & Kolb (1951).

Thin-layer chromatograms were prepared by coating glass plates with a film of silica gel G (E. Merck A.-G., Darmstadt, West Germany) of 0·25 mm. thickness. The chromatograms were developed for 10 cm. with (*a*) hexane containing 5% (v/v) of benzene, or (*b*) benzene, or (*c*) benzene containing 5% (v/v) of ethanol, or (*d*) benzene containing 10% (v/v) of ethanol. Two-dimensional thin-layer chromatograms were used in the examination of acid-labile compounds. They were developed in the first direction with solvent (*c*) or (*d*), sprayed with conc. HCl and heated in an oven to 80° for 10 min. and developed in the second direction with solvent (*a*), (*b*) or (*c*). The chromatograms were examined in u.v. light while still wet, both before and after exposure to NH_3, and sprayed with a 0·5% solution of 2,6-dichloroquinonechloroimide in ethanol followed by aq. 10% (w/v) Na_2CO_3. The properties of the metabolites on paper and on thin-layer chromatograms are recorded in Tables 1 and 2 respectively. It was not possible to find suitable solvents for the chromatography of 9,10-anthraquinone either on paper or thin-layer chromatograms. The presence of the quinone in the various hydrolysates described below was detected by means of the red colour produced when portions of the hydrolysates were heated to 100° for 5 min. with 2 N-NaOH and zinc dust (cf. Houben, 1929). As some of the hydrolysates were themselves coloured brown, and as the test could not be carried out in the presence of 2-hydroxyanthraquinone, which gave an immediate red colour with 2 N-NaOH, it was sometimes necessary to apply the ether extracts of the hydrolysates to the base lines of thin-layer chromatograms that were then developed in solvent (*c*). The silica gel at the base line was removed and heated with zinc and 2 N-NaOH, when the appearance of a red colour indicated the presence of 9,10-anthraquinone.

Enzymic and acid hydrolyses

Hydrolyses with the sulphatases of Taka-diastase (Parke, Davis and Co. Ltd.) were carried out in 0·1 M-acetate buffer, pH 5·7, and hydrolyses with β-glucuronidase (Ketodase; Warner–Chilcott Laboratories) in 0·1 M-acetate buffer, pH 5·0. The mixtures were incubated overnight at 37°. Acid hydrolyses were carried out by heating the material under examination with 5 N-HCl at 100° for 15 min. The products of the hydrolyses were extracted from the reaction mixtures with ether and examined on thin-layer chromatograms.

Animal experiments

Metabolism of anthracene (*I*). Twenty-four male rats of the Chester Beatty strain (body wt. about 250 g.) were maintained for 3 weeks on a diet containing 5% (by wt.) of commercial anthracene as described by Boyland & Levi (1935). The urines were collected daily, pooled and stored at 0°. The urine was filtered, and the filtrate acidified to pH 4 with acetic acid and treated with 50 g. of activated charcoal (British Drug Houses Ltd.). The charcoal was filtered off, washed with water (2 l.) and the absorbed material eluted by washing the charcoal first with 5 l. of methanol–aq. NH_3

Table 1. *Properties of compounds related to anthracene on paper chromatograms*

Details are given in the text. The entry — indicates that the characteristics of the compound could not be determined because of the presence of larger amounts of other metabolites on the chromatograms.

Compound	R_F	Fluorescence	Colour: With diazotized *p*-nitroaniline and Na_2CO_3	Colour: With diazotized *p*-nitroaniline in 4 N-HCl, heating to 80° and Na_2CO_3	Reaction with the platinic iodide reagent
1-Hydroxyanthracene	0·95	Blue	Red–purple	Red–purple	None
2-Hydroxyanthracene	0·95	Violet	Red–brown	Red–brown	None
Anthrone	0·92	Green	Pale yellow–green	Pale yellow–green	None
cis- and *trans*-9,10-Dihydro-9,10-dihydroxyanthracene	0·88	Dark violet	Yellow, turning green	Yellow	Orange
(+)- and (−)-*trans*-1,2-Dihydro-1,2-dihydroxyanthracene	0·87	Blue	Pale red	Red–purple	None
Metabolite, probably 9-anthryl sulphate*	0·62	Bright violet	None	Pale yellow	None
Metabolite, probably 9-hydroxy-10-anthryl sulphate*	0·62	Bright violet	None	None	None
Metabolite, probably a sulphuric ester of *trans*-1,2-dihydro-1,2-dihydroxyanthracene*	0·62	—	—	—	—
Metabolite, probably a sulphuric ester of 2,9,10-trihydroxyanthracene*	0·62	—	—	—	—
1-Anthrylmercapturic acid	0·55	Dark violet	None	None	Pale yellow
2-Hydroxy-9,10-anthraquinone†	0·52	Orange	Orange	Orange	None
Metabolite, probably the sulphuric ester of 2-hydroxy-9,10-anthraquinone	0·38	Dark absorption	None	Orange	None
N-Acetyl-*S*-(1,2-dihydro-2-hydroxy-1-anthryl)cysteine	0·38	Dark violet	Red–purple	Red–purple	Pale yellow
1-Anthrylglucosiduronic acid	0·35	Violet	Orange after 24 hr.	Red–purple	None
Metabolite, probably 9-anthrylglucosiduronic acid	0·35	Bright violet	None	Pale yellow	None
1,2-Dihydro-2-hydroxy-1-anthrylglucosiduronic acid	0·31	Dark violet	Brown after 24 hr.	Purple–brown	None
Metabolite, probably 9,10-dihydro-9-hydroxy-10-anthrylglucosiduronic acid*	0·31	Dark absorption	Yellow, turning green	Bright yellow	None
Metabolite, probably 9-hydroxy-10-anthrylglucosiduronic acid*	0·31	Bright violet	None	None	None
Metabolite, probably a glucuronic acid conjugate of 2,9,10-trihydroxyanthracene*	0·31	—	—	—	—
Metabolite, possibly a diglucuronic acid conjugate of 9,10-dihydroxyanthracene*	0·05	Bright violet	None	None	None

* The presence of these conjugates in the urines of animals treated with anthracene was detected as described in the text.

† This compound was visible as a red spot when the wet chromatograms were examined in daylight; it changed to pale yellow as the chromatograms dried.

(sp.gr. 0·88) (19:1, v/v) and then with 5 l. of methanol–benzene (19:1, v/v) The combined eluates were evaporated, and the residual gum was chromatographed on a cellulose-powder column prepared from 750 g. of cellulose powder (Whatman standard grade). The column was developed with butan-1-ol–cyclohexane–aq. 2N-NH_3 (9:2:1, by vol.), 100 ml. fractions being collected. The fractions were evaporated under reduced pressure and the residues examined on paper chromatograms. Fractions containing similar products were combined to give four main fractions containing dihydrodihydroxy compounds, sulphuric esters, mercapturic acids and glucosiduronic acids, that were treated as described below. Hydroxyanthracenes were not detected in fractions from the column.

(*a*) Dihydrodihydroxy compounds fraction. The fraction formed a semicrystalline mass that, when examined on two-dimensional thin-layer chromatograms, was shown to contain large amounts of a compound that, since it gave a mixture of 1- and 2-hydroxyanthracene with acid, appeared to be 1,2-dihydro-1,2-dihydroxyanthracene (II). Small amounts of a product were detected that appeared to be a 9,10-dihydro-9,10-dihydroxyanthracene (V) because it yielded anthrone (VI) with acid, but, as the large amounts of the 1,2-dihydro-1,2-dihydroxy compound present caused distortion of the chromatogram, the product could not be identified for certain as either the *cis*- or the *trans*-isomer.

The fraction was dissolved in the minimum amount of boiling benzene and the solution allowed to crystallize when (±)-*trans*-1,2-dihydro-1,2-dihydroxyanthracene (II) (25 mg.) separated, which formed plates from benzene, m.p. 183–184°. Booth & Boyland (1949) give m.p. 184° for this compound. An examination of the mother-liquors from the crystallizations showed the presence of 1,2-dihydro-1,2-dihydroxyanthracene, but the substance yielding anthrone with acid could no longer be detected. Instead, the residue obtained by the evaporation of the mother-liquors gave a positive test for 9,10-anthraquinone.

(*b*) Sulphuric ester fraction. This formed a dark gum that could not be crystallized. On paper chromatograms, the gum yielded a spot which had a bright-violet fluorescence in u.v. light and was similar to that of products described below that appeared to be 9-anthryl sulphate and 9-hydroxy-10-anthryl sulphate. A compound indistinguishable on paper chromatograms from 2-hydroxy-9,10-anthraquinone (XI) was also detected. This compound was also indistinguishable from the hydroxyquinone on thin-layer chromatograms developed in solvent (*c*), and its u.v.-absorption spectrum, measured on the solution obtained when spots from the chromatograms were removed and the adsorbed material was eluted from the silica gel with ethanol, showed $\lambda_{max.}$ at 245, 272 and 334 mμ, and the curve was identical with that of the synthetic quinone (see Table 3).

A portion of the gum was hydrolysed with acid to yield products indistinguishable on thin-layer chromatograms from 1- and 2-hydroxyanthracene, 2-hydroxy-9,10-anthraquinone and anthrone. The hydrolysis with sulphatase yielded compounds indistinguishable from 1,2-dihydro-1,2-dihydroxyanthracene, 1,2-anthraquinone, 2-hydroxy-9,10-anthraquinone and anthrone, but 1- and 2-hydroxyanthracene and 9,10-dihydro-9,10-dihydroxyanthracene were not detected. 9,10-Anthraquinone (IX), which was present in both hydrolysates, was therefore presumably formed during the hydrolysis of 9-hydroxy-10-anthryl sulphate.

(*c*) Mercapturic acid fraction. This fraction formed a light-brown gum (850 mg.) that was chromatographed for 18 hr. on six sheets of Whatman no. 3 MM chromatography paper in butan-1-ol–acetic acid–water (2:1:1, by vol.). The mercapturic acid formed dark-violet fluorescent bands when the chromatograms were examined in u.v. light. The bands were cut from the chromatograms and the absorbed material was eluted from the paper with methanol–aq. NH_3 (sp.gr. 0·88) (19:1, v/v). The eluate was evaporated to yield the mercapturic acid as a light-brown gum that could not be crystallized. A small portion of the gum was dissolved in

Table 2. *Properties of compounds related to anthracene on thin-layer chromatograms*

Details of the procedures are given in the text.

Compound	R_F In benzene	R_F In benzene–ethanol (19:1, v/v)	Fluorescence Immediate	Fluorescence After exposure to NH_3	Colour with 2,6-dichloroquinonechloroimide and Na_2CO_3
1-Hydroxyanthracene	0·27	0·62	Blue	Pink	Violet
2-Hydroxyanthracene	0·17	0·58	Violet	Green	Green
Anthrone	0·32	0·90	None	None	Pink, turning yellow
cis-9,10-Dihydro-9,10-dihydroxyanthracene	0·01	0·30	None	Blue	Pink, turning yellow*
trans-9,10-Dihydro-9,10-dihydroxyanthracene	0·01	0·25	None	Blue	Pink, turning yellow*
trans-1,2-Dihydro-1,2-dihydroxyanthracene	0·01	0·15	None	None	Grey*
1,2-Anthraquinone	0·22	0·89	Dark absorption	—	(Brown)†
2-Hydroxy-9,10-anthraquinone	0·01	0·62	Orange	—	Orange (yellow)†
Methyl ester of *N*-acetyl-*S*-(1,2-dihydro-2-hydroxy-1-anthryl)-cysteine	(0·38)‡		Dark violet	—	—
Anthracene	(0·82)§		Violet	—	—

* These colours were obtained only if the chromatograms were first heated with conc. HCl as described in the text.
† These colours were visible on untreated chromatograms.
‡ R_F measured in benzene–ethanol (9:1, v/v).
§ R_F measured in hexane–benzene (19:1, v/v).

Table 3. *Ultraviolet absorption of some compounds related to anthracene*

Where measured, the molecular extinction coefficient is shown in parentheses (as log $\epsilon_{max.}$). Spectra of the compounds for which this was done were measured on a Unicam SP. 500 spectrophotometer. Other spectra were measured on a Perkin–Elmer model 137 ultraviolet spectrophotometer. Measurements were made in ethanol unless otherwise stated.

Compound	$\lambda_{max.}$ (mμ)
Anthrone†	266 and 304*
Anthrone‡	253, 270* and 323
1-Anthrylglucosiduronic acid	253 (4·97), 315 (3·00), 330 (3·32), 345 (3·53), 363 (3·67) and 384 (3·57)
Methyl (1-anthryltri-*O*-acetyl-D-glucosid)uronate	253 (4·90), 315 (3·28), 330 (3·49), 345 (3·74), 362·5 (3·90) and 382·5 (3·80)
1-Anthrylmercapturic acid	250, 350, 367 and 385
(±)-*trans*-1,2-Dihydro-1,2-dihydroxyanthracene§	244 (4·67), 277·5 (4·06), 287 (4·15) and 298 (4·13)
1,2-Dihydro-2-hydroxy-1-anthrylglucosiduronic acid†	244 (4·72), 277 (4·13), 287 (4·24) and 298 (4·20)
(−)-Methyl (2-acetoxy-1,2-dihydro-1-anthryltri-*O*-acetyl-D-glucosid)uronate	245 (4·74), 277 (4·17), 287 (4·26) and 298 (4·22)
(−)-Methyl (2-methoxy-1,2-dihydro-1-anthryltri-*O*-acetyl-D-glucosid)uronate	245 (4·60), 277·5 (3·97), 287 (4·06) and 298 (4·01)
N-Acetyl-*S*-(1,2-dihydro-2-hydroxy-1-anthryl)cysteine†‖	248, 281, 290 and 301
cis-9,10-Dihydro-9,10-dihydroxyanthracene	256 (2·73), 261·5 (2·79) and 269 (2·72)
trans-9,10-Dihydro-9,10-dihydroxyanthracene	256 (2·66), 262·5 (2·72) and 270 (2·48)*
2-Hydroxy-9,10-anthraquinone	245 (4·42), 272 (4·56), 334 (3·55), 362 (3·52) and 375 (3·55)
9-Anthryl acetate	247*, 253 (5·21), 315 (2·23), 330 (2·39), 346 (2·69), 364 (2·87) and 383·5 (2·81)
9,10-Diacetoxyanthracene	249*, 255 (5·26), 352 (2·81) and 371·5 (3·00)

* Inflexion.
† Measured in methanol.
‡ Measured in methanol containing 0·1% (v/v) of aq. NH_3 (sp.gr. 0·88).
§ The (−)-dihydrodihydroxy compound gave an identical spectrum.
‖ The spectrum of the methyl ester of the mercapturic acid was similar.

water, and the solution acidified with conc. HCl and extracted with ether. The ether extract was examined on thin-layer chromatograms and was found to contain compounds indistinguishable from anthracene and 1- and 2-hydroxyanthracene, the last two compounds appearing to be present in about equal amounts. A small amount of anthrone was also detected. In a second experiment, the gum (100 mg.), in water (1 ml.), was acidified with a few drops of conc. HCl. The precipitate was filtered off and treated with aq. saturated $NaHCO_3$ (1 ml.). The mixture was filtered and the filtrate washed twice with ether (5 ml.) and acidified with conc. HCl. 1-Anthrylmercapturic acid (52 mg.) separated as a pale-green solid, m.p. 189–192° (decomp.). Boyland & Levi (1936*b*) give m.p. 195° for the mercapturic acid isolated from the urine of rats treated with anthracene. A little of the original mercapturic acid gum was heated with aq. 2N-NaOH at 100° for 4 hr. The pH of the solution was adjusted to 4 with acetic acid and the solution was extracted with ether. An examination of the ether extract on thin-layer chromatograms showed the presence of a compound indistinguishable from 2-hydroxyanthracene. 1-Hydroxyanthracene could not be detected.

The remainder of the mercapturic acid gum was dissolved in methanol and esterified with diazomethane in ether. The ester was applied to the base lines of thin-layer chromatograms, which were developed with solvent (*d*). The methyl ester was located on the chromatograms as opaque bands, seen when the wet chromatograms were examined in daylight. These bands, and bands 1 cm. wide lying immediately next to them in the direction of the solvent front, were removed and the absorbed materials eluted from the silica gel with methanol. The combined eluates from the slower-moving bands were evaporated to yield a gum that failed to crystallize. With cold conc. HCl the gum yielded compounds indistinguishable on thin-layer chromatograms from anthracene and 1- and 2-hydroxyanthracene, and with aq. 2N-NaOH at 100° 2-hydroxyanthracene was formed. It is likely therefore that the gum consisted mainly of the methyl ester of *N*-acetyl-*S*-(1,2-dihydro-2-hydroxy-1-anthryl)cysteine (IV).

The material eluted from the faster-moving bands was shown on two-dimensional thin-layer chromatograms to contain a small amount of a substance (R_F 0·42 in solvent *d*) that yielded anthrone with conc. HCl. No other evidence for the structure of this substance was obtained, but it could be the methyl ester of *N*-acetyl-*S*-(9,10-dihydro-9-hydroxy-10-anthryl)cysteine.

(*d*) Glucosiduronic acid fraction. During the evaporation of many of the fractions from the column which contained glucosiduronic acid, crystals separated out. These were collected and combined and were examined separately. The crystals, which appeared to be the ammonium salt of one of diastereoisomers of *trans*-1,2-dihydro-2-hydroxy-1-anthrylglucosiduronic acid, were dissolved in water (2 ml.), and conc. HCl (2 ml.) was added. The solid that separated was collected and crystallized from water to yield (−)-*trans*-1,2-dihydro-2-hydroxy-1-anthrylglucosiduronic acid (2·5 g.) in pale-pink flat needles, m.p. 203° (decomp.), $[\alpha]_D^{25}$ −205° (*c* 0·5 in dioxan) (Found: C, 61·85; H, 5·4. Calc. for $C_{20}H_{20}O_8$: C, 61·85; H, 5·2%). Boyland & Levi (1936*a*) give m.p. 199–200° for this acid. The acid was methylated with diazomethane in ether and the product acetylated with acetic

anhydride in pyridine to yield (–)-*methyl (2-acetoxy-1,2-dihydro-1-anthryltri-O-acetyl-D-glucosid)uronate* that, after three recrystallizations from ethanol, formed needles, m.p. 191–192°, $[\alpha]_D^{25}$ –184° (*c* 1·0 in $CHCl_3$) (Found: C, 60·9; H, 5·5. $C_{29}H_{30}O_{12}$ requires C, 61·05; H, 5·3%). Both the glucosiduronic acid and its derivative yielded compounds indistinguishable from 1- and 2-hydroxyanthracene after hydrolysis with HCl as described above. Hydrolysis of the glucosiduronic acid with dilute HCl as described by Boyland & Levi (1936*a*) yielded *1-anthrylglucosiduronic acid* in light-green needles from water, m.p. 209° (decomp.), $[\alpha]_D^{25}$ –66° (*c* 0·48 in dioxan) (Found: C, 65·2; H, 5·2. $C_{20}H_{18}O_7$ requires C, 64·9; H, 4·9%) and small amounts of 1- and 2-hydroxyanthracene, detected in thin-layer chromatograms. When the acid was esterified and acetylated as described above, *methyl (1-anthryltri-O-acetyl-D-glucosid)uronate* was obtained, separating from ethanol in flat greenish needles, m.p. 162°, $[\alpha]_D^{25}$ –122° (*c* 0·57 in $CHCl_3$) (Found: C, 63·3; H, 5·3. $C_{27}H_{26}O_{10}$ requires C, 63·1; H, 5·1%). Both the glucosiduronic acid and its derivative yielded a compound indistinguishable from 1-hydroxyanthracene after hydrolysis with acid, and the glucosiduronic acid yielded this phenol after hydrolysis with β-glucuronidase.

(–)-*trans*-1,2-Dihydro-2-hydroxy-1-anthrylglucosiduronic acid (100 mg.) was hydrolysed with β-glucuronidase as described above and the solid that separated from the reaction mixture was recrystallized from benzene to yield (–)-*trans*-1,2-dihydro-1,2-dihydroxyanthracene (30 mg.) in flat needles, m.p. 162°, $[\alpha]_D^{25}$ –160° (*c* 0·48 in dioxan). Booth & Boyland (1949) give m.p. 162° for this isomer.

The glucosiduronic acid fraction that remained after the removal of the solid formed a dark-brown gum that did not crystallize. An examination of the gum on paper chromatograms showed the presence of a compound that had a bright-blue fluorescence in u.v. light and that was indistinguishable from the conjugate described below, which is probably 9-hydroxy-10-anthrylglucosiduronic acid. Portions of the gum were hydrolysed with acid and with β-glucuronidase. The product from the hydrolysis with acid contained compounds indistinguishable from 1- and 2-hydroxyanthracene, anthrone and 2-hydroxy-9,10-anthraquinone, and that from the enzymic hydrolysis contained compounds indistinguishable from *trans*-1,2-dihydro-1,2-dihydroxyanthracene, 1,2-anthraquinone, 2-hydroxy-9,10-anthraquinone and anthrone. A compound lying in the region of the chromatogram where the isomeric 9,10-dihydro-9,10-dihydroxyanthracenes were expected and that yielded anthrone after being treated with acid was also detected in the enzymic hydrolysis, but the amount of this compound was small compared with that of *trans*-1,2-dihydro-1,2-dihydroxyanthracene. 1- and 2-Hydroxyanthracene were not detected in the products of the enzymic hydrolysis. 9,10-Anthraquinone, which was detected as a product of both hydrolyses, could have arisen from the conjugates of both 9,10-dihydro-9,10-dihydroxyanthracene and 9,10-dihydroxyanthracene.

The gum, in methanol, was esterified with diazomethane in ether, and the product that remained after the removal of the solvent was dissolved in pyridine and treated with an excess of acetic anhydride. The mixture was kept overnight at room temperature and poured into a large volume of water. The gummy product that separated was dissolved in the minimum amount of boiling ethanol and the solution allowed to crystallize. The crystals that separated were recrystallized twice from ethanol to yield a product that appeared to be (–)-*methyl (1,2-dihydro-2-methoxy-1-anthryltri-O-acetyl-D-glucosid)uronate* (280 mg.) in needles, m.p. 221–222°, $[\alpha]_D^{25}$ –171° (*c* 0·5 in $CHCl_3$) (Found: C, 61·85; H, 5·4. $C_{28}H_{30}O_{11}$ requires C, 62·01; H, 5·6%). The derivative yielded a compound on acid hydrolysis indistinguishable on thin-layer chromatograms from 1-hydroxyanthracene, but 2-hydroxyanthracene was not detected. The u.v.-absorption maxima of the derivative are in agreement with the proposed structure (see Table 3).

The mother-liquors from the isolation of the derivative were allowed to evaporate at room temperature, and the solid that separated was recrystallized twice from ethanol to yield (–)-methyl (2-acetoxy-1,2-dihydro-1-anthryltri-*O*-acetyl-D-glucosid)uronate (52 mg.) in needles, m.p. and mixed m.p. 191–192°.

Attempts to obtain more crystalline materia from the mother-liquors were not successful, but a hydrolysis of a portion with acid yielded compounds indistinguishable from 1- and 2-hydroxyanthracene and anthrone on thin-layer chromatograms. The ethanol was evaporated and the gummy residue was dissolved in a solution of 0·1 N-barium methoxide in methanol (5 ml.) (Bollenback, Long, Benjamin & Lindquist, 1955), and the solution was kept overnight at room temperature. The precipitated barium salts of the glucosiduronic acids were filtered off and dissolved in aq. 2 N-acetic acid (10 ml.), and charcoal (5 g.) was added. The charcoal was filtered off and washed with water and the absorbed material eluted with 500 ml. of methanol–aq. NH_3 (sp.gr. 0·88) (19:1, v/v). The methanol was evaporated and the residual gum examined on paper chromatograms, when compounds indistinguishable from the conjugates of 1,2-dihydro-1,2-dihydroxyanthracene and 9,10-dihydro-9,10-dihydroxyanthracene were detected but the conjugate of 9,10-dihydroxyanthracene was not present. The gum was hydrolysed with β-glucuronidase to give a product that contained substances indistinguishable on thin-layer chromatograms from *trans*-1,2-dihydro-1,2-dihydroxyanthracene, *cis*- and *trans*-9,10-dihydro-9,10-dihydroxyanthracene and anthrone, but 2-hydroxy-9,10-anthraquinone was not detected. The product of the hydrolysis was chromatographed on six thin-layer chromatograms developed with solvent (*c*), and the bands that contained the 9,10-dihydro-9,10-dihydroxyanthracenes, located by the pale-yellow colour produced when test strips were sprayed with conc. HCl, were removed from the plates. The absorbed material was eluted from the silica gel with methanol and rechromatographed on three thin-layer chromatograms. Bands corresponding to the *cis*- and *trans*-isomers were located and the material was eluted with methanol as above to give two solutions, both of which showed u.v.-absorption maxima at 256 and 262 mμ and inflexions at 270 mμ. The solution of the faster-moving compound was evaporated to yield a small amount of a solid that was shown on thin-layer chromatograms to consist mainly of a compound indistinguishable from *cis*-9,10-dihydro-9,10-dihydroxyanthracene. A small amount of the *trans*-isomer was also present. The material had m.p. 155–159°, which was not depressed in admixture with the *cis*-isomer. The slower-moving fraction was shown on thin-layer chromatograms to contain mainly *trans*-9,10-dihydro-9,10-dihydroxyanthracene. Removal of the solvent yielded a pale-yellow solid (5 mg.), m.p. 133–135°, raised to 137–139° on admixture with the

synthetic isomer. Because of the small amounts of materials obtained it was not possible to purify the compounds further.

In a second experiment, two rats were each treated by intraperitoneal injection with anthracene (50 mg.), which had been purified as described above and suspended in arachis oil (2 ml.), and the urines were collected for 48 hr. after the injection. The combined urines were treated with charcoal and the eluate was chromatographed on Whatman no. 3 MM chromatography paper in the manner described below for the investigation of 9,10-anthraquinone metabolism. The results obtained were essentially those described above, but, in addition, a small amount of anthrone was detected when the phenolic fraction was examined on thin-layer chromatograms. In particular, the presence of 2-hydroxy-9,10-anthraquinone and conjugates of 9,10-dihydro-9,10-dihydroxyanthracene, 9-hydroxyanthracene, 9,10-dihydroxyanthracene and 2,9,10-trihydroxyanthracene was demonstrated, showing that these compounds are metabolic products of anthracene and are not derived from 9,10-anthraquinone present as an impurity in the hydrocarbon.

Also detected in these experiments was a compound that had a bright-blue fluorescence in u.v. light and was indistinguishable on chromatograms from a product described below which is possibly a diglucuronic acid conjugate of 9,10-dihydroxyanthracene.

Metabolism of 9,10-anthraquinone (IX). Four rats were maintained for 4 days on a diet containing 5% (w/w) of 9,10-anthraquinone, the urines being collected daily. The combined urines were acidified with acetic acid and treated with charcoal (25 g.). The charcoal was filtered off and washed with water (500 ml.) and the absorbed material was eluted with 1 l. of methanol–aq. NH_3 (sp.gr. 0·88) (19:1, v/v). The solvent was evaporated and the residue chromatographed on six sheets of Whatman no. 3 MM chromatography paper for 18 hr. with butan-1-ol–propan-1-ol–2 N-NH_3 (2:1:1, by vol.). A number of fluorescent bands, whose R_F values are given below, were seen when the chromatograms were examined in u.v. light, and these were cut out and the absorbed materials were eluted from the paper with methanol–aq. NH_3 (sp.gr. 0·88) (19:1, v/v). Removal of the solvent gave a number of fractions as gums that were examined on paper chromatograms. The products obtained after chemical and enzymic hydrolyses of the fractions were examined on thin-layer chromatograms.

Fraction 1, R_F 0·90, had a green fluorescence in u.v. light and was indistinguishable from anthrone on paper chromatograms. The gum was dissolved in a little water, the solution acidified with conc. HCl and extracted with ether. Examination of the ether-soluble material on thin-layer chromatograms showed the presence of a compound indistinguishable from anthrone: no other compounds were detected.

Fraction 2, R_F 0·65, had a bright-blue fluorescence in u.v. light, and was indistinguishable on paper chromatograms from the compound described below that is probably 9-hydroxy-10-anthranyl sulphate. The hydrolyses of this fraction with acid or with sulphatase gave small amounts of compounds indistinguishable from anthrone and 2-hydroxy-9,10-anthraquinone on thin-layer chromatograms. 9,10-Anthraquinone was also detected.

Fraction 3, R_F 0·58, had a pale-green fluorescence in u.v. light. The amount of material in this fraction was very small and no phenols could be detected after hydrolyses either with acid or with sulphatase.

Fraction 4, R_F 0·52, had an orange fluorescence in u.v. light and was indistinguishable on paper and thin-layer chromatograms from 2-hydroxy-9,10-anthraquinone. The u.v.-absorption spectrum of the metabolite, a solution of which was obtained by removing spots from thin-layer chromatograms developed with solvent (*c*) and eluting the absorbed material from the silica gel with ethanol, showed $\lambda_{max.}$ at 245, 272 and 334 mμ, identical with that of the synthetic compound (see Table 3).

Fraction 5, R_F 0·36, showed dark absorption in u.v. light. After hydrolysis with acid it gave a compound indistinguishable from 2-hydroxy-9,10-anthraquinone on thin-layer chromatograms, and the u.v.-absorption spectrum of a solution obtained by eluting the absorbed material from the spots with ethanol showed $\lambda_{max.}$ at 245 and 272 mμ. The metabolite is possibly the sulphuric ester of the hydroxyquinone described by Sato *et al.* (1959).

Fraction 6, R_F 0·33, had a bright-violet fluorescence in u.v. light. It was indistinguishable from a compound described below that is believed to be 9-hydroxy-10-anthrylglucosiduronic acid. The products of the hydrolyses, both with acid and with β-glucuronidase, yielded 9,10-anthraquinone together with small amounts of compounds indistinguishable on thin-layer chromatograms from anthrone and 2-hydroxy-9,10-anthraquinone.

Fraction 7, R_F 0·21, had a pale-green fluorescence in u.v. light. No phenolic products were obtained when the fraction was treated with acid or with β-glucuronidase. The metabolite is possibly related to that present in fraction 3, but no evidence could be obtained as to its structure.

Fraction 8, R_F 0·04, had a violet fluorescence in u.v. light. It was indistinguishable on paper chromatograms from compounds described below that were detected in the metabolism of *trans*-9,10-dihydro-9,10-dihydroxyanthracene and anthrone.

Metabolism of trans-*9,10-dihydro-9,10-dihydroxyanthracene (V).* Two rats were each given *trans*-9,10-dihydro-9,10-dihydroxyanthracene (100 mg.) suspended in arachis oil (2 ml.) by intraperitoneal injection. The urines were collected for 48 hr., pooled, treated with charcoal and chromatographed as described above to yield eight fractions.

Fraction 1, R_F 0·90, was indistinguishable from anthrone on paper and thin-layer chromatograms. The u.v.-absorption spectrum of the solution obtained by eluting the absorbed material with methanol from a paper chromatogram developed with butan-1-ol–propan-1-ol–2 N-NH_3 (2:1:1, by vol.) had $\lambda_{max.}$ at 253 mμ and an inflexion at 270 mμ and was identical with that of authentic anthrone similarly chromatographed ($\lambda_{max.}$ at 253 mμ, inflexion at 270 mμ). The spectrum of a solution of anthrone in methanol to which was added 1 drop of aq. NH_3 (sp.gr. 0·88) was similar to the above spectra but they differed from that of a solution of anthrone in methanol (see Table 3).

Fraction 2, R_F 0·63, had a bright-violet fluorescence in u.v. light. Hydrolysis with acid yielded small amounts of compounds indistinguishable on thin-layer chromatograms from anthrone and 2-hydroxy-9,10-anthraquinone, and hydrolysis with sulphatase yielded small amounts of compounds indistinguishable from the hydroxyquinone, anthrone and *trans*-9,10-dihydro-9,10-dihydroxyanthracene. Large amounts of 9,10-anthraquinone were present in both these hydrolysates. The u.v.-absorption spectrum

of the solution in methanol obtained when a portion of the fraction was rechromatographed as described for fraction 1 had $\lambda_{max.}$ at 256 mμ and an inflexion at 249 mμ, and the curve was similar to that of 9,10-diacetoxyanthracene (see Table 3). The fraction probably consisted mainly of 9-hydroxy-10-anthryl sulphate.

Fraction 3, R_F 0·57, had a pale-green fluorescence in u.v. light and appeared to be the same as the 9,10-anthraquinone metabolite present in fraction 3 above. It was not identified.

Fraction 4, R_F 0·50, was indistinguishable from 2-hydroxy-9,10-anthraquinone on paper and thin-layer chromatograms. The u.v.-absorption spectrum of a solution of the metabolite in ethanol had $\lambda_{max.}$ at 273 mμ, and the curve was identical with that of the synthetic quinone (see Table 3).

Fraction 5, R_F 0·35, had a bright-blue fluorescence in u.v. light. 9,10-Anthraquinone and small amounts of 2-hydroxy-9,10-anthraquinone and anthrone were detected after hydrolyses both with acid and with β-glucuronidase. Its u.v.-absorption spectrum, measured on a solution of the fraction in methanol obtained as described above, had $\lambda_{max.}$ at 255, 352 and 371 mμ and an inflexion at 249 mμ, and the curve was similar to that of 9,10-diacetoxyanthracene (see Table 3), and to that of the product in fraction 2 which is probably the sulphuric ester of 9,10-dihydroxyanthracene. The fraction, therefore, probably consists mainly of 9-hydroxy-10-anthrylglucosiduronic acid. The presence of 2-hydroxy-9,10-anthraquinone in the hydrolysates suggests the presence of a glucuronic acid conjugation of either 2-hydroxy-9,10-anthraquinone or, more likely, 2,9,10-trihydroxyanthracene. A small amount of 9-anthrylglucosiduronic acid is probably also present.

Fraction 6, R_F 0·29, formed a dark absorbing spot in u.v. light. After acid hydrolysis of the fraction, anthrone was detected on thin-layer chromatograms, whereas after hydrolysis with β-glucuronidase a mixture of *cis-* and *trans-*9,10-dihydro-9,10-dihydroxyanthracene, anthrone and 9,10-anthraquinone was formed. The fraction, therefore, appears to consist mainly of the glucuronic acid conjugate of 9,10-dihydro-9,10-dihydroxyanthracene.

Fraction 7, R_F 0·21, had a pale-green fluorescence in u.v. light. Metabolites present in this fraction could not be identified because no phenolic products were identified after hydrolysis, but the fraction was indistinguishable on paper chromatograms from the product in fraction 7 of the metabolism of 9,10-anthraquinone. The u.v.-absorption spectrum of the fraction, measured in methanol, showed a flat maximum at 265 mμ.

Fraction 8, R_F 0·04, had a bright-violet fluorescence in u.v. light. The u.v.-absorption spectrum of the compound showed $\lambda_{max.}$ at 258 mμ and an inflexion at 250 mμ and the curve was similar to but not identical with that of 9,10-diacetoxyanthracene (see Table 3). 9,10-Anthraquinone was the only product identified after the fraction was treated with acid or with β-glucuronidase. It is possible that the fraction consists mainly of the diglucosiduronic acid of 9,10-dihydroxyanthracene.

Metabolism of anthrone (VI). Two rats were each given anthrone (75 mg.) in arachis oil (1 ml.) by intraperitoneal injection. The urines were collected for 2 days after the injection and treated in the manner described above. The fractions were examined as before.

Fraction 1, R_F 0·67, had a bright-blue fluorescence in u.v. light. Only anthrone was detected when the fraction was treated either with acid or with sulphatase. The u.v.-absorption spectrum was similar to that of 9-anthryl acetate (see Table 3) and showed maxima at 253, 346, 364 and 383 mμ and an inflexion at 247 mμ. The fraction probably contained mainly 9-anthryl sulphate.

Fraction 2, R_F 0·52, consisted mainly of material that was indistinguishable on paper and on thin-layer chromatograms from 2-hydroxy-9,10-anthraquinone.

Fraction 3, R_F 0·38, had a bright-blue fluorescence in u.v. light. It yielded a compound indistinguishable from anthrone on thin-layer chromatograms after treatment both with acid and with β-glucuronidase. The u.v.-absorption spectrum, which had maxima at 253, 330, 346, 364 and 383 mμ and an inflexion at 247 mμ, was similar to that of 9-anthryl sulphate described above and to that of 9-anthryl acetate (see Table 3). The fraction therefore probably consisted mainly of 9-anthrylglucosiduronic acid.

Fraction 4, R_F 0·33, had a bright-violet fluorescence in u.v. light. The u.v.-absorption spectrum showed $\lambda_{max.}$ at 256 mμ and an inflexion at 258 mμ and the curve was similar to that of 9,10-diacetoxyanthracene. The products from the hydrolysis of the fraction both with acid and with β-glucuronidase yielded mainly 9,10-anthraquinone together with small amounts of 2-hydroxy-9,10-anthraquinone. The fraction therefore appears to be a mixture of the glucosiduronic acids of 9,10-dihydroxy- and 2,9,10-trihydroxy-anthracene.

Fraction 5, R_F 0·02, contained a metabolite indistinguishable from the metabolites described above of similar R_F values, which are presumed to be the diglucosiduronic acid of 9,10-dihydroxyanthracene. No additional evidence for its structure was obtained.

DISCUSSION

The probable pathways in anthracene metabolism, as indicated by the present work, are shown in Scheme 1.

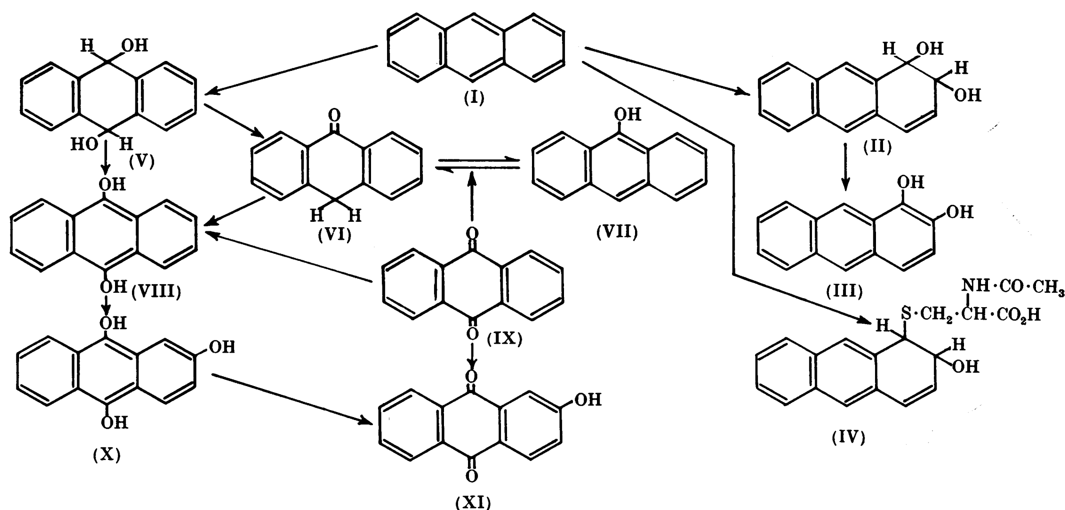
The isolation of *trans*-1,2-dihydro-1,2-dihydroxyanthracene (II) and its derivatives from the urine of rats treated with anthracene (I) confirms the earlier findings of Boyland & Levi (1935, 1936*a*), except that in the present work the free dihydrodihydroxy compound appeared to consist of roughly equal amounts of the (±)- and the (−)-isomers. There was, however, a large excess of the (−)-form of the glucosiduronic acid. Some of the dihydrodihydroxy compound was present in conjugation with sulphuric acid, but the configuration of this could not be determined. Small amounts of 1,2-anthraquinone were formed when the sulphuric ester and glucuronic acid fractions were hydrolysed. It is probable that the quinone was formed by the oxidation with air of 1,2-dihydroxyanthracene (III), released from conjugation in the hydrolyses. The catechol could have arisen in the body from the dehydrogenation of the corresponding dihydrodihydroxy compound, but the amounts formed appear to be small as compared with, for example, the amounts of 1,2-dihydroxynaphthalene formed

in the metabolism of naphthalene (Boyland & Sims, 1957).

After it was kept for some weeks, the glucosiduronic acid gum contained small amounts of 1-anthrylglucosiduronic acid, but 1- and 2-hydroxyanthracene, either free or in conjugation, were not detectable in fresh samples of urine. In this respect the metabolism on the 1,2-bond of anthracene resembles that on the 9,10-bond of phenanthrene (Boyland & Sims, 1962*b*), the 4,5-bond of pyrene (Boyland & Sims, 1964*a*) and the 5,6-bond of benz[*a*]anthracene (Boyland & Sims, 1964*b*), where no phenolic products have been detected. In the oxidation of anthracene in the microsomal enzyme system and in the ascorbic acid–ferrous sulphate model systems, 1,2-dihydro-1,2-dihydroxyanthracene (II) was formed, but 1- and 2-hydroxyanthracene were not detected (Boyland, Kimura & Sims, 1964).

Although the mercapturic acid has not been obtained in crystalline form, the chemical evidence suggests that it is *N*-acetyl-*S*-(1,2-dihydro-2-hydroxy-1-anthryl)cysteine (IV), a structure analogous to other mercapturic acids previously isolated. The mercapturic acid resembles that derived from naphthalene (Boyland & Sims, 1958) in that phenols as well as the parent hydrocarbon and the corresponding arylmercapturic acid are formed on acidification, whereas those derived from phenanthrene (Boyland & Sims, 1962*a*), pyrene (Boyland & Sims, 1964*a*) and benz[*a*]anthracene (Boyland & Sims, 1964*b*), where reaction has occurred on the so-called '*K* region' of the hydrocarbon, only the parent hydrocarbon and the corresponding arylmercapturic acid are formed. The formation of anthracene (I) by the acid decomposition of the mercapturic acid accounts, at least in part, for the anthracene which separates out when the urines of animals treated with anthracene are acidified (Boyland & Levi, 1936*b*; Chang & Young, 1943). There was no evidence for the presence in the glucosiduronic acid fraction of compounds yielding anthracene with acid analogous to the glucosiduronic acid isolated in naphthalene metabolism by Boyland & Solomon (1955) that yielded naphthalene with acid.

The formation of metabolic products at the 9- and 10-positions on the anthracene nucleus is of special interest as little is known of metabolism at these so-called '*meso*' positions of aromatic hydrocarbons. Although work on the metabolism of benz[*a*]anthracene (Boyland & Sims, 1964*b*) suggested that this hydrocarbon was hydroxylated at the 7- and 12-positions, the ease with which the metabolites were oxidized to benz[*a*]anthracene-7,12-quinone made their identification impossible. Dibenz[*a,h*]anthracene is converted into dibenz[*a,h*]anthracene-7,14-quinone by animals (Heidelberger, Hadler & Wolf, 1953) but the mechanism of its formation is not known. The 6-position of benzo[*a*]pyrene, which may be regarded as equivalent to the 7-positions of benz[*a*]anthracene and dibenz[*a,h*]anthracene, is hydroxylated by animals (Falk, Kotin, Lee & Nathan, 1962). With anthracene, the metabolic formation of *trans*-9,10-dihydro-9,10-dihydroxyanthracene (V) is now established. The formation of the corresponding *cis*-isomer in the body is less certain, because of the ease with which the *trans*-isomer is converted into the *cis*-isomer under the conditions used in the enzymic hydrolyses



Scheme 1. Probable pathways in anthracene metabolism.

of glucuronic acid conjugates. The instability of the free dihydrodihydroxy compound detected in the isolation experiments described above suggests that here, at least, only the *trans*-isomer was present. With 1,2-dihydroxyindane and 1,2-dihydroxyacenaphthene it has been shown by Hopkins, Lewis & Young (1964) that the corresponding *cis*- and *trans*-isomers are interconvertible in the body.

It is evident from the later experiments described above that a large proportion of the first-formed 9,10-dihydro-9,10-dihydroxyanthracene (V) is further metabolized in the body. The first stage is presumably a dehydrogenation of the type described above to give 9,10-dihydroxyanthracene (VIII) and some of this compound is then hydroxylated to give 2,9,10-trihydroxyanthracene (X). 9,10-Dihydroxyanthracene (VIII) is excreted in conjugation with sulphuric acid and glucuronic acid and is presumably oxidized by air to 9,10-anthraquinone (IX) during the hydrolyses of its conjugates. 2,9,10-Trihydroxyanthracene (X) is presumably present in the urine as conjugates involving the hydroxyl groups on either the 9- or the 10-position. The detection of 2-hydroxy-9,10-anthraquinone (XI) in the hydrolysates of these conjugates can be explained if it is assumed that the hydroxyl groups in the '*meso*' positions are oxidized by air during the hydrolyses. In the metabolism of 9,10-anthraquinone (IX), a second conjugate was detected which appeared to be the sulphuric ester of 2-hydroxy-9,10-anthraquinone (XI) itself. It is not known whether the conjugate was excreted as such or as the corresponding reduced form, 9,10-dihydroxy-2-anthryl sulphate, which was oxidized during the working-up procedures. The conjugate of 2-hydroxy-9,10-anthraquinone (XI) was not detected as a metabolite of any of the other compounds examined, but Sato *et al.* (1959) report that the sulphuric ester of 2-hydroxy-9,10-anthraquinone (XI) is readily hydrolysed, even in water. This would explain why, in the present work, comparatively large amounts of the free hydroxyquinone (XI) are detected.

The small amount of anthrone detected in the metabolism of anthracene, presumably present as 9-hydroxyanthryl sulphate, could also have arisen from 9,10-dihydro-9,10-dihydroxyanthracene (V), since the ester was also detected in the urine of animals treated with the dihydrodihydroxy compound. The presence of the corresponding glucuronic acid conjugate is not so certain since the glucuronic acid conjugate of the dihydrodihydroxy compound also yielded anthrone (VI) after hydrolysis with β-glucuronidase.

The formation of anthrone in the metabolism of 9,10-anthraquinone is of interest, since it involves the removal of oxygen from the quinone. This could be brought about in the body, either by a reduction of one of the oxo groups to give anthrone directly (a reaction which chemically proceeds very readily) or by a reduction to 9,10-dihydroxyanthracene followed by a rearrangement to the tautomeric 10-hydroxy-9-anthrone (oxanthrone), which might then dehydroxylate in the body to yield anthrone. A related dehydroxylation of 7,12-dihydro-7,12-dihydroxybenz[*a*]anthracene has been described (Boyland & Sims, 1964*b*).

The conversion of anthrone (VI) into 9,10-dihydroxyanthracene (VIII) in the body is presumably brought about by hydroxylation of the methylene group to give oxanthrone which then rearranges to give the dihydroxy compound. The methylene group in the 9-position of fluorene is similarly hydroxylated by animals (Grantham, 1963).

It is difficult to estimate the relative amounts of the metabolites arising by reactions at the 1- and 2-positions as compared with those arising by reactions at the 9- and 10-positions on the anthracene nucleus, because of the number of metabolites formed. It is probable, however, that the 1- and 2-positions are the more active biologically since relatively large amounts of some of the metabolites arising from reactions at these positions were isolated. The theory of Pullman & Pullman (1955) requires that, for an aromatic hydrocarbon to be a carcinogenic agent, it must have an active '*K* region' and a relatively inactive '*L* region'. With anthracene, the 1,2-bond is similar to bonds of the '*K* region' type, both chemically, in that here the additions of osmium tetroxide (Cook & Schoental, 1948) and ethyl diazoacetate (Badger, Cook & Gibb, 1951) take place, and biologically, in that here dihydrodihydroxy compounds and a mercapturic acid, but not phenols, are formed. The 9- and 10-positions of anthracene, which form the '*L* region', are active chemically and, as the present work has shown, are oxidized by metabolic processes.

SUMMARY

1. Anthracene is converted by rats into *trans*-1,2-dihydro-1,2-dihydroxy- and 1,2-dihydroxyanthracene which are excreted mainly as sulphuric acid and glucuronic acid conjugates. 1- and 2-Hydroxyanthracene, either free or conjugated, are not present as urinary metabolites.

2. *N*-Acetyl-*S*-(1,2-dihydro-2-hydroxy-1-anthryl)cysteine is excreted and this compound is decomposed by mineral acid to give 1-anthrylmercapturic acid, 1- and 2-hydroxyanthracene and anthracene.

3. Anthracene is converted into *trans*-9,10-dihydro-9,10-dihydroxyanthracene by rats. The *cis*-dihydrodihydroxy compound was also identified,

but it could have been formed as an artifact during the enzymic hydrolysis of the glucuronic acid conjugate of the *trans*-isomer. 2-Hydroxy-9,10-anthraquinone, anthrone and conjugates of 9-hydroxy-, 9,10-dihydroxy- and 2,9,10-trihydroxy-anthracene, which were shown to be metabolic products of the *trans*-isomer in rats, were also detected in the urine of rats treated with anthracene.

4. 9,10-Anthraquinone and anthrone were metabolized by rats to 2-hydroxy-9,10-anthraquinone and conjugates of 9-hydroxy-, 9,10-dihydroxy- and 2,9,10-trihydroxy-anthracene; 9,10-anthraquinone also yielded anthrone and the sulphuric ester of 2-hydroxy-9,10-anthraquinone.

I thank Professor E. Boyland for his interest, Dr D. Manson for the gift of *cis*- and *trans*-9,10-dihydro-9,10-dihydroxyanthracene, and Mr J. W. Gorrod and Miss S. Gowers for technical assistance. This investigation was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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Metabolism of Polycyclic Compounds

26. THE HYDROXYLATION OF SOME AROMATIC HYDROCARBONS BY THE ASCORBIC ACID MODEL HYDROXYLATING SYSTEM AND BY RAT-LIVER MICROSOMES

By E. BOYLAND, M. KIMURA and P. SIMS
Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W. 3

(*Received* 30 *January* 1964)

The ascorbic acid–Fe^{2+} ion–oxygen model hydroxylating system was first described by Udenfriend, Clark, Axelrod & Brodie (1954), and Brodie, Axelrod, Shore & Udenfriend (1954) showed that with many aromatic compounds, e.g. aniline, acetanilide and salicylic acid, phenols are formed. Boyland & Manson (1958), however, found that 2-acetamidonaphthalene was converted by the hydroxylating system into 2-acetamido-5,6-dihydro-5,6-dihydroxy-, 2-acetamido-1-hydroxy- and 2-acetamido-6-hydroxy-naphthalene. In the present work it was found that, of a number of aromatic hydrocarbons examined, most are converted into mixtures of phenols and dihydrodihydroxy compounds. A comparison of the products obtained in the chemical oxidation with those