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THE METABOLISM OF SOME AROMATIC HYDROCARBONS BY MOUSE EMBRYO CELL CULTURES

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Abstract—7,12-Dimethylbenz[a]anthracene was converted by mouse embryo cells into its 8,9-dihydrodiol and into the 8,9-dihydrodiols of the corresponding hydroxymethyl derivatives. Carboxylic acids, arising from oxidation of the methyl groups were also formed. 7-Methylbenz[a]anthracene was similarly metabolized into the 7-hydroxymethyl derivative, the 8,9-dihydrodiol both of the hydrocarbon and of the 7-hydroxymethyl derivative and benz[a]anthracene-7-carboxylic acid. 7-Bromomethylbenz[a]-anthracene yielded mainly 7-hydroxymethylbenz[a]anthracene, probably by a non-enzymic reaction. The metabolites of benzo[a]pyrene were 3-hydroxybenzo[a]pyrene, 1,2-dihydro-1,2-dihydroxybenzo[a]pyrene and 9,10-dihydro-9,10-dihydroxybenzo[a]pyrene. No 'K-region' dihydrodiols were detected with any of the above substrates. Benzo[e]-pyrene was converted by embryo cells into a phenol and into the 'K-region' dihydrodiol, 4,5-dihydro-4,5-dihydroxybenzo[e]pyrene. The phenol and the dihydrodiol were metabolites of the hydrocarbon in rat-liver homogenates. With embryo cells all the hydrocarbons yielded unidentified water soluble metabolites.

THE METABOLISM of 7,12-dimethylbenz[a]anthracene (Fig. 1, I; R = Me)[1–3] and of 7-methylbenz[a]anthracene (I; R = H)[4] has been studied in rat-liver homogenates: hydroxylation of the methyl groups, to yield the products (I; R = CH_2OH), (IV; R = Me or H) and (V; R = Me or H) and ring-hydroxylation to yield products (II; R = Me or H) occurs. The hydroxymethyl compounds are further metabolised by ring hydroxylation to yield the products (III; R = Me or H). In hamster embryo cells, 7,12-dimethylbenz[a]anthracene is converted into small amounts of the hydroxymethyl derivatives (I; R = CH_2OH and IV; R = Me) and an unidentified water-soluble product.[5]

The carcinogen, benzo[a]pyrene (Fig. 2; VI), is converted by rat-liver homogenate into 3-hydroxybenzo[a]pyrene (VII), 1,2-dihydro-1,2-dihydroxybenzo[a]pyrene (VIII) and 9,10-dihydro 9,10-dihydroxybenzo[a]pyrene (IX).[6, 7] Homogenised hamster embryo cells contain the enzyme 'benzopyrene hydroxylase', which converts benzo[a]pyrene into the 3-hydroxy derivative:[8] the levels of the enzymes are raised if the cells are allowed to grow in the presence of benz[a]anthracene. The metabolism of the non-carcinogen, benzo[e]pyrene (Fig. 3; X), has not previously been studied either in tissue preparations or in cell cultures. The work now described is concerned with the metabolism of these hydrocarbons by mouse embryo cells grown in culture.

EXPERIMENTAL

Materials. 7,12-Dimethylbenz[a]anthracene (DMBA) was obtained from Eastman

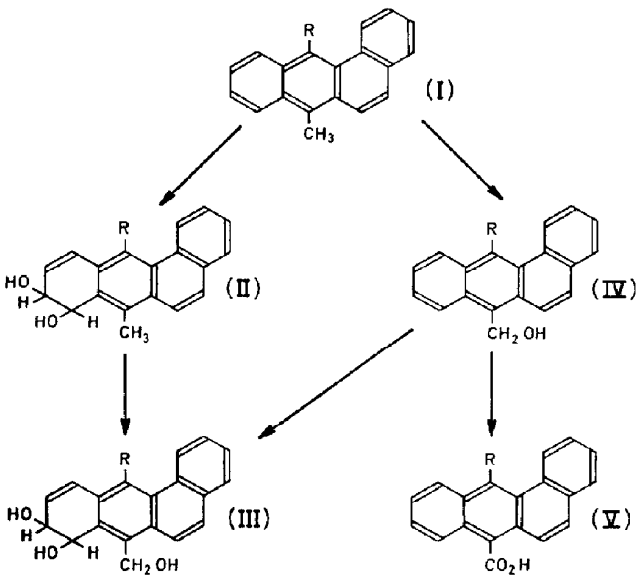

FIG. 1. Summary of principal metabolic reactions of 7,12-dimethylbenz[a]anthracene (I; R = Me_3) and 7-methylbenz[a]anthracene (I; R = H) in mouse embryo cells.

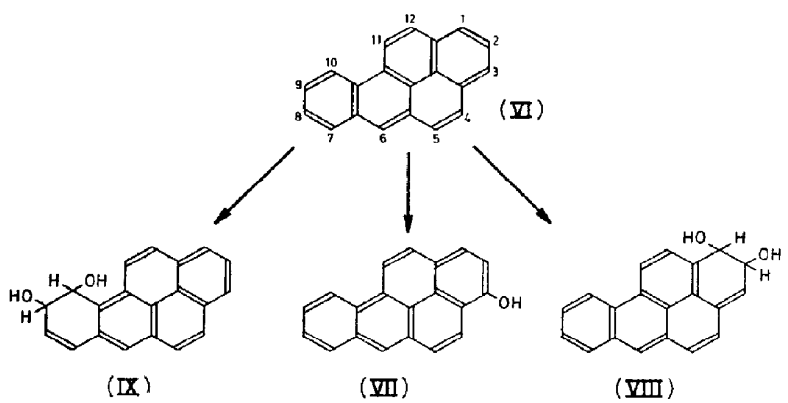

FIG. 2. Metabolic pathways of benzo[a]pyrene.

Kodak Ltd. (Kirkby, Liverpool). 7-Hydroxymethyl-12-methylbenz[a]anthracene (7-OHM-12-MBA), 12-hydroxymethyl-7-methylbenz[a]anthracene (12-OHM-7-MBA), 7,12-dihydroxymethylbenz[a]anthracene (7,12-DiOHMBA), 12-methylbenz-[a]anthracene-7-carboxylic acid (12-MBA-7-COOH) and 5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene (5,6-DiOHDMBA) were prepared as described.[1, 2]

The preparations of 7-methylbenz[a]anthracene (7-MBA),[9] 7-hydroxymethyl-benz[a]anthracene (7-OHMBA),[10] benz[a]anthracene-7-carboxylic acid (BA-7-COOH),[11] 5,6-dihydro-5,6-dihydroxy-7-methylbenz[a]anthracene (5,6-Di-HOHMBA),[12] S-(5,6-dihydro-6-hydroxy-7-methyl-5-benz[a]anthracenyl)-L-cysteine[13]

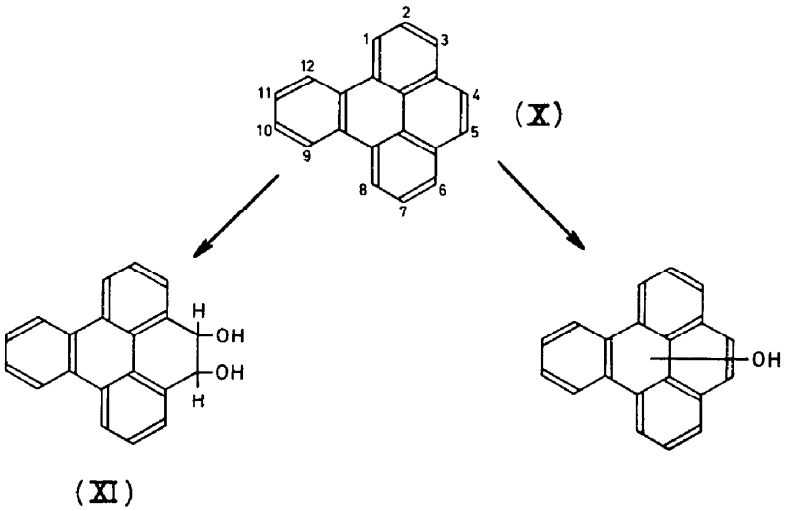

FIG. 3. Metabolic pathways of benzo[e]pyrene.

and -glutathione[13] and *N*-acetyl-S-(5,6-dihydro-6-hydroxy-7-methyl-5-benz[a]anthracenyl)-L-cysteine[13] have been described. S-(7-benzanthracenylmethyl)-glutathione, S-(7-benzanthracenylmethyl)-L-cysteine and *N*-acetyl-S-(7-benz[a]anthracenylmethyl)-L-cysteine were prepared from the reaction of 7-OHMBA in acetic acid with glutathione, L-cysteine and *N*-acetylcysteine respectively in the presence of boron trifluoride (P. Sims, unpublished experiments).

Benzo[a]pyrene (B[a]P) was obtained from Eastman Kodak Ltd. and benzo[e]pyrene (B[e]P) from Koch-Light Ltd. (Colnbrook, Bucks). trans-4,5-*Dihydro*-4,5-*dihydroxybenzo*[a]*pyrene* (4,5-DiHOHB[a]P) was prepared by the reduction of benzo[a]pyrene 4,5-quinone with lithium tetrahydroaluminate in ether: it separated from benzene in needles, m.p. 215° (Found: C, 83·7; H, 5·0. $C_{20}H_{14}O_2$ requires C, 83·9; H, 4·9%). cis-4,5-*Dihydro*-4,5-*dihydroxybenzo*[e]*pyrene* (XI) (4,5-DiHOHB[e]P) was prepared from B[e]P by the action of OsO_4 using the general method described for the preparation of 'K region' dihydrodiols:[14] it separated from benzene in needles, m.p. 208° (Found: C, 83·8; H, 5·1%), λ_{max} at 254, 262, 276 (infl) and 288 mμ (log ε 4·83, 4·98 and 4·16). When heated with 5N-HCl, the dihydrodiol yielded a phenol presumed to be 4-hydroxybenzo[e]pyrene (4-OHB[e]P), λ_{max} at 227, 237, 255 (infl), 264, 286, 299, 335 and 381 mμ.

Chromatography. Paper chromatography was carried out by downward development overnight on Whatman No. 1 chromatography paper in butanol-1-ol-propan-1-ol-aq. 2N-NH_3 (2:1:1, by vol.). Thin-layer chromatograms of 0·25 mm thickness were prepared from silica gel G: they were developed for 15 cm in either (a) benzene, (b) benzene–ethanol (19:1, v/v), or (c) benzene–ethanol (9:1, v/v). The chromatographic properties of the DMBA derivatives,[2] of the 7-MBA derivatives[4] and of the B[a]P derivatives[6] on thin-layer chromatograms have been described.

Experiments with rat-liver homogenates. Rat-liver homogenates prepared from the livers (30 g) of male Chester Beatty strain rats that had been pretreated with 3-methyl-

cholanthrene (5 mg in 0·5 ml of arachis oil) 48 hr previously, were incubated for 15 min with 1 mg of either DMBA, 7-OHM-12-MBA, 7-MBA, 7-OHMBA or B[a]P as previously described.[2, 4, 6] The mixtures were extracted with ethyl acetate (50 ml) to yield solutions containing metabolites that have not been synthesised for use as chromatographic standards. DMBA yielded 8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz[a]anthracene (8,9-DiHOHDMBA), 7-OHM-12-MBA yielded 8,9-dihydro-8,9-dihydroxy-7-hydroxymethyl-12-methylbenz[a]anthracene (8,9-DiHOH-7-OHM-12-MBA), 7-MBA yielded 8,9-dihydro-8,9-dihydroxy-7-methylbenz[a]anthracene (8,9-DiHOHMBA) and 7-OHMBA yielded 8,9-dihydro-8,9-dihydroxy-7-hydroxymethylbenz[a]anthracene (8,9-DiHOH-7-OHMBA).

Benzo[a]pyrene similarly yielded a mixture shown previously[6, 7] to contain 3-hydroxybenzo[a]pyrene (3-OHB[a]P), 1,2-dihydro-1,2-dihydroxybenzo[a]pyrene (1,2-DiHOHB[a]P) and 9,10-dihydro-9,10-dihydroxybenzo[a]pyrene (9,10-DiHOHB[a]P).

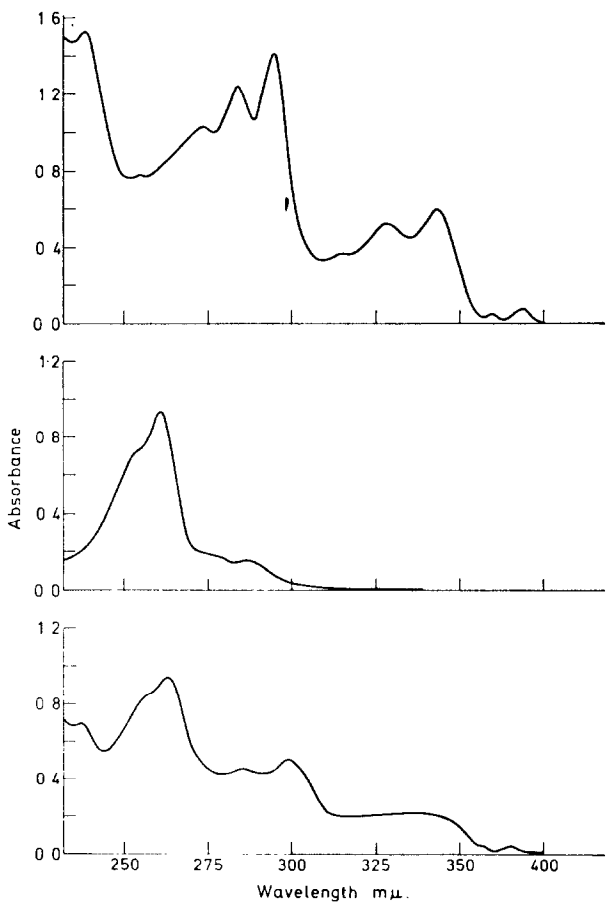

FIG. 4. Ultra-violet absorption spectra of products related to benzo[e]pyrene: upper curve, phenolic metabolite; middle curve, dihydrodiol metabolite; lower curve, phenol formed from the dihydrodiol metabolite with acid. The spectra were obtained as described in the text.

B[e]P (1·5 mg) was incubated with homogenate from rat-liver (30 g). The ethyl acetate-soluble products were applied to the base-line of a thin-layer chromatogram (20 × 20 cm), which was developed for 15 cm with solvent (b). Two violet fluorescent bands were detected in u.v. light: the faster moving band had R_f 0·64, and its fluorescence changed to blue when the chromatogram was exposed to NH_3. The u.v. spectrum, obtained on material eluted with ethanol from the silica gel, is shown in Fig. 4. The slower-moving metabolite had R_f 0·20 and its u.v. spectrum (Fig. 4) was identical with that of 4,5-DiHOHB[e]P. On a two-dimensional chromatogram that was developed for 10 cm in solvent (b), sprayed with conc. HCl and heated in an oven to 110° for 5 min, and developed in the second direction with solvent (a) the metabolite yielded a phenol identical in its chromatographic properties and u.v. spectrum (Fig. 4) with the product believed to be 4-OHB[e]P.

Experiments with mouse embryo cells. The culture media from primary mouse embryo cells that had been incubated with the tritiated substrates described below were kindly provided by Dr. P. Brookes of this Institute. The cells were incubated in a medium containing DMBA (0·1 μg/ml; 2·4 μc/ml) for 47 hr or in a medium containing either 7-MBA (0·06 μg/ml; 8·5 μc/ml), 7-bromomethylbenz[a]anthracene (7-BrMBA) (0·47 μg/ml; 2·0 μc/ml), B[a]P (0·30 μg/ml; 0·24 μc/ml) or B[c]P (0·30 μg/ml; 0·13 μc/ml) for 30 hr. Portions (5 ml) of the media were extracted with ethyl acetate (2 × 50 ml) and the extracts dried over Na_2SO_4 and evaporated and the residues applied as 2 cm bands to the base-lines of thin-layer chromatograms which were developed in solvent (b). To assist in the location on the plates of bands containing metabolites, small amounts of 7-OHM-12-MBA, 12-OHM-7-MBA, 7,12-DiOHMBA, 5,6-DiHOHDMBA and products from the incubation of DMBA and of 7-OHM-12-MBA with rat-liver homogenate were added to the extract of the medium from cells treated with tritiated DMBA and small amounts of 7-OHMBA and 5,6-DiOHMBA and products from the incubation of 7-MBA and 7-OHMBA with rat-liver homogenates were added to that of the medium from cells treated with both tritiated 7-MBA and 7-BrMBA. Similarly 4,5-DiHOHB[a]P and 4,5-DiHOHB[e]P together with rat-liver homogenate experiments were added to the ethyl acetate extracts of the media from the B[a]P and B[e]P incubations respectively. The chromatograms were examined in u.v. light and fluorescent bands and intermediate bands were removed, the silica gel transferred to glass vials and the radioactivity present determined by liquid scintillation counting as previously described.[3]

The aqueous layers were acidified to pH 4 with acetic acid and treated with activated charcoal (1 g). The charcoal was filtered off and washed with water (50 ml) and the absorbed material eluted with methanol containing 5% (v/v) of NH_3 (sp. gr. 0·88). The eluates were evaporated and the residues applied as bands of 5 cm width to paper chromatograms which were developed as described above. The chromatograms were cut into convenient transverse strips, which were immersed in scintillation fluid in glass vials and the radioactivity determined. Methyl red, used as an indicator in the culture medium was absorbed and eluted in the above procedure: it proved a convenient marker on the paper chromatograms at R_f 0·26.

To obtain sufficient material for confirmatory and other tests, the main bulks of the media were processed in a similar manner to those described above, except that thin-layer chromatograms of 20 cm width and whole sheets of chromatography paper were used in the separations. Products were eluted with ether from bands of silica gel

removed from thin-layer and with methanol 5% (v/v) NH_3 from bands cut from paper chromatograms.

RESULTS

Metabolism of 7,12-*dimethylbenz*[a]*anthracene*. The results of the examination of the ethyl acetate extract and of the aqueous phase of mouse embryo cells treated with DMBA are shown in Fig. 5. Attempts to confirm the presence of 12-OHM-7-MBA and 7-OHM-12-MBA in fractions 4 and 6 by re-chromatography of the material

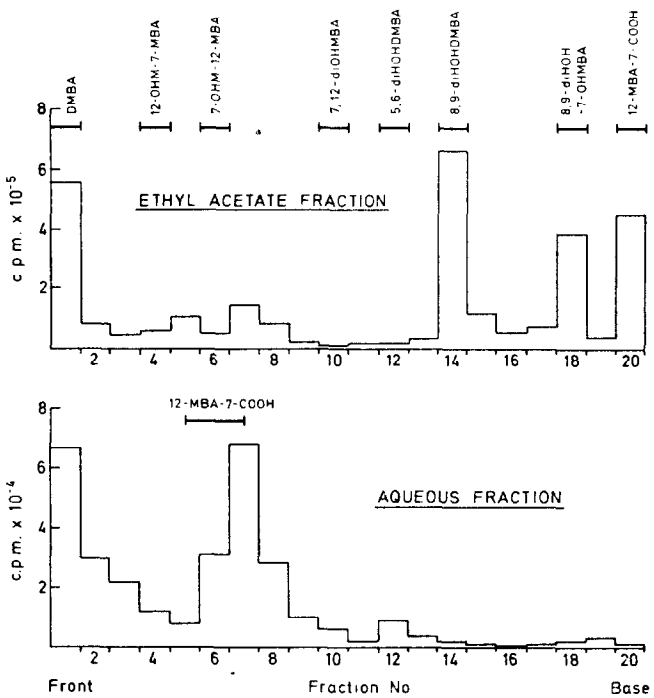

FIG. 5. The metabolism of 7,12-dimethylbenz[a]anthracene in mouse embryo cells. The positions of the metabolites on the chromatograms are shown thus: ——; abbreviations are those given in the text. Chromatography was carried out as described in the text.

obtained from the appropriate fractions from the large scale extractions in solvent (a) or (b) were unsuccessful. The products in fractions 5, 7 and 8 are probably the results of non-enzymic oxidation of DMBA, since products running in these regions of the chromatograms were obtained when DMBA was incubated with medium alone. Because of this, it was not possible to determine if the phenolic metabolites previously described,[1] were present in fraction 5.

The main products in the ethyl acetate fraction were identical in their chromatographic behaviour with 8,9-DiHOHDMBA and 8,9-DiHOH-7-OHMBA. Presumably, the 12-hydroxymethyldihydrodiol is also formed but it cannot be separated from the 7-hydroxymethyl derivative in the chromatographic systems used. Confirmation of

the presence of the two types of dihydrodiols was provided by treating mixtures of the appropriate fractions of material from the cell medium and from the liver homogenate incubations with acetic anhydride in pyridine and examining the products on thin-layer chromatograms developed in solvent (c): the radioactivity was then associated with new fluorescent bands (seen when the chromatograms were examined in u.v. light) at R_f 0·94 from the acetylation of 8,9-DiHOHDMBA and at R_f 0·87 from the acetylation of 8,9-DiHOH-7-OHMBA. The material in fraction 15 probably contained 12-MBA-7-COOH because when the products in this fraction were chromatographed on paper together with the carboxylic acid, most of the radioactivity was associated with the carboxylic acid spot. The isomeric 7-methylbenz[a]anthracene-12-carboxylic acid is probably also present.

Only one major metabolite was present in the aqueous fraction of the cell medium. In its chromatographic properties it was similar to, but not identical with, 12-MBA-7-COOH (see Fig. 5). Treatment of solutions of the metabolite with hot HCl, with sulphatase or with β-glucuronidase failed to produce any recognisable products. The identity of the product therefore remains unknown.

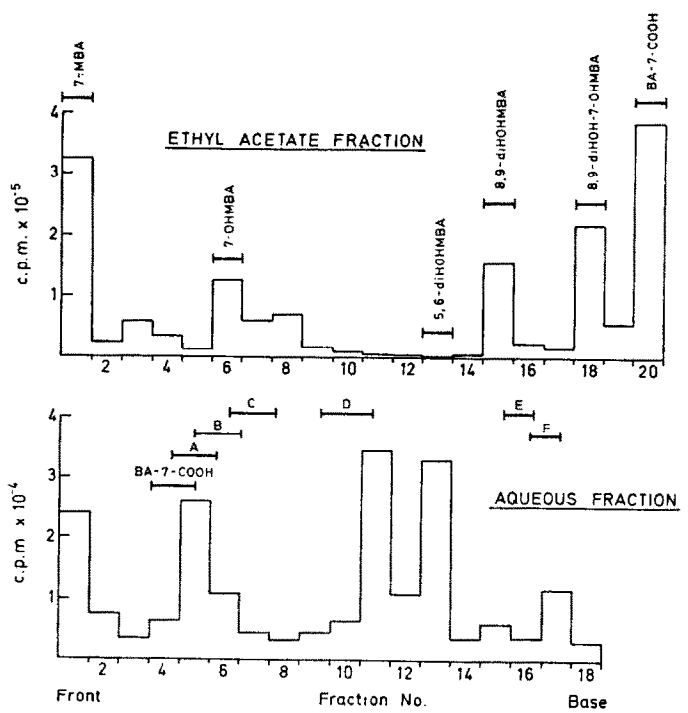

FIG. 6. The metabolism of 7-methylbenz[a]anthracene in mouse embryo cells. The position of the metabolites on the chromatograms are shown thus: ——; abbreviations are as used in the text except that A = *N*-acetyl-S-(7-benz[a]anthracenylmethyl)-L-cysteine, B = S-(7-benz[a]anthracenylmethyl)-L-cysteine, C = *N*-acetyl-S-(5,6-dihydro-6-hydroxy-5-benz[a]anthracenyl)-L-cysteine, D = S-(5,6-dihydro-6-hydroxy-5-benz[a]anthracenyl)-L-cysteine, E = S-(7-benz[a]anthracenylmethyl)glutathione and F = S-(5,6-dihydro-6-hydroxy-5-benz[a]anthracenyl)glutathione. The chromatograms were developed as described in the text.

Metabolism of 7-methylbenz[a]anthracene. The results of the examination of the ethyl acetate and aqueous phases are shown in Fig. 6. The presence of 7-OHMBA in fraction 6 was confirmed by treating the material obtained in this fraction with acetic anhydride in pyridine and examining the products on thin-layer chromatograms developed in solvent (a) in the presence of the acetyl derivative of 7-OHMBA. Most of the radioactivity was associated with the violet fluorescent band of the acetoxy derivative, at R_f 0·62, seen when the chromatogram was examined in u.v. light. The product in fraction 8 was not identified: it could be an oxidation product of the hydrocarbon since DMBA forms an oxidation product with similar chromatographic properties.

The products in fractions 15 and 18 were identical in their properties to 8,9-DiHOHMBA and 8,9-DiHOH-7-OHMBA obtained from the rat-liver homogenate experiment. Acetylation of the fractions in the presence of the products from the rat-liver homogenate incubation yielded products on chromatograms developed in solvent (b), each of which showed a violet fluorescence in u.v. light, at R_f 0·96 for that derived from 8,9-DiHOHMBA and 0·84 for that derived from 8,9-DiHOH-7-OHMBA: most of the radioactivity was associated with these fractions.

The product in fraction 20 was mainly BA-7-COOH, since when the product was chromatographed on paper together with the synthetic acid, most of the radioactivity was associated with the violet fluorescent spot formed by the acid.

There appeared to be three major products in the aqueous phase. The product in

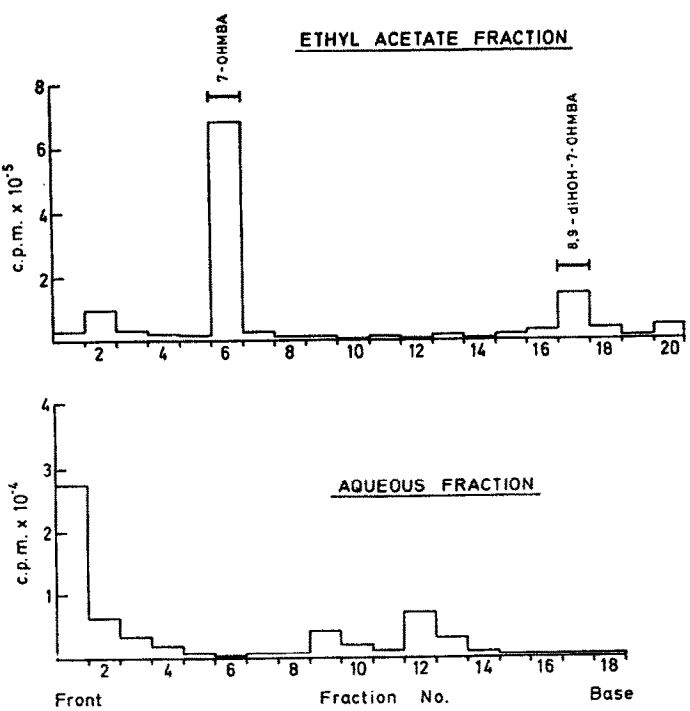

FIG. 7. The metabolism of 7-bromomethylbenz[a]anthracene in mouse embryo cells. The positions of the metabolites are shown thus: ——; abbreviations are those given in the text. Chromatography was carried out as described in the text.

fraction 5 was not attacked by HCl, by sulphatase or by β-glucuronidase and in its chromatographic properties it differed from BA-7-COOH or any of the mercapturic acids or cysteine derivatives described above. The product in fraction 10 was similarly resistant to acid and enzyme treatment and was not identified. The product in fraction 12, however, was hydrolysed with β-glucuronidase to yield mainly a product with the properties on thin-layer chromatograms of 8,9-DiHOH-7-OH-MBA. A small amount of a product with the properties of 8,9-DiHOHMBA was also formed. The product in the aqueous phase, therefore, probably contains a mixture of the glucuronic acid conjugates of these dihydrodiols.

Metabolism of 7-bromomethylbenz[a]anthracene. The results are shown in Fig. 7. The major product in the ethyl acetate phase was 7-OHMBA: this probably arises from the non-enzymic reaction of the bromomethyl compound with the aqueous

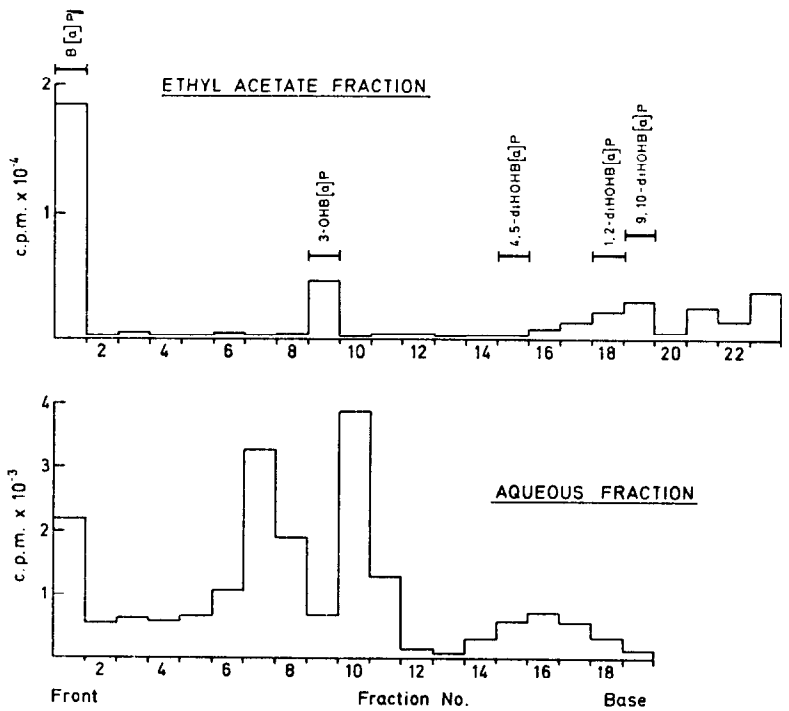

FIG. 8. The metabolism of benzo[a]pyrene in mouse embryo cells. The positions of metabolites on the chromatograms are shown thus: ——; abbreviations are as used in the text.

medium, since the compound is readily converted into the hydroxymethyl derivative by water. Small amounts of products with the chromatographic properties of 8,9-DiHOH-7-OHMBA and BA-7-COOH were also formed, presumably arising from the further metabolism of the hydroxymethyl derivative.

Very little radioactivity was detected in the aqueous phase: the small amount of product in fraction 12 may be the glucuronic acid conjugate of 8,9-DiHOH-7-OHMBA but there was insufficient to permit a full investigation.

Metabolism of DMBA in other cells. Examination of the ethyl acetate-soluble

products from the culture media of human leucocytes, lymphocytes and melanoma cells and of rat hepatoma cells incubated with DMBA failed to show the presence of any of the metabolites described above. The media from all these incubations yielded a mixture of unidentified oxidation products which were also found when DMBA was incubated with culture medium alone.

Metabolism of benzo[a]pyrene. Three products were identified in the ethyl fraction, with the chromatographic properties of 3-OHB[a]P, 1,2-DiHOHB[a]P and 9,10-DiHOHB[a]P (see Fig. 8). There was no evidence for the presence of the K-region dihydrodiol, 4,5-DiHOHB[a]P, in the incubation media. The B[a]P molecule possesses two 'K-regions': the second dihydrodiol, 11,12-Dihydro-11,12-dihydroxybenzo[a]-pyrene (11,12-DiHOHB[a]P), has been shown to move slightly faster on thin-layer chromatograms, developed with solvent (b), than 4,5-DiHOHB[a]P.[6] No significant radioactivity was detected in this region of the chromatogram.

The presence of 1,2-DiHOHB[a]P and 9,10-DiHOHB[a]P in the cell culture medium was confirmed by two-dimensional thin-layer chromatography carried out as described above in the presence of the products from the incubation of B[a]P with rat-liver homogenate. The spots of the phenols obtained by the acid decomposition of the dihydrodiols on the chromatograms were detected as previously described and removed and the radioactivity counted. 1-Hydroxybenzo[a]pyrene, derived from 1,2-DiHOHB[a]P, had R_f 0·42 and gave $1{\cdot}0 \times 10^3$ cpm and 9-hydroxybenzo[a]-pyrene, derived from 9,10-DiHOHB[a]P had R_f 0·32 and gave $15{\cdot}9 \times 10^3$ cpm, indicating that the latter compound was the major dihydrodiol formed by the cells.

Products in fractions 21 and 23 were not identified: they could be 1,6- and 3,6-dihydroxybenzo[a]pyrene which are metabolites of the hydrocarbon in whole animals.[15]

The aqueous fraction contained three products. The faster-running products in fractions 7 and 10 failed to yield any recognisable products when they were either heated with 2N-HCl or incubated with sulphatase or β-glucuronidase. With β-glucuronidase, the metabolite in fractions 15, 16 and 17 yielded a product with the mobility of 9,10-DiHOHB[a]P on a thin-layer chromatogram developed in solvent (b). The fractions are therefore presumed to contain a glucuronic acid conjugate of this dihydrodiol.

Metabolism of benzo[e]pyrene. Four major metabolites of B[e]P were detected in the ethyl acetate fraction (see Fig. 9). Two were identical in their chromatographic properties with the phenol and 4,5-DiHOHB[e]P detected as metabolites of B[e]P in rat-liver homogenate. The identity of the dihydrodiol was confirmed on a two dimensional chromatogram of the type described above in which the metabolite from the medium and synthetic 4,5-DiHOHB[e]P were chromatographed simultaneously. The spot containing the phenol, presumed to be 4-OHB[e]P, liberated from the dihydrodiol by acid was removed from the chromatogram and counted for radioactivity: $24{\cdot}2 \times 10^3$ cpm were obtained.

The products in fractions 17 and 19 were not identified. No products with the chromatographic characteristics of these metabolites were detected when B[e]P was incubated with rat-liver homogenates: the product in fraction 17 may be a dihydroxy compound.

The aqueous fraction contained at least four metabolites, but no recognisable products were obtained when the fractions were either heated with 2N-HCl or incubated with sulphatase or with β-glucuronidase.

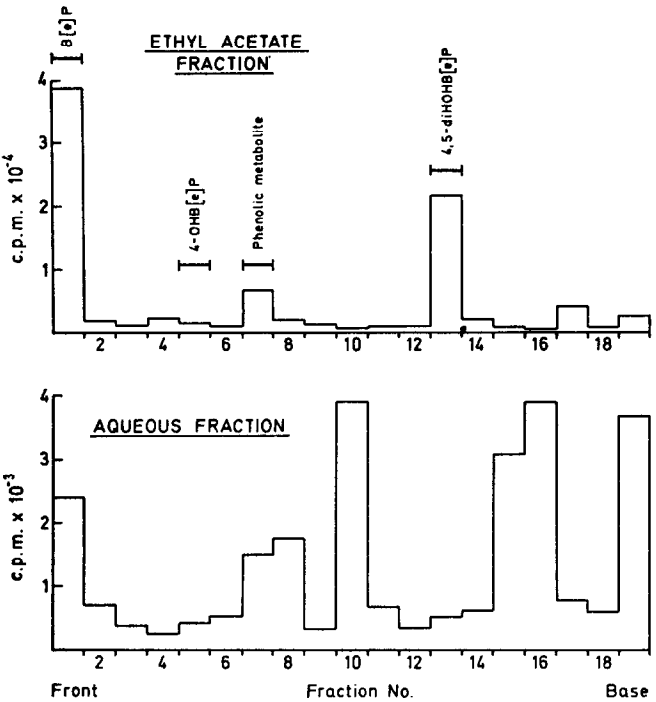

FIG. 9. The metabolism of benzo[e]pyrene in mouse embryo cells. The positions of metabolites on the chromatograms are shown thus: ——; abbreviations are as used in the text.

DISCUSSION

With both DMBA and 7-MBA, the ethyl acetate-soluble metabolites formed by embryo cells are similar to those formed by rat-liver homogenates.[1–4] The principal routes (see Fig. 1) involve either ring-hydroxylation or hydroxylation of the methyl groups, the latter being followed by further oxidation of the hydroxymethyl compounds either to carboxylic acids or to ring-hydroxylated products. Ring-hydroxylation occurs mainly in the 8,9-positions, with both the parent hydrocarbons and the hydroxymethyl derivatives to yield dihydrodiols. The absence of 7-OHM-12-MBA and 12-OHM-7-MBA in the medium from cells treated with DMBA may be because these products are further metabolized under the conditions used. The presence of small amounts of the hydroxymethyl derivatives has been demonstrated in the medium from secondary hamster embryo cultures.[5] Although phenols derived both from the hydrocarbons and the hydroxymethyl derivatives have been found as metabolites of the hydrocarbons in rat-liver homogenates,[1, 2, 4] their presence was not demonstrated in the work now described. In liver homogenates, however, they represent only small proportions of the metabolites formed,[3] so that they may have escaped detection. The 8,9-dihydrodiols form the largest proportions of metabolites present in the media from cells treated with both DMBA and 7-MBA: in this respect the pattern of ethyl acetate-soluble products formed resembles those formed when liver homogenates from animals pretreated with enzyme inducers are used.[2] The hydroxymethyl deri-

vatives are probably intermediates which are further metabolized to their 8,9-dihydrodiols both in activated liver preparations and with embryo cells: the cells were incubated for longer periods of time than were the liver homogenates. It is also possible that the hydrocarbons themselves induce drug metabolising enzymes in the cells during the incubations: it has been shown[8] that benz[a]anthracene and 3-methylcholanthrene will induce 'benzpyrene hydroxylase' in embryo cell cultures.

The major ethyl acetate-soluble metabolites of B[a]P and B[e]P formed by mouse embryo cells or from rat-liver homogenates are similar. The identity of the phenolic metabolite of B[e]P is not certain: analogy with pyrene[16] and B[a]P[6] metabolism suggests that it may be the 3-hydroxy compound. The metabolic reactions of B[a]P and B[e]P are shown in Fig. 2 and 3 respectively.

The absence of the 'K-region' dihydrodiols in the metabolic products of 7-MBA, DMBA and B[a]P (which are powerful carcinogens) and the presence of the K-region dihydrodiol, 4,5-DiHOHB[e]P, as the major metabolite in the metabolism of B[e]P (which is not a carcinogen) is of interest. The bonds of the K-regions are those with the highest double bond characters in the molecule and might be expected to undergo addition reactions more readily than the other bonds. It is probable that the dihydrodiols arise from the enzymic addition of water to epoxides, which themselves arise by the enzymic addition of oxygen to aromatic bonds.[17] It is difficult to understand why the K-region bonds of B[e]P readily undergo these reactions whilst the K-region bonds of the other hydrocarbons are inactive. In a rat-liver microsomal system, DMBA and B[a]P yield active intermediates that react both with protein and DNA.[18] It is possible, therefore, that the absence of K-region metabolites with these hydrocarbons in the present work is due to the reaction of the active intermediates (which may be epoxides) with cellular macromolecules: protein in the case of the liver homogenates and protein, RNA and DNA with the cells in culture.

Embryonic rodent cells, which are susceptible to the toxicity of DMBA, exhibit a higher level of binding to cell constituents such as DNA than do transformed rodent cells, which are resistant to the action of the hydrocarbon.[19] If the binding to DNA involves the formation of active metabolic intermediates then resistant cells should exhibit a lower metabolic activity than sensitive cells. With DMBA, the conversion of the hydrocarbon to water-soluble products is less in resistant than in sensitive cells.[5] In the present work, human melanoma cells and rat hepatoma cells were devoid of metabolising activity towards DMBA. A similar result has been found with benzo[a]pyrene:[20] the hydrocarbon is metabolised by normal rodent fibroblast cultures but not by cultures of tumour fibroblasts.

The absence of detectable amounts of glutathione, cysteine or *N*-acetylcysteine conjugates in the cell media is of interest. Glutathione conjugates have been detected, although with some difficulty, in the aqueous phases from rat-liver homogenates incubated with aromatic hydrocarbons.[13, 21] Conjugates of these types might also be expected in the incubation of 7-bromomethylbenz[a]anthracene with embryo cells: possibly the chemical reaction of the bromomethyl group with water in the medium is too fast to allow the bromomethyl compound to react with SH-groups within the cells. In general, the tritium counted in the aqueous phases was less by a factor of 10 than that counted in the ethyl acetate: if it is assumed that the recoveries and counting efficiencies are the same for both fractions then the unidentified water-soluble products are comparatively minor metabolites.

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