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METABOLISM OF ADRENOCHROME IN EXPERIMENTAL ANIMALS

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Abstract—Crystalline adrenochrome, adrenolutin, and 5:6-dihydroxy-N-methylindole (DHMI) were administered to rats. Three metabolites were found in the urine after administration of adrenochrome; these have been shown to be conjugates of DHMI and adrenolutin. One metabolite, a yellow fluorescent product, is a sulfate conjugate of adrenolutin; another is a sulfate conjugate of DHMI. The third metabolite also appears to be a conjugate of DHMI. The evidence suggests that adrenochrome is metabolized via two pathways, one involving adrenolutin, the other DHMI. The same metabolites were excreted by mice, rats, guinea pigs, and rabbits.

INTRODUCTION

THE indolization of epinephrine to form adrenochrome, adrenolutin, and 5:6-dihydroxy-N-methylindole (DHMI) has been known to occur *in vitro* for many years.[1, 2] The structures of these compounds are given in Fig. 1. Although animal enzymes are known which catalyze the oxidation of epinephrine to adrenochrome, it has not been conclusively established that the reaction occurs *in vivo*. A number of physiological, pharmacological, and biochemical effects have been attributed to adrenochrome. Among the most interesting and provocative is the report by Hoffer[3] that adrenochrome and adrenolutin can cause psychotic episodes in man and his suggestion that they may play a role in schizophrenia. These substances have been considered as possible metabolites of epinephrine,[4] but more recently other workers have challenged this view.[5, 11]

Adrenochrome, adrenolutin, and DHMI are relatively unstable substances and are rapidly metabolized when administered to an animal. Previous studies[6] have revealed only part of these processes. It may be necessary more fully to understand the metabolic reactions that these substances undergo before a role can be assigned to them *in vivo*. If a stable metabolite were identified, such a compound might be useful in investigations of a possible metabolic role of adrenochrome *in vivo*.

The present study is concerned with the metabolic products which appear in the urine of animals upon administration of adrenochrome, adrenolutin, and DHMI.

EXPERIMENTAL

Preparation of adrenochrome, adrenolutin, and DHMI

Crystalline adrenochrome was prepared from l-epinephrine (Mann) according to

the method of Heacock *et al.*[7] This procedure was modified in the last step to facilitate crystallization: after passage through Dowex-1, two volumes of peroxide-free ether were added to each volume of methanol–adrenochrome solution. Crystallization occurred rapidly at — 20 °C, with yields of about 60 per cent.

Adrenolutin and its monohydrate were prepared by the procedure of Heacock and Mahon.[8] DHMI was prepared by adding 2·4 g of powdered zinc to 1 g of adrenochrome in 50 ml of water and bubbling nitrogen through the reaction mixture. After the mixture turned yellow, it was extracted three times with 100 ml of peroxide-free ether. The ether extracts were dried with sodium sulfate, then evaporated to dryness with a stream of nitrogen. The residue was extracted with 400 ml of heptane and the extract was concentrated to 20 ml. On cooling, DHMI crystallized out. The crystals were filtered and washed with petroleum ether; the yield was 30 mg and the melting point was 134 °C. Recently, Heacock *et al.*[9] have reported a method of preparing DHMI by reduction of adrenochrome with sodium borohydride with much better yields.

Preparation of ^{14}C-*labeled adrenochrome*

To 193 mg of l-epinephrine bitartrate (Nutritional Biochemicals Corp), 7 mg of β-^{14}C-*dl*-epinephrine bitartrate, specific activity, 1 mc per m-mole (Tracerlab) was added, and the compounds were dissolved in 33 ml of water. Approximately 1·5 ml of concentrated ammonium hydroxide were added; free epinephrine precipitated after standing a few minutes and was removed by filtration. The epinephrine was dissolved in 1 ml of methanol by the addition of two drops of formic acid. Silver oxide was freshly prepared by reacting 295 mg of silver nitrate with 0·35 ml of 10 N sodium hydroxide and 0·75 ml of water. The precipitate of silver oxide was washed with distilled water until the washings were neutral, then with methanol and finally with absolute methanol. The epinephrine solution was added to the silver oxide. The reaction mixture was kept in an ice bath, stirred, and then filtered through Reeve Angel SB-1, ion-exchange paper. The volume of methanol filtrate was measured and two volumes of peroxide-free ether were added; crystallization was allowed to occur at — 20 °C for 2 hr. The crystals were filtered and washed with ether; yield, 16 mg of adrenochrome.

Purity and identity of synthesized compounds

The adrenochrome, adrenolutin, and DHMI prepared by us were compared chromatographically and spectrophotometrically with samples of these compounds which were kindly sent to us by Dr. Heacock. The compounds behaved identically on descending chromatography on Whatman 54 paper, using either 2 per cent acetic acid or methanol as the solvent system. Spectrophotometrically, the adrenochrome, adrenolutin and DHMI samples also were found to behave identically with the reference materials.

Both DHMI samples melted sharply at 134 °C and no depression was observed in the melting point when a mixed melting point was taken.

Administration of compounds and collection of urine

Adrenochrome and DHMI were usually administered in doses of 20 mg/kg, dissolved in physiological saline. Adrenolutin was administered at the same dosage, suspended in a 4 per cent solution of gum arabic. Urine was collected at intervals

from 30 min to 3 hr in iced vessels and used immediately or stored at − 20 °C. The usual route of injection was intravenous, although similar results were obtained with intraperitoneal injection. Most of the work was done with Sprague–Dawley rats weighing from 200 to 400 g.

Chromatographic procedures

Instability of adrenochrome and its derivatives and metabolites requires that the time needed for chromatographic separation be kept to a minimum. Although the substances seem to be stable during short exposures to acidic solvent systems, prolonged exposure causes definite decomposition. Alkaline solvent systems cause extensive decomposition even on short exposures. For this reason we have used neutral or acidic solvent systems which could effect separation in less than 3 hr. For the separation of adrenochrome, adrenolutin and DHMI, descending chromatography on Whatman 54 paper with 2 per cent acetic acid or methanol as solvent systems proved most satisfactory. These solvent systems, however, did not give adequate separations of the urinary metabolites. Circular chromatography using solvent systems composed of butanol:acetic acid:water gave satisfactory separations of these metabolites. The chromatograms were run on circles of Whatman 1 paper and the following two solvent systems were most often employed—butanol:acetic acid:water, 25:3:5, and butanol:acetic acid:water, 12:3:5. For pure compounds, 10 lambda of a 1 per cent solution was usually spotted: for neat urine samples, 30–60 lambda were spotted. Indoles were located with Erhlich's reagent: 0·5 per cent *p*-dimethylaminobenzaldehyde in 1 per cent ethanolic hydrochloric acid.

Radioactivity on paper chromatograms was detected by radioautography. The chromatogram was cut in half, one-half was dipped in Ehrlich's reagent, while the other half was exposed to Kodak Blue Brand X-ray film for 2 weeks. The radiogram was then compared to the chromatogram.

TABLE 1. CHROMATOGRAPHIC BEHAVIOUR OF ADRENOCHROME AND ITS METABOLITES

Compound	Butanol: acetic* acid:water (25:3:5)	Butanol: acetic* acid:water (12:3:5)	2% Acetic† acid
Adrenochrome	54‡	61	92
Adrenolutin	58	80	41
DHMI	86	90	33
Fluorescent compound (*F*)	12	39	69
Erhlich-reacting compound (*E*-1)	28	44	50
Erhlich-reacting compound (*E*-2)	40	70	64

* Circular system on Whatman No. 1 paper.
† Descending system on Whatman No. 54 paper.
‡ $R_f \times 100$.

RESULTS

Adrenochrome metabolism in the rat

Administration of adrenochrome to the rat resulted in the appearance of three metabolites in the urine. The R_f-values of these metabolites in three solvent systems are presented in Table 1. One of the metabolites (designated *F*) exhibited bright

yellow fluorescence in ultraviolet light and had an R_f of 0·12 in butanol:acetic acid:water, 25:3:5. Adrenolutin had a similar fluorescence, but had an R_f of 0·58 in this solvent system. The compound reacted slowly and weakly with Ehrlich's reagent and was very unstable, disappearing from urine samples within 48 hr at 4 °C.

The other two metabolites were colorless, but reacted immediately with Ehrlich's reagent giving a blue-violet color and have been designated *E*-1 and *E*-2. These compounds were more stable than the fluorescent compound. Compound *E*-2 moved and behaved similarly to indican in a number of solvent systems. The data in Table 1 indicate that compounds *E*-1, *E*-2 and *F* are clearly distinct from adrenochrome, adrenolutin, and DHMI; the latter three compounds were not observed in the urine.

The derivation of *E*-1, *E*-2 and *F* from adrenochrome was confirmed by the administration of ^{14}C-labeled adrenochrome at a dose of 15 mg/kg. The urine was treated and chromatographed in the usual manner; radiograms were prepared which showed radioactivity in areas corresponding to the metabolites.

Adrenochrome metabolism in other animals

Adrenochrome was administered to rabbits, guinea pigs, mice, and hooded rats. The dosages employed and the strains of animals used are listed in Table 2. In rats, mice and guinea pigs, *E*-1, *E*-2 and *F* were the major metabolites found. In the rabbit, in addition to these compounds, a second fluorescent compound was present in the urine; this substance is believed to be adrenolutin.

TABLE 2. URINARY METABOLITES OF ADRENOCHROME FORMED BY VARIOUS SPECIES

Species	Strain	Average weight	Dose	Major urinary metabolites			
				F	*E*-1	*E*-2	Others
Rat	Sprague–Dawley	450 g	20 mg/kg	+	+	—	—
Rat	Hooded	300 g	20 mg/kg	+	—	—	—
Mice	Swiss	40 g	20 mg/kg	+	—	+	—
Guinea pig	Hartley	350 g	20 mg/kg	+	+	+	—
Rabbit	English spotted	3·2 kg	20 mg/kg	+	+	+	+

Metabolism of adrenolutin and DHMI

Adrenolutin, 20 mg/kg, was given intravenously to rats and the urine was collected and chromatographed. Only the yellow fluorescent product was found in the urine; neither of the Ehrlich-reacting compounds could be detected.

Administration of DHMI, 20 mg/kg, resulted in only the Ehrlich-reacting compounds, *E*-1 and *E*-2, appearing in the urine; the yellow fluorescent product was not detected.

Metabolism of 5:6-*diacetoxy-N-methylindole*

5:6-diacetoxy-N-methylindole (Wyeth Laboratories) was administered intravenously to Sprague–Dawley rats at a dosage level of 20 mg/kg. The urine, which was collected and chromatographed, contained only compounds *E*-1 and *E*-2, the same metabolites that were found when DHMI was administered. This finding indicated that the compound is deacetylated in the rat.

Conjugation of urinary metabolites

The urinary metabolites of adrenochrome appear to be conjugates of adrenolutin and DHMI. This was demonstrated by treating 0·2 ml of urine with 0·1 ml of a preparation ("Glusulase") of the Endo laboratories (Richmond Hill, N.Y.) containing 50,000 units of sulfatase activity and 100,000 units of glucuronidase activity per ml; after 30 min the enzyme reaction was stopped by the addition of 0·5 ml of acetone. Precipitated protein was removed by centrifugation, and 0·2-ml samples of the supernatant fraction were chromatographed; control mixtures of acetone-inactivated enzyme and urine alone were treated similarly.

The glusulase-treatment resulted in the disappearance of compounds *E*-1, *E*-2 and *F*, and the appearance of two new substances. One of these was identified as adrenolutin by its fluorescence and behavior in three solvent systems. The other product of the reaction was identified as DHMI by its reaction with Ehrlich's reagent and its chromatographic behavior.

Nature of conjugation

To establish whether the metabolites were sulfate conjugates, experiments were performed to determine whether the isotope of ^{35}S-sulfate was incorporated into the urinary metabolites. Ten minutes before giving a dose of adrenochrome, rats were injected intraperitoneally with 7 mg of ^{35}S-labeled sodium sulfate, specific activity 1 mc/m-mole, and the urine was collected, and chromatographed. Radiograms were prepared of the chromatograms and radioactivity was found only in areas corresponding to the yellow fluorescent compound (*F*) and compound *E*-2.

Demonstration of the conjugation of adrenolutin and DHMI in vitro

The *in vitro*-conjugation of adrenolutin and DHMI with sulfate by rat liver supernatant preparations yielded products identical with those found in rat urine. The reaction mixture contained 4·45 m-moles of substrate (adrenolutin or DHMI), 7·8 m-moles of ATP, 7·5 m-moles of $MgCl_2$, and 1·95 m-moles of Na_2SO_4, in 0·9 ml of Tris buffer (0·15 M, pH 7·27–7·4), to which was added 1·0 ml of rat liver supernatant, prepared in the buffer described by Grimes.[10] After incubation at 37 °C for 30 min, 80 lambda of the mixture were spotted and chromatographed. The adrenolutin reaction mixture showed the disappearance of the substrate and the appearance of a fluorescent band with R_f-values in two solvent systems (butanol:acetic acid:water (25:3:5) and (12:3:5)) similar to those of the urinary fluorescent compound *F*. The DHMI reaction mixture resulted in a product with R_f-values in two systems similar to those of compound *E*-2. When a heat-inactivated rat liver supernatant preparation was added to the otherwise complete reaction mixtures, neither product was formed. Metabolite *E*-2 was not formed in the DHMI reaction mixture in the absence of ATP; similarly, in the absence of added sulfate, only a trace of *E*-2 was formed. In the absence of ATP, the reaction resulting in the formation of melanin from DHMI appears to be favored, since the rate of melanin formation was markedly increased, as compared to that which occurred in the complete reaction mixture.

DISCUSSION

The findings that adrenochrome has two metabolites in common with DHMI and one in common with adrenolutin, and that the products formed from adrenolutin

differ from those formed from DHMI, indicate that adrenochrome is metabolized via two divergent pathways. One is by way of adrenolutin to give the yellow fluorescent urinary product, the other by way of DHMI, which gives the two Ehrlich-positive compounds. These pathways are illustrated in Fig. 1.

The association of radioactivity with two of the metabolites on chromatograms of urines from animals which had received adrenochrome and ^{35}S-sulfate and the finding of DHMI and adrenolutin after incubation of the urine with a sulfatase–glucuronidase preparation indicates that two of the urinary products are simple conjugates of DHMI and adrenolutin. The yellow fluorescent substance is a sulfate-conjugate of adrenolutin, while *E*-2 is a sulfate-conjugate of DHMI. The nature of the conjugate of compound *E*-1 has not been established, but it may be a glucuronide of DHMI, since it disappears on treatment with the sulfatase–glucuronidase preparation, but ^{35}S-sulfate is not incorporated into this metabolite.

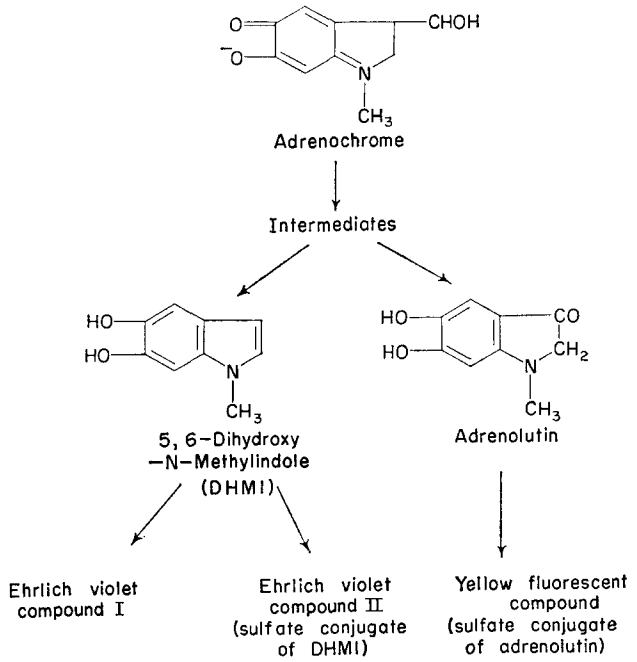

FIG. 1. Pathways of adrenochrome metabolism in the rat.

The sulfate conjugation in the case of compounds *E*-2 and *F* was further demonstrated by the *in vitro* experiments in which the metabolites were formed on incubation of DHMI or adrenolutin with liver homogenate, ATP, and sulfate. The requirement for ATP indicates that these compounds are sulfated by an active sulfate mechanism similar to that involved in the case of phenol sulfate.

After the administration of adrenochrome to rodents, the yellow fluorescent product and compounds *E*-1 and *E*-2 are the principal metabolites found in the urine. The products formed after the administration of adrenochrome were reported to vary from species to species by Fisher and Le Comte,[6] who found that in cats and dogs

some adrenochrome is excreted unchanged, while the major portion is excreted as a reduction product, whereas in the rabbit, adrenolutin and a sulfo-conjugate of adrenolutin were excreted. These results in the rabbit agree with our own in that both adrenolutin and a sulfate-conjugate of adrenolutin were excreted. In most of the species investigated, however, we did not find free adrenochrome, adrenolutin or DHMI excreted in the urine.

Schayer and Smiley[11] administered 3-^{14}C-*dl*-adrenochrome to rats. In the urine of these rats, they found an unstable yellow pigment that produced a broad band of radioactive material in their chromatograms; this substance was not identified. These authors considered that extensive decomposition of the urinary metabolites of adrenochrome occurred with the chromatographic procedures which they used; this suggestion may explain the differences between their results and ours.

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