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The Metabolism of *cis*- and *trans*-Indane-1,2-diol

By D. A. LEWIS*
Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E. 1

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1. The metabolism of *cis*-indane-1,2-diol, *trans*-indane-1,2-diol, indene epoxide and 2-hydroxyindan-1-one in rats has been studied. The substances were administered to the animals by subcutaneous injection. 2. The urine of the dosed animals was examined for the presence of free and conjugated *cis*- and *trans*-dihydrodiols, and for each compound it was possible to isolate both *cis* and *trans* forms of indane-1,2-diol from the urine. 3. The urines were also examined by paper chromatography for ketones and two ketonic metabolites were detected in the urine of rats dosed separately with *cis*-indane-1,2-diol, *trans*-indane-1,2-diol, 2-hydroxyindan-1-one and indene epoxide. The ketones were provisionally identified as (1-oxoindan-2-yl glucosid)uronic acid and 1-oxoindan-2-yl sulphuric acid. 4. (1-Oxoindan-2-yl glucosid)uronic acid was isolated as the 2,4-dinitrophenylhydrazone from the urine of rats dosed separately with *cis*-indane-1,2-diol and *trans*-indane-1,2-diol. 5. Possible mechanisms for the interconversion of *cis*- and *trans*-indane-1,2-diol are discussed.

When cyclic hydrocarbons are administered to animals, hydroxylated metabolites such as phenols and dihydrodiols are often found in the urine in the free form or conjugated with glucuronic acid or sulphuric acid. The formation of dihydrodiols is of particular interest as these compounds may act as intermediates in the formation of phenols and ring-fission products of the parent hydrocarbons. Most of the dihydrodiols isolated from the urine of animals to which cyclic hydrocarbons have been administered have been shown to have the *trans* configuration, but two cyclic hydrocarbons, namely indene (Brooks & Young, 1956) and acenaphthylene (Hopkins, Brooks & Young, 1962), are metabolized to both *cis*- and *trans*-dihydrodiols. Hopkins, Lewis & Young (1964) in a preliminary communication reported that after the separate administration of *cis* or *trans* forms of acenaphthene-1,2-diol or indane-1,2-diol to rats it was possible to detect both *cis* and *trans* forms of the dihydrodiol in the urine. The work described below is concerned with the metabolism of *cis*- and *trans*-indane-1,2-diol and an examination of the possibility that metabolic interconversion of *cis* and *trans* forms of the dihydrodiol occurs.

MATERIALS

All melting points are uncorrected. Elementary microanalyses were carried out by Weiler and Strauss, Oxford.

* Present address: Department of Physiological Chemistry, University of Birmingham.

Indene epoxide. This compound (m.p. 31–32°) was prepared by the modification of the procedure of Whitmore & Gebhart (1942) described by Brooks & Young (1956).

cis- *and* trans-*Indane-1,2-diol.* *cis*- and *trans*-Indane-1,2-diol were prepared by the method described by Brooks & Young (1956). *trans*-Indane-1,2-diol was also prepared by the reaction of Prévost (1933*a*,*b*). To a suspension of 101g. of dry silver benzoate (i.e. 10%, w/v, excess of 2mol.prop.) in 800ml. of boiling benzene (previously dried over anhydrous $CaCl_2$ and redistilled) were added 23·2g. of indene (1mol.prop.) and 50·8g. of iodine (2mol.prop.). The mixture was shielded from direct light by dark paper and refluxed for 8hr. The mixture was then cooled and the precipitated AgI was removed by centrifuging. The residual benzene solution was washed with water and then successively with solutions of 0·02M-$Na_2S_2O_3$ and 0·02M-Na_2CO_3. The aqueous washings were each extracted once with benzene and the benzene washings were added to the original benzene solution. Anhydrous Na_2SO_4 was added to the solution, which was kept overnight. After filtration the benzene was removed by evaporation under reduced pressure. A pale-yellow oil was obtained that after drying over P_2O_5 *in vacuo* crystallized as a cream-coloured solid. The solid was dissolved in boiling methanol and treated with charcoal. The filtrate was allowed to cool and white crystals of *trans*-indane-1,2-dibenzoate were obtained. The compound had m.p. 77–78°, unchanged by admixture with *trans*-indane-1,2-dibenzoate, prepared by treating *trans*-indane-1,2-diol with benzoyl chloride in pyridine at room temperature (Brooks & Young, 1956) (Found: C, 77·3; H, 5·1. Calc. for $C_{23}H_{18}O_4$: C, 77·1; H, 5·1%).

The dibenzoate was converted into *trans*-indane-1,2-diol by hydrolysis with alkali. *trans*-Indane-1,2-dibenzoate (21g.) was dissolved in dioxan (50ml.) and 25ml. of methanol was added to the solution. The solution was transferred to a dark stoppered bottle and 1ml. (2mol.prop.)

of 60% (w/v) KOH was added, in small portions, over a period of 20min. The bottle was shaken continuously between additions of KOH. The solution was kept overnight at room temperature and was then neutralized with acetic acid. The mixture was extracted exhaustively with ether and the combined ether extracts were dried overnight by the addition of anhydrous Na_2SO_4. After filtration the ether was evaporated off under reduced pressure, and the white crystalline residue obtained was recrystallized from boiling $CHCl_3$. The *trans*-indane-1,2-diol had m.p.158–159°; the mixed m.p. with *trans*-indane-1,2-diol prepared from indene epoxide was 158–159° (Found: C, 72·4; H, 6·8; Calc. for $C_9H_{10}O_2$: C, 72·0; H, 6·7%). The infrared spectrum of the compound (0·5% in a KBr disk) was indistinguishable from that of *trans*-indane-1,2-diol prepared by the method of Brooks & Young (1956). About 50% yields of *trans*-indane-1,2-diol from indene were obtained by this method, compared with 13% obtained by the method used by Brooks & Young (1956).

Potassium trans-*hydroxyindanyl sulphate. trans*-Indane-1,2-diol (1·5g.) (1mol.prop.) was dissolved in the minimum volume of dry pyridine and 0·2g. of anhydrous $CaSO_4$ was added. The mixture was cooled to 0° and 1·17g. (1mol.-prop.) of cholorosulphonic acid diluted with 9ml. of dry $CHCl_3$ was added, with constant stirring, to the mixture over a period of 30min. The mixture was then kept for 1hr. at 0° to allow the reaction to go to completion. Dry ether (400ml.) was added with stirring and the mixture was kept overnight at 0°. Ammonia was passed through the solution for 10min. and the precipitated $(NH_4)_2SO_4$ and $CaSO_4$ were filtered off at the pump. A solution of potassium acetate (1·08g.; 10% excess of 1mol.prop.) in methanol (15ml.) was added to the filtrate, followed by an equal volume of ether. After standing overnight at 0° the precipitate was filtered off. The salt at this stage was found to be free from inorganic sulphate but contaminated with chloride. This was removed by partition chromatography. The impure salt was applied to a cellulose column and the compound was eluted with butan-1-ol–cyclohexane–2N-NH_3 (9:2:1, by vol.) (Boyland & Sims, 1962). Fractions (10ml.) were collected and examined for the presence of ethereal sulphate by a modification of the method of Burma (1953). A drop of the eluate from each fraction was spotted on to filter paper and the spots were allowed to dry. The filter paper was kept for 5min. in an atmosphere of HCl and it was then sprayed with 1% (w/v) $BaCl_2$ solution, followed by aq. 1% (w/v) sodium rhodizonate. The presence of sulphate was revealed as white spots on a red background. Ester sulphate was detected in fractions 16–24, and these fractions were combined and evaporated to a small volume under reduced pressure. White needles of the potassium salt were precipitated. The crystals were filtered off and rinsed on the filter with a few millilitres of ether. The potassium hydroxyindanyl sulphate was recrystallized from methanol. The compound had m.p.193–195° (decomp.) (Found: C, 40·9; H, 3·6; S, 12·1. $C_9H_9KO_5S$ requires C, 40·3; H, 3·4; S, 11·9%). The product when examined by paper chromatography (see Table 3) behaved as a single compound. The compound was obtained in 37% yield.

Attempts to prepare potassium 2-hydroxyindan-1-yl sulphate from 2-bromoindan-1-ol and 2-aminoindan-1-ol were not successful, and it was not possible to characterize fully the compound prepared from *trans*-indane-1,2-diol.

Potassium cis-*hydroxyindan*-1-*yl sulphate*. This compound was prepared from *cis*-indane-1,2-diol and chlorosulphonic acid by the method described for the preparation of potassium *trans*-hydroxyindanyl sulphate. The compound had m.p.174° (decomp.). The compound behaved as a single substance when examined by paper chromatography (see Table 3). The compound was obtained in low yield (10%).

2-*Hydroxyindan*-1-*one*. The preparation of this compound from 2-bromoindan-1-ol has been described by Ishiwara (1924). The compound (m.p.40–41°) has low stability, but may be stored satisfactorily for 3–4 weeks in the dark at −20°. The 2,4-dinitrophenylhydrazone was prepared, m.p.230° (decomp.) (Found: C, 54·6; H, 3·5; N, 16·4. Calc. for $C_{15}H_{12}N_4O_5$: C, 54·9; H, 3·6; N, 17·1%).

Indan-2-*one*. This compound (m.p.58–60°) was prepared from *cis*- or *trans*-indane-1,2-diol by the method described by Brooks & Young (1956). The 2,4-dinitrophenylhydrazone was prepared and obtained as light-orange needles, m.p.204–205° (decomp.) (Suter & Milne, 1940) (Found: C, 57·6; H, 4·3; N, 17·5. Calc. for $C_{15}H_{12}N_4O_4$: C, 57·7; H, 3·9; N, 18·0%).

METHODS

Animals and dosing

Male rats (body wt. 150–200g.) were used. They were housed in metabolism cages designed to permit the collection of urine separate from the faeces. The rats were given a diet of rat cake [J. Murray and Sons (London) Ltd., London, S.E. 8] and they had access to water at all times. *cis*- and *trans*-Indane-1,2-diol were administered to the animals as a 40% (w/v) suspension in arachis oil by subcutaneous injection in the lumbar region. For paper-chromatographic experiments a single injection of 1ml. of the suspension was administered to individual rats. For isolation purposes the rats were each given a second injection of 1ml. of the suspension 24hr. after the first. Urine was collected daily during the period of dosing and for 3 days after the final injection. The urines were stored at −20°.

Chromatography

Paper chromatography. Paper chromatograms were developed at room temperature on Whatman no. 1 paper by the ascending or descending technique. Separation of dihydrodiols was achieved with the following solvent systems: A, benzene–water–aq. NH_3 (sp.gr.0·88) (5:4:1, by vol.); B, benzene–water–ethanol (5:4:1, by vol.); C, benzene–water–ethyl acetate–acetic acid (4:4:1:1, by vol.). Separation of ketones was achieved with the following solvents: D, pyridine–ethanol–water (1:2:2, by vol.); E, butan-1-ol–aq. NH_3 (sp.gr.0·88)–ethanol–water (40:1:10:49, by vol.); F, butan-1-ol–acetic acid–water (31:6:13, by vol.); G, butan-2-one saturated with water; H, benzene–butan-1-ol–pyridine–water (1:5:3:3, by vol.). Separation of ester sulphates was achieved with the following solvent systems: I, butan-1-ol saturated with 2N-NH_3; J, 3N-NH_3–3N-$(NH_4)_2CO_3$–butan-1-ol (3:3:4, by vol.); K, 3N-NH_3–3N-$(NH_4)_2CO_3$–propan-2-ol (3:3:4, by vol.). Where two-phase solvent systems were used the upper layer was used as the mobile phase and the lower layer as the stationary phase. Dihydrodiols were detected by spraying either with 2% (w/v) $NaIO_4$ followed by Schiff's reagent (Brooks & Young, 1956) or with ethanol–conc. H_2SO_4–water (18:1:1,

by vol.) (Hopkins *et al.* 1962), followed by heating the paper at 100° for 1 min. Ketones were detected by spraying the dried paper with a saturated solution of 2,4-dinitrophenylhydrazine in 2N-HCl. Ester sulphates were detected by the modified procedure of Burma (1953). Glucuronides were detected by spraying with a 2% (w/v) solution of naphtharesorcinol in 20% (w/v) trichloroacetic acid, followed by heating the paper at 100° until blue spots appeared on a pink background.

Radiochromatography. The radioactive compounds on paper chromatograms were located by automatic scanning with the equipment and procedure described by Morrison & Young (1959).

Isolation procedures

cis- *and* trans-*Indane*-1,2-diol. The frozen urine was allowed to thaw at room temperature and filtered through glass wool if necessary. The urine at pH 7–8 was extracted by mechanically shaking the urine with an equal portion of peroxide-free ether for 30 min. Five successive extractions were carried out. The ether extracts were pooled and the ether was evaporated off under reduced pressure. The residue was dissolved in a few millilitres of N-NaOH and the alkaline solution was exhaustively extracted with ether. The ether extracts were again pooled and dried over anhydrous Na_2SO_4. The filtrate was evaporated to dryness under reduced pressure. The crystalline residue was extracted repeatedly with small portions of boiling cyclohexane, which on cooling deposited white needles of *cis*-indane-1,2-diol. The residue left after the cyclohexane extraction was extracted with portions of boiling $CHCl_3$, which on cooling deposited white needles of *trans*-indane-1,2-diol. The *cis*-dihydrodiol was recrystallized from cyclohexane and the *trans*-dihydrodiol from $CHCl_3$.

(1-*Oxoindan*-2-*yl glucosid*)*uronic acid.* The frozen urine was allowed to thaw and filtered through glass wool if necessary. The urine was adjusted to pH 4·5 and percolated through a column of activated charcoal–Celite 535 (Johns–Manville Co., New York, N.Y., U.S.A.) (1:1, w/w; 20 g. of the mixture was used for each 10 ml. of urine). The column was washed through with water until the washings gave negative results when tested for inorganic sulphate and chloride. The column was developed with methanol–aq. NH_3 (sp.gr. 0·88) (19:1, v/v) and 25 ml. fractions were collected. The presence of (1-oxoindanyl glucosid)uronic acid in the fractions was determined by spotting small samples of each fraction on to filter paper and spraying the dried paper with the 2,4-dinitrophenylhydrazine reagent. The compound was detected as a characteristic deep-red spot on a yellow background. The presence of the compound in the fractions was confirmed by spraying a second filter sheet spotted with small samples from each fraction with the naphtharesorcinol reagent. The fractions giving positive tests for both glucosiduronic acid and ketone were pooled and the solvent was evaporated off under reduced pressure. The residue, usually a brown gum, was dissolved in a little water (10 ml.) and an equal volume of 2,4-dinitrophenylhydrazine dissolved in ethanolic H_2SO_4 (Books & Young, 1956) was added. The reaction mixture was kept overnight at 0° and the red precipitate that formed was filtered off. The substance was recrystallized from aq. ethanol. The (oxoindanyl glucosid)uronic acids isolated from urine as the 2,4-dinitrophenylhydrazones were rather unstable and were stored at 0° over P_2O_5.

Potassium hydroxyindanyl sulphate. The frozen urine was allowed to thaw and the pH was adjusted to 4·5. Activated charcoal (the charcoal was preheated to a dull red and allowed to cool in a desiccator over P_2O_5) was added to the urine (10 g. of charcoal for 100 ml. of urine), the urine being stirred continually during the addition. The mixture was kept for 1 hr. and the charcoal filtered off at the pump. The charcoal was well washed on the filter with water and was then extracted exhaustively with portions of methanol–aq. NH_3 (sp.gr. 0·88) (19:1, v/v). The ammoniacal-methanol extracts were pooled and evaporated to dryness under reduced pressure. The residual gum was dissolved in a few millilitres of methanol and applied to a cellulose column. The column was eluted with butan-1-ol–cyclohexane–2N-NH_3 (9:2:1, by vol.) and 15 ml. fractions were collected. The presence of sulphate ester in the fractions was determined by the modified Burma reagent test. The fractions containing the sulphate ester were pooled and the solvent was evaporated off under reduced pressure. The residual gum was dissolved in a little methanol and ethanol was added until precipitation ceased. The white precipitate was removed by centrifuging and the ethanol was removed by evaporation under reduced pressure. The white precipitate gave a strong naphtharesorcinol test but a negative reaction when tested for ketones with the 2,4-dinitrophenylhydrazine reagent. The substance was probably a (hydroxyindanyl glucosid)uronic acid. The ethanolic precipitation procedure was repeated until no further precipitation occurred. The residual gum obtained by the removal of the ethanol was dissolved in the minimum volume of methanol, and 0·5 g. of potassium acetate dissolved in a minimum volume of methanol followed by 1 ml. of ether added. The mixture was kept overnight at 0° and the precipitated potassium salt was filtered off. The salt was recrystallized from methanol.

Enzyme experiments

The urine of animals dosed separately with *cis*- and *trans*-indane-1,2-diol was extracted with ether to remove free dihydrodiols and was then treated either with a crude rat-liver β-glucuronidase preparation (Corner, Billett & Young, 1954) or a β-glucuronidase–arylsulphatase preparation of bacterial origin [Boehringer Corp. (London) Ltd., London, W. 5]. The method of enzymic hydrolysis was that described by Hopkins *et al.* (1962). After the enzymic treatment the urine was centrifuged to remove solid matter and adjusted to pH 7–8. The urine was exhaustively extracted with ether and the pooled ether extracts were evaporated to dryness under reduced pressure. The residue was examined for the presence of dihydrodiols and hydroxyindanones by the methods described above.

Determination of the purity of the synthetic cis- *and* trans-*indane*-1,2-*diol*

The purity of the *cis* and *trans* isomers was determined by paper chromatography. By using the solvent systems and the ethanolic-H_2SO_4 reagent described above it was possible to detect 5 μg. of one isomer in the presence of 1 mg. of the other.

Infrared spectroscopy. Infrared spectra were measured with the Perkin–Elmer model 237 or the Unicam SP. 200

spectrophotometer. Compounds were examined in KBr disks.

RESULTS

Paper chromatography

Detection of cis- *and* trans-*indane*-1,2-*diol*. Both *cis*- and *trans*-indane-1,2-diol were detected in the urine or ether extracts of the urine of rats dosed separately with *cis*-indane-1,2-diol, *trans*-indane-1,2-diol, indene epoxide and 2-hydroxyindan-1-one (see Table 1).

Detection of (1-*oxoindan*-2-*yl glucosid*)*uronic acid*. Urine from rats dosed separately with arachis oil, *cis*-indane-1,2-diol, *trans*-indane-1,2-diol, indene epoxide and 2-hydroxyindan-1-one was examined for ketone metabolites. The results (see Table 2) show that two ketone metabolites were excreted in the urine of the animals to which the four indane derivatives had been administered. One ketone gave a strong permanent reaction with the 2,4-dinitrophenylhydrazine reagent and the other a weak reaction with the red colour of the spot rapidly darkening to brown, indicating a rapid

Table 1. *Excretion of* cis- *and* trans-*indane*-1,2-*diol*

The urine of the dosed rats, or an ether extract of it, was examined for dihydrodiols by paper chromatography. The descending method was used. The detecting reagents and solvent systems are described in the text.

		R_F value					
		Solvent A		Solvent B		Solvent C	
Substance given	Dihydrodiol detected	Biosynthetic	Synthetic	Biosynthetic	Synthetic	Biosynthetic	Synthetic
trans-Indane-1,2-diol	*cis*	0·29	0·30	0·40	0·40	0·60	0·62
	trans	0·07	0·07	0·10	0·11	0·40	0·41
cis-Indane-1,2-diol	*cis*	0·28	0·28	0·39	0·40	0·62	0·65
	trans	0·04	0·05	0·15	0·15	0·41	0·43
Indene epoxide	*cis*	0·30	0·31	0·40	0·41	0·60	0·61
	trans	0·07	0·05	0·12	0·15	0·38	0·40
2-Hydroxyindan-1-one	*cis*	0·28	0·28	0·42	0·40	0·62	0·62
	trans	0·05	0·04	0·12	0·13	0·04	0·04

Table 2. *Excretion of ketols*

Expt. A. The urine of the dosed rats was examined for ketols by paper chromatography. The intensity of the reaction between the ketol and the detecting reagent is indicated as either s (strong) or w (weak). The descending method was used. The detecting reagent and solvent systems are described in the text. None of the ketols was detected when arachis oil only was given. Expt. B. Portions of the urines examined by paper chromatography for ketols (Expt. A) were incubated for 24hr. with β-glucuronidase (for method see the text). The urines, or ether extracts of the urines, were then re-examined for ketols by paper chromatography. It was found that the ketols originally present in the untreated urine had either disappeared or were present in much lower concentration. A new ketol was detected and its chromatographic behaviour compared with that of 2-hydroxyindan-1-one.

	R_F values of metabolites				
Substance given	Solvent D	Solvent E	Solvent F	Solvent G	Solvent H
Expt. A (before treatment with β-glucuronidase)					
trans-Indane-1,2-diol	0·53 (s)	0·08 (s)	0·31 (s)	0·16 (s)	0·52 (s)
	0·15 (w)	0·43 (w)	0·26 (w)	0·02 (w)	0·15 (w)
cis-Indane-1,2-diol	0·53 (s)	0·08 (s)	0·31 (s)	0·18 (s)	0·52 (s)
	0·15 (w)	0·43 (w)	0·26 (w)	0·01 (w)	0·13 (w)
Indene epoxide	Not tested	Not tested	0·30 (s)	Not tested	Not tested
			0·22 (w)		
2-Hydroxyindan-1-one	0·52 (s)	0·08 (s)	0·31 (s)	0·21 (s)	0·52 (s)
	0·15 (w)	0·44 (w)	0·22 (w)	0·01 (w)	0·15 (w)
Expt. B (after treatment with β-glucuronidase)					
trans-Indane-1,2-diol	0·86	0·88	0·88	0·96	0·87
cis-Indane-1,2-diol	0·87	0·89	0·90	0·95	0·86
Indene epoxide	Not tested	Not tested	0·90	Not tested	Not tested
2-Hydroxyindan-1-one	0·87	0·90	0·90	0·96	0·88
2-Hydroxyindan-1-one (reference substance)	0·87	0·88	0·90	0·96	0·88

decomposition of the substance on the paper. The ketone giving a strong reaction with the 2,4-dinitrophenylhydrazine reagent also gave a positive reaction with the naphtharesorcinol reagent when a second chromatogram developed in an identical solvent system was sprayed with this reagent. The ketone giving a weak reaction followed by decomposition did not give a positive reaction with the naphtharesorcinol reagent but gave a positive reaction for sulphate when sprayed with the Burma reagent after pretreatment with hydrochloric acid and barium chloride. The evidence suggests that the ketone giving a weak reaction was a sulphuric acid conjugate. No free 2-hydroxyindan-1-one was detected in the urines. Paper chromatography of ethereal extracts of the urines described above failed to detect the presence of ketone metabolites in the urine. Portions of the urines were buffered at pH 6·4 and incubated for 24hr. with a bacterial β-glucuronidase–arylsulphatase preparation. The samples were then examined by paper chromatography for ketone metabolites. The results (see Table 2) show that pretreatment of the experimental urines with β-glucuronidase–arylsulphatase resulted in a change in the chromatographic pattern of metabolites. A new ether-soluble metabolite with paper-chromatographic behaviour identical with that of synthetic 2-hydroxyindan-1-one was present. At the same time the ketonic metabolites in the untreated urines were either absent or present only in much lower concentrations.

Detection of hydroxyindanyl sulphates in urine. The urines from rats dosed separately with *cis*- and *trans*-indane-1,2-diol were examined for the presence of ester sulphate (Table 3). The presence of ester sulphate was detected by the Burma reagent in both urines. The sulphates did not react with a phenolic detecting reagent consisting of 1% (w/v) ferric chloride and 1% (w/v) potassium ferricyanide (Barton, Evans & Gardner, 1952) after preliminary hydrolysis on the paper by hydrochloric acid.

Radiochromatography

Urines from pairs of rats dosed by subcutaneous injection in the right lumbar region with (*a*) 150mg. of *trans*-indane-1,2-diol suspended in 1ml. of arachis oil, followed immediately by a second injection in the left lumbar region of aq. $Na_2{}^{35}SO_4$, (*b*) 150mg. of *cis*-indane-1,2-diol suspended in 1ml. of arachis oil, followed immediately by an injection of $Na_2{}^{35}SO_4$, and (*c*) 1ml. of arachis oil, followed immediately by an injection of $Na_2{}^{35}SO_4$, were examined by paper chromatography. The papers were scanned by the apparatus described by Morrison & Young (1959).

In addition, equal portions of urines from the rat pairs dosed as in (*a*) and (*b*) were mixed and examined. Two major radioactive components were found in the urines from rat pairs (*a*) and (*b*), and these were absent from the control urine from rat pair (*c*). The spots were not of equal density, the component with R_F 0·42 (solvent I) being the stronger in urine (*a*) and the component with R_F 0·40 being the stronger in urine (*b*). Examination of a mixed sample of the two urines from the rat pairs (*a*) and (*b*) and the mixed sample were prepared and the results confirmed the presence of two identical pairs of radioactive metabolites in each experimental urine. Similar paper-chromatographic results were obtained with solvent system J.

Metabolism of cis-*indane*-1,2-*diol*

Isolation of cis- *and* trans-*indane*-1,2-*diol.* In one experiment ten rats were each dosed with 150mg. of *cis*-indane-1,2-diol, the purity of the dihydrodiol having been shown by paper chromatography to be at least 99%. The dose was repeated after 24hr. The urine was extracted with ether and 47mg. of *trans*-indane-1,2-diol was isolated. The dihydrodiol had m.p. 154–157°, unchanged by admixture with synthetic *trans*-indane-1,2-diol, $[\alpha]^{21}+5°$ (*c* 1·0 in ethanol) (Found: C, 72·1; H,

Table 3. *Excretion of hydroxyindanyl sulphates*

The substances were either isolated as the potassium salts or tested for directly in experimental urines by paper chromatography. The detecting reagents and solvent systems are described in the text. The ascending method was used.

	R_F values					
	Solvent I		Solvent J		Solvent K	
Indane-1,2-diol given..... Compound	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*
Biosynthetic sulphate	0·41	0·40	0·39	0·40	0·88	0·90
Synthetic potassium *cis*-hydroxyindanyl sulphate	0·40	0·40	0·40	0·40	0·90	0·90
Synthetic potassium *trans*-hydroxyindanyl sulphate	0·42	0·42	0·40	0·40	0·90	0·89

6·6. Calc. for $C_9H_{10}O_2$: C, 72·0; H, 6·7%). The dihydrodiol was chromatographically identical with the synthetic compound in solvent systems A, B and C. The rate of reaction of the isolated dihydrodiol and the synthetic dihydrodiol with lead tetra-acetate was also determined. The isolated compound reacted at the same rate as the synthetic compound (K_{19} 0·4441./mole/min.); Criegee, Kraft & Rank (1933) recorded K_{20} 0·4671./mole/min. for *trans*-indane-1,2-diol.

In addition to the *trans* isomer, 241mg. of optically inactive *cis*-indane-1,2-diol was isolated, m.p. 96–98°, unchanged by admixture with synthetic *cis*-indane-1,2-diol (Found: C, 71·5; H, 6·6. Calc. for $C_9H_{10}O_2$: C, 72·0; H, 6·7%). The compound was chromatographically identical with synthetic *cis*-indane-1,2-diol in solvent systems A, B and C. The residual urine after the initial ether extraction was treated with a rat-liver β-glucuronidase preparation. From this urine a further batch of *trans*-indane-1,2-diol crystals (26mg.) was obtained, m.p. 151–155° and mixed m.p. 153–155° with synthetic *trans*-indane-1,2-diol. *cis*-Indane-1,2-diol (34mg.), m.p. 93–96° and mixed m.p. 94–96° with synthetic *cis*-indane-1,2-diol, was also isolated from the urine. The amounts of *trans*-indane-1,2-diol isolated represent a total interconversion of 2·5% of the *cis* isomer.

Isolation of (1-oxoindan-2-yl glucosid)uronic acid. Fifteen rats were each dosed with 200mg. of *cis*-indane-1,2-diol, followed by a second equal dose 24hr. later. (1-Oxoindan-2-yl glucosid)uronic acid was isolated as the 2,4-dinitrophenylhydrazone. The derivative was crystallized from boiling ethanol and 112mg. of small red needles was obtained, m.p. 210–211° (decomp.) (Found: C, 49·4; H, 4·3; N, 11·2. $C_{21}H_{20}N_4O_{11}$ requires C, 50·0; H, 4·0; N, 11·2%). The yield represents 0·6% of the dihydrodiol administered. The evidence for the proposed structure of this compound is presented below.

Separation from urine of potassium hydroxyindanyl sulphates detected by radiochromatography. Ten rats were each dosed with 200mg. of *cis*-indane-1,2-diol suspended in arachis oil (1ml.). A second equal dose was administered 24hr. later. The urine was examined for the sulphate esters by paper chromatography. The sulphates were separated as the potassium salts (47mg.). The infrared spectrum of the salts was unlike that of either the synthetic potassium *cis*- or *trans*-hydroxyindanyl sulphate. This was consistent with the chromatographic evidence that a sulphate mixture was isolated from the urine, rather than a single compound. The presence of carbonyl absorption at 1730cm.$^{-1}$ was unexpected. To confirm the presence of this group 100mg. of the biosynthetic sulphate mixture obtained after dosing with *cis*-indane-1,2-diol was dissolved in water and an excess of the 2,4-dinitrophenylhydrazine reagent added. A red precipitate formed immediately. The precipitate was filtered off and dried in a vacuum desiccator. The precipitate decomposed rapidly, the deep-red colour darkening visually to a deep brown. The solid was dried *in vacuo* over phosphorus pentoxide at room temperature and 8mg. obtained. This compound was probably a potassium oxoindanyl sulphate. Evidence for the presence of this compound in the experimental urines has been described in the paper-chromatographic experiments. Attempts to separate the components of the sulphate mixture by partition chromatography on cellulose and Celite 535 columns were not successful.

Metabolism of trans-*indane*-1,2-*diol*

Isolation of cis- *and* trans-*indane*-1,2-*diol.* Ten rats were dosed with 150mg. of *trans*-indane-1,2-diol, the purity of the dihydrodiol having been shown by paper chromatography to be at least 99·5%. The dose was repeated after 24hr. The urine was extracted with ether and 152mg. of *cis*-indane-1,2-diol was isolated. The compound had m.p. 96–98°, unchanged by admixture with synthetic *cis*-indane-1,2-diol, $[\alpha]_D^{21}+41°$ (*c* 0·9 in chloroform) (Found: C, 71·5; H, 6·9. Calc. for $C_9H_{10}O_2$: C, 72·0; H, 6·7%). The biosynthetic compound also reacted with lead tetra-acetate at the same rate as the synthetic compound (K_{21} 30800l./mole/min.); Criegee *et al.* (1933) recorded K_{20} 27800l./mole/min. for *cis*-indane-1,2-diol. In addition to the *cis*-indane-1,2-diol, 264mg. of optically inactive *trans*-indane-1,2-diol was recovered from the urines. The compound had m.p. 156–159°, not depressed by admixture with synthetic *trans*-indane-1,2-diol (Found: C, 71·6; H, 6·8. Calc. for $C_9H_{10}O_2$: C, 72·0; H, 6·7%). The compound was chromatographically identical with *trans*-indane-1,2-diol. The residual urine from the initial ether extraction was incubated with a rat-liver β-glucuronidase preparation. A further 10mg. of *cis*-indane-1,2-diol was isolated from the urine by ether extraction, m.p. 92–97° and mixed m.p. 95–98° with a sample of synthetic *cis*-indane-1,2-diol. The biosynthetic diol was chromatographically identical with the synthetic compound (see Table 1). In addition, 98mg. of *trans*-indane-1,2-diol was also recovered from the urine, m.p. 155–159°, not depressed by admixture with synthetic *trans*-indane-1,2-diol, and the compound was also chromatographically identical with the synthetic compound. The dihydrodiols recovered from the urine represent a 5·4% conversion of the *trans* into the *cis* isomer and a 12·1% recovery of the original dose of *trans*-dihydrodiol.

Isolation of (1-*oxoindan*-2-*yl glucosid*)*uronic acid*. Fifteen rats were each dosed with 200mg. of *trans*-indane-1,2-diol and the dose was repeated after 24hr. The compound was isolated as the 2,4-dinitrophenylhydrazone (47mg.), m.p. 168–172° (decomp.). The compound, like the corresponding derivative isolated from the urine of rats dosed with *cis*-indane-1,2-diol, was rather unstable. The evidence for the proposed structure of this compound is given below.

Separation from urine of potassium hydroxyindanyl sulphates detected by radiochromatography. Ten rats were each dosed with 200mg. of *trans*-indane-1,2-diol and a second equal dose was administered 24hr. later. From rat urine 116mg. of the mixture of impure potassium hydroxyindanyl sulphates was isolated and when this was recrystallized from methanol 73mg. of white platelets was obtained, m.p. 174–176° (decomp.), $[\alpha]_D^{21} + 39°$ (*c* 1·0 in water) (Found: C, 40·3; H, 3·5; S, 12·0. $C_9H_9KO_5S$ requires C, 40·3; H, 3·4; S, 11·9%). The infrared spectrum of this mixture was identical with that of the mixture of salts isolated from the urine of rats to which *cis*-indane-1,2-diol had been administered. This was consistent with the chromatographic evidence that both *cis*- and *trans*-dihydrodiols are metabolized to identical sulphuric acid conjugates. The presence of a small amount of a carbonyl compound in the mixture was confirmed by treatment of the mixture with the 2,4-dinitrophenylhydrazone reagent. Attempts to separate the individual sulphates from the mixture by partition chromatography on cellulose and Celite 535 columns were not successful.

Metabolism of 2-*hydroxyindan*-1-*one*

Isolation of cis- *and* trans-*indane*-1,2-diol. Twenty-four rats were each dosed with 100mg. of optically inactive 2-hydroxyindane-1-one and a second equal dose was administered 24hr. later. The urine was examined for dihydrodiols by ether extraction and 97mg. of *cis*-indane-1,2-diol was obtained, m.p. 98–99°, unchanged by admixture with synthetic *cis*-indane-1,2-diol. The biosynthetic dihydrodiol was chromatographically identical with the synthetic compound. In addition to the *cis*-isomer, 9mg. of the *trans*-dihydrodiol was obtained, m.p. 181–182°, $[\alpha]_D + 30°$ (*c* 0·56 in ethanol). The m.p. was not depressed by admixture with a sample of (+) *trans*-indane-1,2-diol (m.p. 185–186°) isolated by Brooks & Young (1956) from the urine of rats dosed with indene. The biosynthetic diol was chromatographically identical with synthetic *trans*-indane-1,2-diol in solvent systems A, B and C. The infrared spectra of biosynthetic and synthetic *trans*-indane-1,2-diol were identical. After incubation of the extracted urine with a rat-liver β-glucuronidase preparation a further 12mg. of *cis*-indane-1,2-diol (m.p. 95–97°) was isolated. No further *trans*-indane-1,2-diol could be obtained but the substance was detected in the urine by paper chromatography. The amount of dihydrodiols isolated represents a 2·3% yield of *cis*-indane-1,2-diol from 2-hydroxyindane-1-one.

Metabolism of indene epoxide

Eighteen rats were each dosed with 250mg. of indene epoxide, suspended in 1ml. of arachis oil, a second dose being administered 24hr. later. The urine (1050ml.) was divided into two portions. One portion (800ml.) was examined for dihydrodiols. *cis*-Indane-1,2-diol (139mg.) (m.p. 88–91°) and *trans*-indane-1,2-diol (11mg.) (m.p. 153–155°) were isolated from the urine. The identity of the biosynthetic dihydrodiols was confirmed by mixed m.p. and paper chromatography. The dihydrodiols isolated represented a yield of 1·8% of *cis*-indane-1,2-diol and 0·2% of *trans*-indane-1,2-diol. A portion (50ml.) of the extracted urine was acidified to 7% (v/v) sulphuric acid by the addition of 50% (v/v) sulphuric acid. The urine was refluxed for 1½hr. and was then steam-distilled. By this procedure indan-2-one was prepared from hydrolysed dihydrodiol conjugates. The indan-2-one was recovered from the distillate as the 2,4-dinitrophenylhydrazone by the method described by Brooks & Young (1956). The indan-2-one 2,4-dinitrophenylhydrazone (17mg.) had m.p. 204–205°, unchanged by admixture with the synthetic compound. In a control experiment indan-2-one was added to urine and recovered by steam-distillation as the 2,4-dinitrophenylhydrazone. A 93% yield was obtained. Assuming a 7% loss of indan-2-one from the experimental urine the recovery of dihydrodiol from the acid hydrolysis of conjugates was 2·0%.

Hydrolysis of indene epoxide in urine at pH 7·4. Indene epoxide (874mg.) was dispersed in rat urine buffered at pH 7·4 (0·1M-sodium phosphate). The urine was kept at room temperature for 3 days and was then exhaustively extracted with ether. The ether extracts were pooled and examined for dihydrodiols. *cis*-Indane-1,2-diol (128mg.) was isolated, m.p. 91–93°, mixed m.p. 93–95° with synthetic *cis*-indane-1,2-diol. *trans*-Indane-1,2-diol (24mg.) was also isolated, m.p. 143–146°, not depressed by admixture with synthetic compound. The identity of both dihydrodiols was confirmed by paper chromatography. The recovery of dihydrodiols represents a conversion of 12·9% of the epoxide into *cis*-isomer and 2·4% into the *trans*-isomer.

Hydrolysis of the 2,4-dinitrophenylhydrazones of (1-oxoindan-2-yl glucosid)uronic acids isolated from urine

Acid hydrolysis of the 2,4-dinitrophenylhydrazones separately isolated from the urines of rats dosed with *trans*-indane-1,2-diol and *cis*-indane-1,2-diol was carried out. A small sample (5mg.) of each of the 2,4-dinitrophenylhydrazones was hydrolysed with 6N-hydrochloric acid (0·5ml.) for 6hr. in a sealed tube at 100°. The hydrolysate was evaporated to dryness under reduced pressure and the residue was extracted with 2ml. of warm water. This extract was examined by paper chromatography for the presence of free glucuronic acid. This substance was found to be present, indicating that the substance isolated was a glucuronic acid conjugate.

Stability of the synthetic and biosynthetic sulphate esters

The experimental urines were collected for periods of 24hr. To examine the possibility that hydrolysis of sulphate conjugates might have occurred during the collection period their stability to hydrolysis was investigated. Quantities (80mg.) of synthetic potassium *cis*- and *trans*-hydroxyindanyl sulphate, potassium hydroxyindanyl sulphate isolated from the urine of rats dosed with *trans*-indane-1,2-diol and potassium hydroxyindanyl sulphate isolated from the urine of rats dosed with *cis*-indane-1,2-diol were dissolved separately in portions (20ml.) of normal urine and the total and inorganic sulphate contents of each urine were determined. The urines were kept for 24hr. and the total and inorganic sulphate contents were again determined (Fiske, 1921). A second experiment to test the stability of biosynthetic sulphate esters was performed. Each of a pair of rats was injected subcutaneously with 150mg. of *trans*-indane-1,2-diol suspended in 1ml. of arachis oil. The urine was collected 24hr. later and analysed for total and inorganic sulphate. The urine was then kept for 24hr. at room temperature and the analysis was repeated. Closely similar analytical results were obtained. The average values of inorganic sulphate (0·15mg. of S/24hr.) and total sulphate (6·8mg. of S/24hr.) show that most of the sulphate detected in the urine 24hr. after administration of the *trans*-dihydrodiol was in ester form. This suggests that it was extremely unlikely that breakdown of sulphate esters occurred in the rat bladder. The experiment was repeated with another pair of rats to which the *cis*-indane-1,2-diol had been administered and similar results were obtained.

Hydrolysis of potassium hydroxyindanyl sulphates

Portions (5mg.) of mixtures of biosynthetic potassium hydroxyindanyl sulphates isolated respectively from the urines of rats dosed with *cis*- or *trans*-indane-1,2-diol, and synthetic potassium *cis*- and *trans*-hydroxyindanyl sulphate, were each dissolved in 1ml. of water in a test tube. The test tubes were heated in a boiling-water bath for 3hr. and the cooled solutions were examined by paper chromatography for dihydrodiols. *cis*- and *trans*-Indane-1,2-diol were detected in each of the four solutions examined, the *cis*-dihydrodiol being present in larger quantity than the *trans*-dihydrodiol. The formation of a mixture of *cis*- and *trans*-indane-1,2-diol from the synthetic potassium hydroxyindanyl sulphates is of some interest. It suggests that carbon–oxygen fission occurred during hydrolysis rather than sulphur–oxygen fission. The same observation cannot be made for the hydrolysis of the biosynthetic material since the radiochromatography results had shown that the material isolated was a sulphate mixture.

DISCUSSION

Boyland (1950) has suggested that epoxides may serve as intermediates in the metabolic oxidation of cyclic hydrocarbons. Indene epoxide was administered to rats in the present work and *cis*- and *trans*-indane-1,2-diol were isolated from the urine. Indene epoxide is hydrolysed, however, in aqueous media to a mixture of *cis*- and *trans*-indane-1,2-diol. Boyland & Sims (1960) administered 1,2-epoxy-1,2,3,4-tetrahydronaphthalene to rabbits and obtained *trans*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene from the urines. This epoxide is rapidly hydrolysed by water to the *trans*-dihydrodiol only. The hydrolysis products of the epoxides are therefore consistent with their possible role as intermediates in the formation of dihydrodiols from the parent cyclic hydrocarbon. The direct metabolic formation of epoxides from cyclic hydrocarbons has not yet been demonstrated, but Davidow & Radomski (1953*a*,*b*) detected an epoxide of Heptachlor in the tissues and body fat of dogs that had been given the insecticide Heptachlor. The interconversion of *cis* and *trans* forms of indane-1,2-diol suggests the additional possibility that, where two isomeric forms of dihydrodiols are excreted in the urine as metabolites of a cyclic hydrocarbon, one form may have arisen from the other by a metabolic process. The detection of conjugated ketols as metabolites of dihydrodiols, and the demonstration that a synthetic ketol is metabolized by the rat to a mixture of the two hydrodiols, suggests that a

ketol may act as an intermediate in the interconversion of the two hydrodiol forms. This type of isomerization has been reported in the metabolic interconversion of steroid hormones. The conversion *in vivo* of 16α-hydroxyoestrone into oestriol (Brown & Marrian, 1957), 16-epioestriol and 17-epioestriol (Nocke, Breuer & Knuppen, 1961) has been demonstrated. The latter workers also demonstrated the conversion of 16-β-hydroxyoestrone into oestriol, 16-epioestriol and 16,17-epioestriol. Some interest attaches to the fact that the shape of the indene molecule is similar to that of the C and D rings of steroids. The presence of sulphuric acid and glucuronic acid conjugates in the urine of rats to which *cis*- and *trans*-indane-1,2-diol had been administered was detected in the present work. The ketol conjugates appear to be more stable than the parent ketol since the free compound was not detected in experimental urines, even after the administration of the compound to rats.

The direct interconversion of the indane-1,2-diols in the urine was shown to be unlikely by Brooks & Young (1956). The interconversion of these dihydrodiols is normally achieved only at low pH and elevated temperature (Hermans, 1924; Suter & Milne, 1940). The possibility does exist that some interconversion may take place by hydrolysis of a labile conjugate such as a sulphate. In the present work it was shown that it is unlikely that decomposition of sulphate ester occurred in the urine before or after conversion. The possibility remains that conjugates may act as metabolic intermediates in the interconversion of the indane-1,2-diols. The degree of optical homogeneity of the isolated dihydrodiols could not be determined, since the two indane-1,2-diols do not appear to have been resolved into their enantiomorphic pairs. Brooks & Young (1956) found a species difference between the rabbit and the rat. The *trans*-indane-1,2-diol isolated as a metabolite of indene in the rabbit was found to have m.p. varying between 155° and 160°, and $[\alpha]_D$ in ethanol varied between $-7°$ and $+5°$. The *trans*-dihydrodiol isolated as a metabolite of indene from the rat had m.p. 184–186°, $[\alpha]_D$ $+31°$ (in chloroform). It was suggested that this compound was (+)-*trans*-indane-1,2-diol. In the present work the *trans*-indane-1,2-diol isolated as a metabolite of *cis*-indane-1,2-diol was identical in properties with the *trans*-dihydrodiol isolated by Brooks & Young (1956) from rabbit urine as a metabolite of indene. This contrasts with the metabolic conversion of 2-hydroxyindan-1-one into the *trans*-dihydrodiol that appeared to be identical with (+)-*trans*-indane-1,2-diol isolated as a metabolite of indene from the urine of dosed rats. A possible explanation is that the dihydrodiols are formed by more than one metabolic pathway.

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