ova were 17–42, 0–28 and 0–6 % when inseminates containing 30,000–44,000 sperms had volumes of 0.1, 0.4 and 1.0 ml. respectively. In seeking an explanation for this effect, Chang (1946*b*) suggested that in the more dilute suspensions fewer sperms were able to enter the cervix uteri and so reach the Fallopian tubes. Though this may well be true it is probable that at least two other factors may apply. In the first place, the evidence presented in this paper shows that the sperms in the more dilute suspensions would contain less hyaluronidase, while in the second, it has been observed that the survival time of sperms in dilute suspensions in Baker's solution is greatly reduced. For example, whereas a suspension containing 10–20 millions/ml. may survive for 36 hr. or more at room temperature, the survival time of a suspension containing 1 million/ml. may be less than 1 hr. No explanation for this phenomenon has yet been found, but it has prevented the carrying out of an experiment which the previous results have obviously suggested, namely, the possibility of sperms whose hyaluronidase content has been depleted being made to reabsorb the enzyme on being suspended in a medium rich in the latter.

SUMMARY

1. Investigation of some of the factors concerned in the liberation of hyaluronidase from rabbit sper-

matozoa has shown that freezing for a short time or standing in aqueous suspension at 0° for 24 hr. or longer allows apparently maximal extraction of the enzyme. Freeze-drying may cause an insignificant loss of potency but the product remains stable for long periods.

2. At least three quarters of the total hyaluronidase content of semen is associated with the spermatozoa, the remainder being in solution in the plasma. In suspensions of live sperms in Baker's solution, the amount of hyaluronidase in solution is roughly proportional to the logarithm of the volume of suspension for a constant number of sperms. The presence of hyaluronidase in solution in the suspending medium reduces the amount of enzyme liberated by the sperms.

3. Spermatozoa do not appear actively to produce hyaluronidase; they merely liberate the preformed enzyme.

4. It is suggested that the mechanism which accounts for the relationship between volume of suspension and hyaluronidase liberated is sufficient to explain the release of enzyme in the Fallopian tubes necessary to bring about disintegration of the cumulus surrounding freshly ovulated ova.

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The Metabolic Conversion of Naphthalene to 1:2-Dihydronaphthalene-1:2-diol

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Studies of the metabolism of naphthalene (Bourne & Young, 1933*a, b*, 1934; Ing, Bourne & Young, 1934) and anthracene (Boyland & Levi, 1935, 1936*a, b*) have revealed certain similarities in the chemical changes which these hydrocarbons undergo in the animal body. Both are converted to mercapturic acids and both give rise to derivatives which yield the parent hydrocarbons when decomposed by acid. The

purpose of the present work was to determine whether the administration of naphthalene to the rat is followed by the excretion of a derivative analogous to 1:2-dihydroxy-1:2-dihydroanthracene, a compound which was isolated by Boyland & Levi (1935) from the urine of rats and rabbits to whose diet anthracene had been added.

Naphthalene, in the form of a solution in corn oil, was injected subcutaneously into rats. From the

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urine excreted by the dosed animals it was possible to isolate a laevorotatory compound, the elementary analysis of which corresponded to $C_{10}H_{10}O_2$. A study of the structure of this compound led to the conclusion that it was 1:2-dihydronaphthalene-1:2-diol. Further work showed that this compound was present also in urine excreted by rats following the administration of naphthalene by stomach tube. Hitherto, 1:2-dihydronaphthalene-1:2-diol has not been shown to be a metabolite of naphthalene. Furthermore, this compound does not appear to have been described in the chemical literature.

EXPERIMENTAL

All the animals to which naphthalene was administered were male white rats. During the experimental period they were housed in cages which permitted the collection of urine separate from the faeces. The urine was collected daily and was stored in the refrigerator. The rats were fed on a diet which consisted of Master Fox Breeding Ration (Toronto Elevators Limited) supplemented with fresh milk daily and whole wheat bread twice a week. In order to avoid contamination of the urine with fallen food, the animals were fed in a separate cage twice daily for periods of 1 hr. each, and any urine excreted during these periods was not collected. The rats had access to water at all times.

Five experiments were conducted in each of which a group of 12 rats received by subcutaneous injection a total of 4.8 g. of naphthalene dissolved in corn oil. The naphthalene solution was prepared by dissolving 6 g. of the pure hydrocarbon in corn oil so as to give a solution the volume of which was 30 ml. Each rat was given an injection of 0.5 ml. of this solution (i.e. 0.1 g. of naphthalene) under the skin of the back on 4 successive days. The collection of urine was started immediately after the administration of the first dose of hydrocarbon and was continued until 2 days had elapsed after giving the fourth dose. The average weights of the rats in the various groups at the time of the first dosing with naphthalene were 416, 219, 414, 229 and 343 g.

Isolation of the naphthalene derivative from urine

The urine obtained in each experiment was treated separately. It was filtered through glass wool and its volume was measured. The urine volumes ranged from 530 to 720 ml. The urine was adjusted to pH 7-8 and it was then extracted with ether in a continuous extractor for a period of 9 hr. on each of 3 successive days. The ether extract obtained each day was evaporated to dryness under reduced pressure. Most of the material removed from the urine was extracted on the first day, a small amount was removed on the second day and almost none on the third day. The residues obtained by evaporation of the ether extracts were either oily or partly crystalline in nature, and they were dark brown in colour. They were combined and treated with 200 ml. of warm water until as much of the material as would dissolve had done so. The aqueous solution was cooled to room temperature and filtered. The material which did not dissolve in water contained most of the pigment. It was dissolved in 50 ml. of ether and the ether solution was shaken with four 25 ml. portions of water.

These aqueous extracts were then combined with the main aqueous solution and extracted with ether in a continuous extractor for two periods of 9 hr. each. The ether extract, when evaporated to dryness under reduced pressure, yielded a light-brown crystalline residue. This material was extracted as completely as possible by refluxing with several 200 ml. portions of cyclohexane. The cyclohexane extracts were filtered while hot and were concentrated by evaporation. The precipitate which formed was separated by filtration and was crystallized from benzene. The material thus obtained was crystallized again from cyclohexane and from benzene and in most cases yielded a colourless crystalline product, the melting-point of which was not raised by further crystallization.

In the five experiments in each of which a group of rats received a total of 4.8 g. of naphthalene, the amounts of the compound isolated were 0.270, 0.635, 0.499, 0.338 and 0.521 g., and they accounted for 4.5, 10.5, 8.2, 5.6 and 8.6% respectively of the naphthalene administered.

Properties of the compound isolated from urine

The compound separated from benzene in the form of colourless plates and from cyclohexane as thin needle-shaped crystals. It melted with decomposition at 127-128° (this and other melting-points reported herein are uncorrected). $[\alpha]_D^{25} = -154^\circ$ (c, 1% in ethanol) and $[\alpha]_D^{24} = -328^\circ$ (c, 0.5% in water). Found: C, 74.1; H, 6.3%; mol. wt. 159. $C_{10}H_{10}O_2$ requires C, 74.0; H, 6.2%; mol. wt. 162.

In solid form the compound appeared to be stable under ordinary conditions, for a sample which was left at room temperature for 3 months showed no discoloration and no change in melting-point or specific rotation. At 23° the compound showed the following solubilities (in 100 ml. of solution): water, 2.26 g.; benzene, 0.27 g.; cyclohexane, 0.01 g. It was very soluble in ethanol and in ether.

A solution (0.5%) in water gave no coloration when treated with dilute ferric chloride solution. No colour was produced when a solution of diazotized sulphanilic acid was added to a 0.25% solution of the compound containing an excess of NaOH. A strong red colour was produced when a 0.25% solution of the compound in N-HCl was boiled for 1 min., cooled, and treated with diazotized sulphanilic acid in the presence of excess NaOH. When a 0.25% solution of the compound in N-HCl was allowed to stand at room temperature and measured samples were withdrawn at intervals and submitted to the diazo test, the following reactions were observed: 1 hr., faintly positive; 4 hr., moderately positive; 24 hr., strongly positive. Colour development in the test just described was found (see below) to result from the coupling of diazotized sulphanilic acid with α -naphthol formed by acid decomposition of the compound from urine. Information on the stability of the compound in alkaline solution was obtained from an experiment in which 0.051 g. of the compound was dissolved in 5 ml. of 2N-NaOH solution and heated in a water-bath at 94-96° for 30 min. At the end of this period the solution was diluted with 10 ml. of water and extracted with four 20 ml. portions of ether. The ether extracts were evaporated to dryness and the residue was extracted with a small volume of dry ether. When evaporated to dryness, this ether extract yielded 0.048 g. of product, m.p. 126-127°, $[\alpha]_D^{25} = -154^\circ$ (c, 1% in ethanol).

Structure of the isolated compound

The conclusion that the compound isolated from the urine of rats dosed with naphthalene (I) was 1:2-dihydronaphthalene-1:2-diol (II) is based on the following evidence. The elementary analysis of the compound corresponded to $C_{10}H_{10}O_2$. The presence of a naphthalene ring structure with two hydroxyl groups, one of which was in position 1, was indicated by the conversion of the compound to a diacetate (IV) and by its decomposition to α -naphthol (III). The presence of both hydroxyl groups on the same ring was suggested by the finding that oxidation of the compound with potassium permanganate yielded *o*-phthalic acid (VI). These findings, in conjunction with the absence of phenolic properties and the fact that the compound was optically active, indicated that the compound was either 1:2-dihydronaphthalene-1:2-diol or 1:4-dihydronaphthalene-1:4-diol. On hydrogenation under suitable conditions, the compound took up two atoms of hydrogen to give a product $C_{10}H_{12}O_2$. This hydrogenated derivative was identified as 1:2:3:4-tetrahydronaphthalene-1:2-diol (V), which led to the conclusion that the compound isolated from urine was 1:2-dihydronaphthalene-1:2-diol.

The recognition of the hydrogenated derivative as 1:2:3:4-tetrahydronaphthalene-1:2-diol was based on the following findings. The analysis of the compound corresponded to $C_{10}H_{12}O_2$. A positive reaction was obtained when this compound was decomposed by acid and submitted to the test described by Straus & Rohrbacher (1921) for 2-keto-1:2:3:4-tetrahydronaphthalene (VII). On oxidation with potas-

sium permanganate at 5–8° the hydrogenated compound yielded β -(2-carboxyphenyl)propionic acid (VIII). This acid was identified by comparison with the synthetic compound obtained by oxidation of 1:2:3:4-tetrahydro-2-naphthylamine (IX) prepared by reduction of 2-naphthylamine. The oxidation of 1:2:3:4-tetrahydronaphthalene-1:4-diol would not be expected to give rise to β -(2-carboxyphenyl)propionic acid. On the other hand, Straus & Rohrbacher (1921) have shown that this dicarboxylic acid is formed when the *trans* form of 1:2:3:4-tetrahydronaphthalene-1:2-diol is oxidized by potassium permanganate under conditions similar to those used in the present work to oxidize the hydrogenated derivative prepared from the compound isolated from urine.

Acid decomposition of the compound. A solution of 0.106 g. of the compound in 50 ml. of 2*N*-HCl was heated in a boiling water-bath for 15 min., cooled, and extracted with three 25 ml. portions of ether. The combined ether extracts were washed with 25 ml. of water, evaporated to dryness, and yielded 0.089 g. of residue. This residue dissolved almost completely when treated with 25 ml. of boiling light petroleum (b.p. 40–60°). The petroleum solution was filtered, concentrated and cooled. It gave a precipitate (0.070 g.), m.p. 95°, mixed m.p. with α -naphthol (m.p. 96° 95°. Found: C, 83.1; H, 5.8. Calc. for $C_{10}H_8O$: C, 83.3; H, 5.6%.

To 0.052 g. of the acid decomposition product were added 2 ml. of pyridine and 1 ml. of acetic anhydride. The solution which formed was refluxed gently for 15 min. and then poured into 10 ml. of water cooled in an ice-bath. The precipitate which separated was filtered and crystallized from aqueous methanol, giving 0.033 g. of product, m.p. 45°, mixed m.p. with α -naphthyl acetate (m.p. 45–46°) 45–46°. Found: C, 77.6; H, 5.5. Calc. for $C_{13}H_{10}O_2$: C, 77.4; H, 5.4%.

Acetylation of the compound. The compound (0.056 g.) was refluxed for 15 min. with 2 ml. of pyridine and 1 ml. of acetic anhydride. The solution was then poured into 10 ml. of water cooled in an ice-bath. The precipitate which formed was crystallized twice from aqueous methanol. The product was obtained as plates (0.052 g.), m.p. 78–79°, $[\alpha]_D^{25} = -423^\circ$ (c, 0.5% in ethanol). Found: C, 68.4; H, 5.8. Calc. for $C_{14}H_{14}O_4$: C, 68.3; H, 5.7%.

Oxidation of the compound. To a solution of 0.098 g. of the compound in 15 ml. of water were added 15 ml. of 4% $KMnO_4$ (aqueous). The mixture was heated in a boiling water-bath for 1 hr. and then cooled. SO_2 was bubbled through the mixture until all the precipitate dissolved, and the acid solution was then extracted with four 50 ml. portions of ether. The combined ether extracts were evaporated to dryness under reduced pressure and the residue thus obtained was crystallized from a small volume of water. The product was filtered and dried over P_2O_5 (0.055 g.). Neutralization equivalent, 84; calculated for *o*-phthalic acid, 83. Found: C, 57.9; H, 3.6. Calc. for $C_8H_6O_4$: C, 57.8; H, 3.6%.

When 0.021 g. of the oxidation product was heated at 130° under reduced pressure in a microsublimation apparatus, 0.017 g. of crystalline product was obtained, m.p. 129°. The mixed m.p. with phthalic anhydride was not depressed.

Hydrogenation of the compound. The compound was hydrogenated in glacial acetic acid solution in the presence of a platinum catalyst prepared by the method of Voorhees & Adams (1922). The catalyst (0.085 g.) was shaken in 15 ml. of glacial acetic acid in an atmosphere of hydrogen and took up 19.8 ml. of hydrogen in 45 min. 0.096 g. of the compound isolated from urine was then added and 15.3 ml. of hydrogen were absorbed in 6 min. at 759 mm. Hg pressure and 21°. The hydrogenation was then stopped. The theoretical uptake of hydrogen for the addition of two H atoms/molecule of compound was 14.3 ml. at the above temperature and pressure. The catalyst was separated by filtration, washed with glacial acetic acid, and the filtrate and washings were evaporated to dryness under reduced pressure. The residue was dissolved in methanol, the solution evaporated to dryness, and the residue then extracted with benzene. The benzene solution was filtered and when evaporated to a small volume it yielded 0.068 g. of crystalline product, m.p. 112–113°. This was dissolved in benzene, the solution filtered to remove traces of catalyst and then concentrated to small volume. This solution was cooled for a short time in the refrigerator, and the crystals which formed were filtered and washed with a little cold benzene, giving 0.050 g., m.p. 115°, $[\alpha]_D^{25} = +62^\circ$ (c, 0.5% in ethanol). Found: C, 73.2, H, 7.4. Calc. for $C_{16}H_{14}O_2$: C, 73.1, H, 7.4%. Two further hydrogenations were carried out in the manner just described. 0.100 g. of the compound isolated from urine was used in each hydrogenation, and together they yielded 0.116 g. of the hydrogenated derivative, m.p. 115°, $[\alpha]_D^{25} = +61^\circ$ (c, 0.5% in ethanol).

A solution of 0.010 g. of the hydrogenated derivative in 2 ml. of 2N-HCl was heated in a boiling water-bath for 15 min., and then cooled. A few drops of ethanol, 2 ml. of ether, and an excess of conc. NaOH were then added. When the mixture was shaken, the ether became a dark blue colour. According to Straus & Rohrbacher (1921), this test is given by 2-keto-1:2:3:4-tetrahydronaphthalene. A negative result was obtained when the hydrogenated derivative was subjected to the above test without first being decomposed by acid.

Oxidation of the hydrogenated derivative. The hydrogenated compound (0.083 g.) was moistened with 0.2 ml. of ethanol and then dissolved by the addition of 8 ml. of water. The solution was cooled to 5° in the refrigerator and 4.0 ml. of 4% $KMnO_4$ (aqueous) were added in 0.5 ml. portions over a period of 2 hr. while the temperature was maintained at 5–8°. The mixture was left overnight in the refrigerator and was then centrifuged. The precipitate was washed twice with water. The supernatant solution and the washings were combined, filtered, and extracted with three 50 ml. portions of ether. The ether extracts were evaporated to dryness and the residue was crystallized twice from benzene. 0.012 g. of crystalline material was obtained, consisting of unreacted hydrogenated derivative, m.p. 114°. The ether-extracted aqueous solution was made acid to Congo red by the addition of 2N- H_2SO_4 , and then extracted with three 50 ml. portions of ether. The combined ether extracts were evaporated to dryness, treated with hot benzene, and the extract filtered. The filtrate was concentrated to small volume and the material which separated was crystallized from benzene (0.031 g.). When crystallized from water containing a little ethanol, it was obtained as long needles, m.p. 164–165° with preliminary sintering (alone, and when mixed with synthetic β -(2-carboxyphenyl)propionic acid

(see below)). Found: C, 61.7; H, 5.1. Calc. for $C_{16}H_{14}O_4$: C, 61.8; H, 5.2%. Neutralization equivalent, 99; calc. for β -(2-carboxyphenyl)propionic acid, 97.

Synthesis of 1:2:3:4-tetrahydro-2-naphthylamine and its oxidation to β -(2-carboxyphenyl)propionic acid. In the course of their study of the structure of 1:2:3:4-tetrahydro-2-naphthylamine, Bamberger & Müller (1888) found that this compound yields β -(2-carboxyphenyl)propionic acid when oxidized. This reaction was used in the present work to prepare a sample of this acid for comparison with the product obtained by oxidation of the hydrogenated derivative of the compound isolated from urine.

1:2:3:4-Tetrahydro-2-naphthylamine was prepared by the reduction of 2-naphthylamine (10 g.) with sodium in boiling isoamyl alcohol by a method based on that of Gilman & Blatt (1941). The hydrogenated base was converted to its hydrochloride and most of the isoamyl alcohol removed by distillation. An excess of aqueous KOH solution was added to the residue, the mixture was extracted with ether, and 1:2:3:4-tetrahydro-2-naphthylamine carbonate (8.7 g.) was precipitated by the passage of CO_2 through the combined ether extracts. To 2 g. of this product in 100 ml. of water were added, with stirring, 50 ml. of 1% NaOH solution. While the temperature of the mixture was maintained at 8–12°, 125 ml. of 4% $KMnO_4$ (aqueous) were added in small portions over a period of 3 hr. The precipitate which formed was separated by centrifuging, washed with water, and the supernatant liquids were filtered. The filtrate was made acid to Congo red by the addition of 10 ml. of conc. HCl, and was extracted with four 200 ml. portions of ether. The ether extracts were combined and evaporated to dryness under reduced pressure. The residue was heated with 75 ml. of water, the solution was decanted from some oily material, and was then heated with charcoal and filtered. The filtrate was concentrated to a small volume and cooled in the refrigerator. The material which separated was filtered, washed with water, and dried (0.101 g.). It was crystallized first from benzene and then from dilute aqueous ethanol. 0.050 g. of long colourless needles was obtained, m.p. 164–165° with preliminary sintering. Found: C, 61.9; H, 5.1. Calc. for $C_{16}H_{14}O_4$: C, 61.8; H, 5.2%. Neutralization equivalent, 100; calc. for β -(2-carboxyphenyl)propionic acid, 97. Melting points recorded in the literature for this acid are 165–166° (Gabriel & Michael, 1877), 165.5° (Bamberger & Müller, 1888), and 165.5–167° (Straus & Rohrbacher, 1921).

Excretion of 1:2-dihydronaphthalene-1:2-diol by rats dosed with naphthalene by stomach tube

Twelve male white rats (average wt., 353 g.) were each given a suspension of 0.1 g. naphthalene in 1 ml. of 1% starch solution by stomach tube daily on 4 successive days. The other experimental conditions were similar to those in the experiments in which naphthalene was administered by subcutaneous injection. Treatment of the urine (vol., 500 ml.) by the method already described yielded 0.284 g. of crystalline material, $[\alpha]_D^{25} = -153^\circ$ (c, 1% in ethanol), m.p. 127–128°. The melting-point was not depressed when the compound was mixed with 1:2-dihydronaphthalene-1:2-diol isolated from the urine of rats dosed with naphthalene by subcutaneous injection. Found: C, 73.7; H, 6.2. Calc. for $C_{16}H_{14}O_2$: C, 74.0; H, 6.2%. 0.071 g. of the compound was acetylated and yielded 0.073 g. of product,

$[\alpha]_D^{24} = -430^\circ$ (c, 0.5% in ethanol); m.p. 78–79°, the mixed m.p. with 1:2-dihydronaphthalene-1:2-diol diacetate (m.p. 78–79°) was unchanged. Found: C, 68.2; H, 5.8. Calc. for $C_{14}H_{14}O_4$: C, 68.3; H, 5.7%.

Other naphthalene derivatives excreted by rats dosed with naphthalene

From the urine of a group of 12 rats (average wt., 219 g.) which had been injected subcutaneously with naphthalene (total amount, 4.8 g.) under the conditions already described, 0.635 g. of 1:2-dihydronaphthalene-1:2-diol was isolated. The urine of these animals was then examined for the presence of *l*-1-naphthylmercapturic acid and a compound which yields naphthalene when decomposed by acid. These compounds have been shown to be present in the urine of rabbits dosed with naphthalene by stomach tube (Bourne & Young, 1933*a*, *b*, 1934) and in the urine of rats after the addition of naphthalene to their diet (Stekol, 1937; Chang & Young, 1943).

Half the urine (vol. 350 ml.) was made just acid to Congo red by the addition of conc. HCl, and 50 ml. of conc. HCl were then added. After being allowed to stand at room temperature for several hours, the acidified urine was extracted with four 400 ml. portions of chloroform. The chloroform extracts were combined, concentrated by evaporation, and extracted with 100 ml. of *m*-NaHCO₃ solution. The bicarbonate extract was made acid to Congo red by the addition of conc. HCl and left in the refrigerator overnight. The precipitate which formed was filtered, washed with water, and crystallized from aqueous ethanol. The pink crystals thus obtained weighed 0.653 g., m.p. 168–169°, mixed m.p. with *l*-1-naphthylmercapturic acid (m.p. 170–171°) 169–170°. The compound was purified further by precipitation as the sodium salt and reversion to the acid by the method described by Bourne & Young (1934). When this material was crystallized from aqueous ethanol, 0.505 g. of *l*-1-naphthylmercapturic acid was obtained, $[\alpha]_D^{28} = -25^\circ$ (c, 1% in ethanol), m.p. and mixed m.p. 170–171°.

The remaining half of the urine was acidified to pH 2 by the addition of conc. HCl and allowed to stand overnight at room temperature. These conditions have been found to be optimal for the decomposition of the acid-decomposable naphthalene precursor excreted by rats following the ingestion of naphthalene (Chang & Young, 1943). The urine was then made alkaline to litmus and extracted with ether in a continuous extractor for 10 hr. The ether extract was washed with 2*N*-HCl, then with 2*N*-NaOH, and finally with water. It was then dried over anhydrous CaCl₂, filtered, and evaporated to dryness. The residue was extracted with hot light petroleum (b.p. 40–60°). The petroleum extract was filtered, evaporated to dryness in a current of air, and yielded 0.098 g. of crude naphthalene, m.p. 75–77°. This was purified by dissolving it in ethanol and adding water. The crystalline precipitate melted at 80° (alone and mixed with naphthalene).

Experiments similar to those just described were conducted with the urine of the 12 rats (average wt. 353 g.) which received a total of 4.8 g. of naphthalene by stomach tube. The urine of these animals yielded 0.284 g. of 1:2-dihydronaphthalene-1:2-diol. From half the urine (250 ml.), 0.431 g. of *l*-1-naphthylmercapturic acid was obtained, m.p. and mixed m.p. 169–170°. This material was pre-

cipitated as the sodium salt, reconverted to the acid, and when crystallized from aqueous ethanol 0.328 g. of the mercapturic acid was obtained, m.p. and mixed m.p. 170–171°, $[\alpha]_D^{28} = -24^\circ$ (c, 1% in ethanol). The other half of the urine yielded 0.151 g. of crude naphthalene, m.p. 76–78°, which when recrystallized from aqueous ethanol melted at 80° (alone and when mixed with naphthalene).

Amounts of compounds excreted

In the experiment in which naphthalene was administered by subcutaneous injection, 10.5% of the hydrocarbon was recovered from the urine as 1:2-dihydronaphthalene-1:2-diol, 12.1% as *l*-1-naphthylmercapturic acid, and 4.1% as naphthalene derived from the acid-decomposable precursor. When the naphthalene was given by stomach tube, 4.7% was obtained from the urine in the form of the diol, 8.0% as the mercapturic acid, and 6.3% as naphthalene liberated from the precursor. The amounts of hydrocarbon converted to these derivatives were certainly greater than the values just given, for the main purpose of the experiments was the separation of the compounds in a form suitable for identification, and some losses occurred during the purification processes.

DISCUSSION

The finding that naphthalene is converted to 1:2-dihydronaphthalene-1:2-diol in the rat provides the second example of a polycyclic hydrocarbon undergoing this type of metabolic change, for Boyland & Levi (1935) have shown that the corresponding anthracene derivative is excreted by rats and rabbits following the addition of anthracene to the diet. It seems probable that other polycyclic hydrocarbons give rise to derivatives of this type in the body. In preliminary experiments with the urine of rats dosed subcutaneously with solutions of phenanthrene in corn oil, the writer isolated small amounts of a crystalline product, m.p. 186–187°, the analysis of which corresponded to $C_{14}H_{12}O_2$. (Found: C, 78.9; H, 5.6. Calc. for $C_{14}H_{12}O_2$: C, 79.2; H, 5.7%.) This compound gave no colour when treated with diazotized sulphanilic acid in the presence of excess NaOH. When the compound was boiled with *N*-HCl for 1 min., cooled, and submitted to the diazo test, an orange-red colour was produced. This behaviour is similar to that shown by 1:2-dihydronaphthalene-1:2-diol, and when considered in conjunction with the analysis of the compound it suggests that in the rat phenanthrene may be converted to a dihydro-phenanthrene-diol.

It has long been recognized that some cyclic hydrocarbons and certain of their derivatives are oxidized in the body to phenolic compounds which are excreted in the urine in the 'free' form or in conjugation with sulphuric acid or glucuronic acid.

Conjugated phenols are hydrolyzed when heated with strong acid, and heating the acidified urine is used commonly as a step in the isolation of phenolic compounds from urine. If the urine of rats which have been dosed with naphthalene is subjected to this treatment without previous removal of the 1:2-dihydronaphthalene-1:2-diol, this compound will be converted to α -naphthol. If this reaction is not known to have taken place, it might be supposed that this naphthol was originally present as such in the urine, or that it had been liberated by the hydrolysis of its sulphuric acid or glucuronic acid derivative:

A similar situation could arise with respect to the isolation of 1-anthrol following the acid hydrolysis of urine excreted by rats or rabbits which had been dosed with anthracene.

The fact that naphthalene and anthracene give rise to dihydroxy-dihydro derivatives which are decomposed by acid to yield monohydroxy compounds suggests that in studies of the metabolism of

cyclic hydrocarbons consideration should be given to the possibility that part, at least, of a phenolic compound isolated from an acid-treated urine may have been formed by the breakdown of a dihydroxy-dihydro derivative of the hydrocarbon.

SUMMARY

1. A study of the structure of a laevorotatory compound isolated from the urine of rats dosed with naphthalene by subcutaneous injection or by stomach tube led to the conclusion that this compound was 1:2-dihydronaphthalene-1:2-diol. The amounts of this metabolite isolated from the urine accounted for 4.5–10.5% of the naphthalene administered.

2. The urine of rats dosed with naphthalene also contained *l*-1-naphthylmercapturic acid and a compound which yielded naphthalene when decomposed by acid.

3. The isolation of phenolic compounds after the acid hydrolysis of urine excreted by animals dosed with cyclic hydrocarbons is discussed in relation to the excretion of compounds of the type of 1:2-dihydronaphthalene-1:2-diol.

All the elementary micro-analyses reported above were carried out by Mr Michael Edson, who also gave valuable assistance in the conduct of the catalytic hydrogenations. A preliminary account of the work described in this paper was presented at a meeting of the Toronto Biochemical and Biophysical Society held on 25 April 1946 (Young, 1946).

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