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CATABOLISM AND EXCRETION OF THE ANTITHYROID SUBSTANCE, DIACETYL-2,6-DIIODOHYDROQUINONE*

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Abstract—The metabolism of the antithyroid compound, diacetyl-2,6-diiodohydroquinone, and its related iodoquinones and iodohydroquinones was studied in rats by means of ^{131}I-labeled derivatives. Chromatography, radioautography, and enzyme studies of the excreted products indicated that the major portion of injected diacetyl-2,6-diiodohydroquinone is deacetylated to form free diiodohydroquinone which is conjugated with glucuronic or sulfuric acid and excreted via the urine. Deiodination of diacetyl-2,6-diiodohydroquinone does occur as a catabolic step since free iodide ion was observed. However, the deiodination does not yield monoiodohydroquinone or its excretion forms. This fact suggests the simultaneous removal of two atoms of iodine from a given molecule. Toxicity studies of the various derivatives were also conducted.

THE iodinated hydroquinone derivative, diacetyl-2,6-diiodohydroquinone (DDIH), has been shown to behave as an antithyroid drug. Its mode of action appears similar to that of the compound n-butyl-4-hydroxy-3,5-diiodobenzoate (BHDB) which, it has been suggested, interferes with the conversion of thyroxine to triiodothyronine.[1, 2] Despite this potential similarity with respect to mode of action, the structures of BHDB and DDIH possess marked differences (Fig. 1). Thus DDIH, unlike BHDB, is a hydroquinone derivative which might readily be catabolized *in vivo* to yield a series of free hydroquinones and quinones. It is conceivable that these catabolic products might considerably influence the antithyroid characteristics of DDIH. This study was undertaken for the purpose of identifying these iodine-containing catabolic products and their excretion forms and to evaluate their toxic effects.

METHODS

Male Sprague–Dawley rats were used to study catabolic and excretory processes. Compounds to be evaluated were labeled with ^{131}I by procedures already described.[3] Animals received the ^{131}I-labeled derivatives by intraperitoneal injection and were maintained in glass metabolism cages where the total excreta could be collected over known time intervals. Preliminary studies revealed that the feces contained negligible quantities of ^{131}I derivatives at the dose levels studied, and hence attention was assigned solely to the urine samples. In excretion-rate studies the volumes of all timed urine samples were measured and an aliquot of each sample dried on a filter paper disc in an aluminum planchet and counted with a Nuclear Chicago γ-ray scintillator detector.

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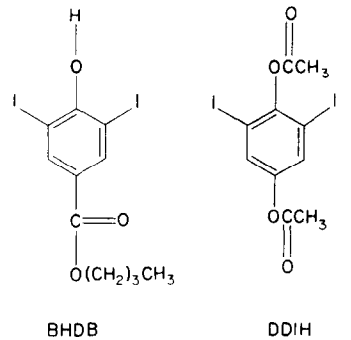
By counting the urine aliquots and an aliquot of the originally injected material at the same time it was possible to express the excretion rate in terms of a percentage of the originally injected material without the necessity of correcting for radioactive decay. An aliquot of the total 12-hr urine sample from a given rat was spotted directly to Whatman No. 1 filter paper and developed with either the organic phase of an n-butanol:glacial acetic acid:water solvent (25:6:25) or a benzene:glacial acetic acid:



BHDB DDIH

FIG. 1. Structures of n-butyl-4-hydroxy-3,5-diiodobenzoate (BHDB) and diacetyl-2,6-diiodohydroquinone (DDIH).

water solvent (2:4:1). ^{131}I compounds on the chromatograms were visualized by means of radioautography. The spots could then be cut from the chromatograms and counted with a Nuclear-Chicago γ-ray crystal scintillation detector. Thus each spot could be expressed as a percentage of the total activity on the chromatogram and the percentage of each component excreted in the urine directly determined.

Iodohydroquinones were separated by means of the organic phase of a benzene: glacial acetic acid:water solvent (2:2:1 or 2:4:1). Iodinated quinones were separated on Whatman No. 1 paper which had been impregnated with silicone fluid as described by Lester and Ramasarma.[4] The solvent system used with the quinone derivatives was the aqueous phase of benzene:glacial acetic acid:water (2:4:1).

Unlabeled reference hydroquinones were visualized by spraying the chromatograms with an alcoholic solution of α,α'-dipyridyl and ferric chloride.[4] Unlabeled quinones were visualized by first spraying the chromatograms with an aqueous solution of sodium borohydride. Excess sodium borohydride was then destroyed by a dilute hydrochloric acid spray. After drying at room temperatures the reduced quinones could be detected by the α,α'-dipyridyl-ferric chloride reagent.

Several of the urine components were isolated by eluting them from their chromatograms with water or alcohol. After removal of the solvent by lyophilization, the respective components were subjected to treatment by dilute acid or by enzymes in order to establish their identity. The enzyme preparations used were β-glucuronidase (Nutritional Biochemicals), and sulfatase (Mylase P; Mann Research Laboratories).

The sulfate conjugates of the iodohydroquinone were synthesized by treatment of the respective ^{131}I-labeled hydroquinone with chlorosulfonic acid. Ten mg of the ^{131}I-labeled hydroquinone was taken up in a solution containing 0·4 ml chloroform and 0·1 ml diethylaniline. This mixture was cooled and maintained at ice-bath temperatures while a solution containing 0·1 ml chloroform, 0·02 ml diethylaniline, and 10 μl

chlorosulfonic acid was slowly added. The reaction mixture was then allowed to stand at room temperature for 3 hr. Excess acid was neutralized with KOH, and samples of the precipitate obtained were separated by paper chromatography in n-butanol: glacial acetic acid:water (25:6:25).

Acute toxicity studies were conducted in white mice (R. F. Beyers, Billings, Mo.) with the procedures described by Thompson.[5] The compounds were injected intraperitoneally as aqueous solutions with the exception of the diiodo-derivatives which were not appreciably water soluble and were injected in cottonseed oil. The median lethal dose and the 95 per cent confidence limit for each compound were determined by the moving average method of Thompson[5] and the tables constructed by Weil.[6]

RESULTS

The most probable iodine-containing degradation products of DDIH are 2,6-diiodohydroquinone, monoiodohydroquinone, and their respective quinones. Thus it was considered advisable to study the excretion rates and forms of these compounds with the thought that they might be compared to those of DDIH.

Typical excretion curves for DDIH and its potential degradation products are given in Fig. 2. It is apparent that the ^{131}I derived from injected hydroquinones is cleared

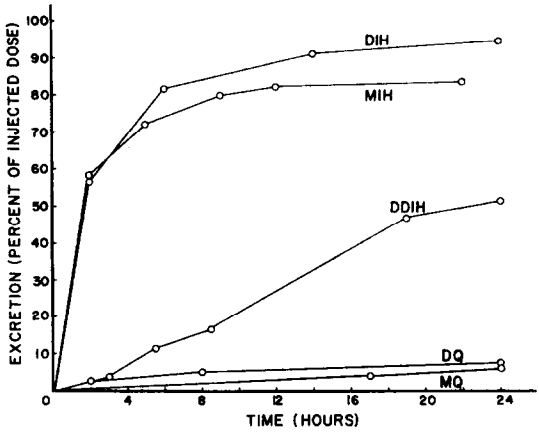


FIG. 2. Typical excretion curves for the iodinated derivatives. The abbreviations are: DIH, diiodohydroquinone (25 mg/kg); MIH, monoiodohydroquinone (22 mg/kg); DDIH, diacetyldiiodohydroquinone (20 mg/kg); DQ, diiodoquinone (10 mg/kg); MQ, monoiodoquinone (12 mg/kg).

from the system much more rapidly than that from the corresponding quinones. Thus 2 hr after injection, over 55 per cent of the ^{131}I from both hydroquinones is excreted via the urine, whereas less than 3 per cent of the radioactivity from the quinones is recovered. DDIH excretion occupies an intermediate position between the excretory rates of hydroquinones and quinones.

Radioautographs of the 12-hr urines from monoiodohydroquinone, 2,6-diiodohydroquinone, and DDIH are shown in Fig. 3. It can be seen that ^{131}I-labeled DDIH does not appear in the urine, in appreciable quantities, in its originally injected form but is excreted in altered forms. The excretion patterns for 2,6-diiodohydroquinone

and DDIH are qualitatively the same. Components A and B of the monoiodohydroquinone urine were eluted and treated separately with dilute hydrochloric acid, β-glucuronidase, or sulfatase. The products of the action of these agents were chromatographed in benzene:acetic acid:water (2:4:1) and radioautographed. Component A (Rf 0·05) yielded a compound with the same Rf as an authentic sample of monoiodohydroquinone (Rf 0·60) with both acid and β-glucuronidase treatment but was unaffected by sulfatase. Component B (Rf 0·23) yielded a compound with the same Rf as an authentic sample of monoiodohydroquinone with acid and with sulfatase but was unaffected by β-glucuronidase. These data would suggest that A is a glucuronide conjugate and B a sulfate conjugate of monoiodohydroquinone. The nature of component B was further established by comparison with a synthetic sulfate conjugate of monoiodohydroquinone which exhibited the same Rf as component B (Rf 0·51) in n-butanol:acetic acid:water (25:6:25) as well as the same response to hydrochloric acid or sulfatase. An attempt to prepare a synthetic glucuronide of monoiodohydroquinone was unsuccessful.

Components D and E were eluted and treated separately with dilute hydrochloric acid, β-glucuronidase, or sulfatase. The products formed were chromatographed in n-butanol:acetic acid:water (25:6:25). Component D yielded a compound with the same Rf as an authentic sample of diiodohydroquinone (Rf 0·90) with dilute hydrochloric acid or β-glucuronidase but was unaffected by sulfatase. Component E released a compound with the same Rf as an authentic sample of diiodohydroquinone with dilute hydrochloric acid or sulfatase but was unaffected by β-glucuronidase. These data are consistent with the possibility that component D is a glucuronide and E a sulfate conjugate of diiodohydroquinone. Further proof of the nature of component E was provided by comparison with a synthetic sulfate conjugate of diiodohydroquinone which exhibited the same Rf as component E (Rf 0·71) in n-butanol: acetic acid:water (25:6:25) and the same response to acid or sulfatase. Attempts to prepare a synthetic glucuronide of diiodohydroquinone were unsuccessful.

The excretion patterns of the quinones, monoiodoquinone and diiodoquinone, were compared to their respective hydroquinones by means of chromatography and radioautography. The quinones were found to manifest a qualitatively similar pattern to their hydroquinones with respect to their major components.

The results of the acute toxicity studies of hydroquinone, monoiodohydroquinone, 2,6-diiodohydroquinone, diacetyl-2,6-diiodohydroquinone, and their respective quinones are summarized in Tables 1 and 2. When considered on a weight basis (mg/kg body weight) the substitution of iodine into the hydroquinone ring system causes a decrease in toxicity as measured by the LD_{50}. However, when these compounds are compared on a mole–weight basis, it is seen that the substitution of iodine causes an increase in the toxicity of the iodine with increased substitution.

In the quinone series, the compounds in this oxidized form were considerably more toxic than the hydroquinones. Again on a weight basis, the substitution of iodine into the molecule causes a decrease in toxicity. When the compounds were compared on a mole–weight basis, 2,6-diiodoquinone was found to be considerably less toxic than quinone or monoiodoquinone, which have the same order of toxicity on a molar basis.

In the hydroquinone series, toxicity symptoms appear to change with increased iodine substitution. The unsubstituted hydroquinone initiates spasmodic muscular twitching, loss of righting reflex, and convulsions. The nonoiodohydroquinone in-

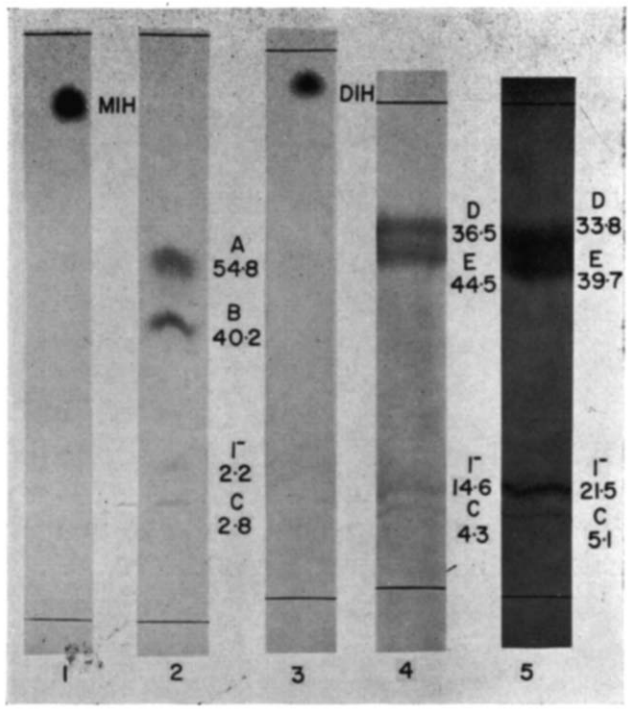


FIG. 3. Radioautographs of chromatograms from the 12-hr urines of rats injected with monoiodohydroquinone at a level of 20 mg/kg,[3] 2,6-diiodohydroquinone at a level of 25 mg/kg[4], and DDIH at a level of 30 mg/kg.[5] Radioautographs 1 and 3 are from reference chromatograms of ^{131}I-labeled monoiodohydroquinone (MIH) and 2,6-diiodohydroquinone (DIH) respectively. The letters A, B, C, and D represent specific ^{131}I-labeled components found in the urines. The figures beneath the letters represent the percentage of that component found in that particular 12-hr urine. I^- indicates iodide ion formed from degradation of the injected derivatives. The solvent system used was n-butanol: acetic acid:water (25:6:25). The Rf of DDIH with this solvent is 0·95. In this figure the transverse lines at the bottom and top of each radioautograph indicate the position of the origin and solvent front respectively.

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TABLE 1. THE ACUTE INTRAPERITONEAL TOXICITIES OF HYDROQUINONE AND ITS IODINATED DERIVATIVES

Compound	Mol. wt.	No. of mice	Mean weight (g)	LD_{50} and 95% confidence interval (mg/kg)	(mmole/kg)
Hydroquinone	110	25	17·5 ± 1·2	186 (152–227)	1·69 (1·38–2·06)
Monoiodo-hydroquinone	236	21	31·9 ± 4·1	208 (167–258)	0·881 (0·71–1·09)
Diiodo-hydroquinone*	362	25	31·0 ± 4·1	237 (183–306)	0·655 (0·51–0·85)
Diacetyl-diiodo-hydroquinone*	446	11	18·1 ± 1·9	>1,350	

* Compounds dissolved in cottonseed oil; all others dissolved in distilled water.

TABLE 2. THE ACUTE INTRAPERITONEAL TOXICITIES OF QUINONE AND ITS IODINATED DERIVATIVES

Compound	Mol. wt.	No. of mice	Mean weight (g)	LD_{50} and 95% confidence interval (mg/kg)	(mmole/kg)
Quinone	108	23	18·6 ± 1·4	8·5 (7·5–9·6)	0·078 (0·07–0·09)
Monoiodoquinone	234	20	17·9 ± 1·1	16·3 (13·3–19·8)	0·07 (0·06–0·085)
Diiodoquinone*	360	20	36·0 ± 4·8	84 (58–121)	0·23 (0·16–0·34)

* Dissolved in cottonseed oil; other compounds dissolved in distilled water.

duces tremors, loss of righting reflex, and convulsions. The diiodohydroquinone seldom induces tremors or convulsions, but the animal loses its righting reflex and enters a state of depression.

In the quinone series the toxic symptoms appear similar with all compounds. Writhing was apparent immediately after injection of the quinones. Paralysis of the hind limbs was apparent several hours after the injection if the animal lived that long-Cyanosis was always observed. Death of animals given the quinone form of the compounds was longer delayed than in those given the hydroquinones.

DDIH exhibited no toxic effects at the highest level tested (Table 1).

DISCUSSION

The catabolic and excretory products of DDIH as determined from this study are outlined in Fig. 4. The acetyl groups of DDIH are apparently completely hydrolyzed *in vivo* to yield free 2,6-diiodohydroquinone. This observation is consistent with other reports of acetyl group cleavage in phenolic derivatives.[7, 8] Since the excretion of

DDIH is much less rapid than that of 2,6-diiodohydroquinone (Fig. 2) it appears that removal of the acetyl groups is the rate-determining metabolic step in DDIH excretion. Presumably the low solubility of DDIH in aqueous media prevents rapid transport of the compound to the necessary enzyme sites.

The major excretion forms of DDIH and 2,6-diiodohydroquinone appear to be their sulphate and glucuronide conjugates. Cohn[9] and Shulz[10] have demonstrated similar conjugates of *p*-hydroquinone and trichloro-*p*-hydroquinone in experimental animals. It is anticipated, as indicated in Fig. 4, that despite the presence of two hydroxyl

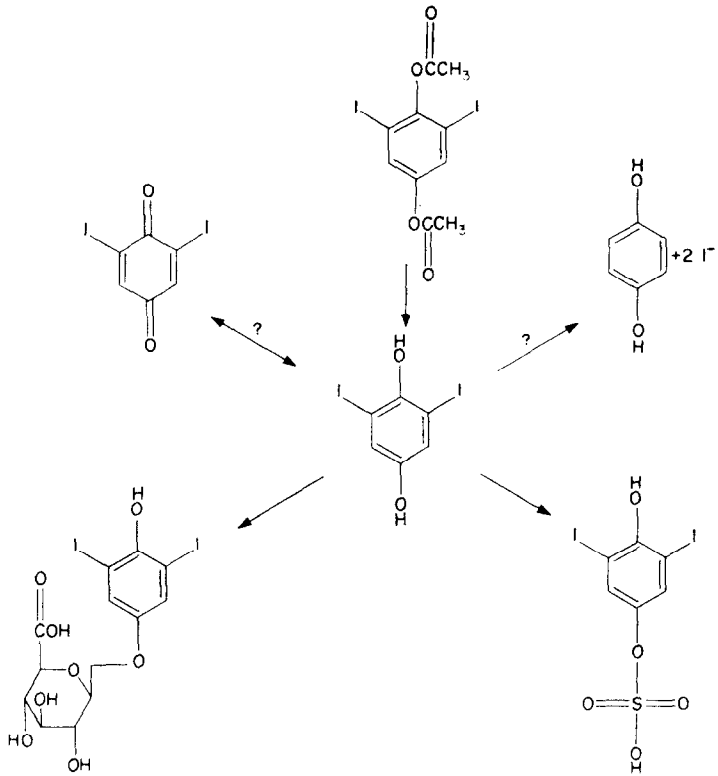

FIG. 4. Catabolic and excretory forms of DDIH.

groups only one group becomes conjugated. This would be expected since as soon as one hydroxyl group is conjugated with sulfuric or glucuronic acid, a relatively strong acid is formed which tends to be rapidly excreted before a second conjugation can occur. The position of the substituted group is most probably at the hydroxyl furthest removed from the bulky iodine atoms. Dodgson and colleagues have shown this to be the preferred orientation with several chlorine-substituted polyphenols.[11–13]

The presence of iodide ion in the urine of DDIH-injected rats indicates deiodination as a catabolic step. Whether this iodide ion is formed directly through deiodination of DDIH itself or after, 2,6-diiodohydroquinone is formed is not known. It is of interest, however, that the deiodination of DDIH or 2,6-diiodohydroquinone does

not lead to the formation of any appreciable quantity of sulfate or glucuronide conjugates of monoiodohydroquinone. Thus deiodination of DDIH or 2,6-diiodohydroquinone must either involve the simultaneous removal of both iodine atoms to yield diacetyl-hydroquinone or hydroquinone or, alternatively, a more extensive breakdown of the molecule must be indicated to yield iodide ion and some unknown iodine-free catabolic product. Support for the former possibility is given by the report of Wynn and Gibbs[14] that a rat liver microsomal preparation deiodinates thyroxine by a simultaneous removal of two iodine atoms to form 3,5-diiodothyronine with no intermediate formation of 3′,3,5-triiodothyronine.

It is possible that some oxidation of 2,6-diiodohydroquinone to its corresponding quinone may occur as a catabolic step. This cannot be established with certainty from these data since the studies presented here show a qualitative similarity in the excretory patterns of both hydroquinones and quinones. The toxicity data of Tables 1 and 2 indicate, however, that if this oxidation does occur it leads to a limited formation of 2,6-diiodoquinone since appreciable generation of the quinone would render DDIH much more toxic than it has been demonstrated to be.

The identity of component C was not determined, but it appeared in close association with iodide ion in all excretion patterns studied. The possibility exists that this component might be the "extraneous" form of iodine described by Doctor[15] and others.

DDIH *per se* may or may not be primarily responsible for the antithyroid response it elicits. The results of this study introduce the possibility that the catabolic product, 2,6-diiodohydroquinone, or its excretion forms, may be an active antithyroid compound. Studies have been conducted in this laboratory to determine the antithyroid potential of 2,6-diiodohydroquinone, but the toxicity of this compound (Table 1) has prevented its direct introduction into experimental animals in sufficiently high concentration to elicit an antithyroid response. It is possible, however, that once DDIH is introduced into the body, the slow, sustained release of 2,6-diiodohydroquinone may permit this compound more effectively to reach inhibiting sites at concentrations that do not initiate its toxic symptons. The evaluation of the antithyroid characteristics of less labile derivatives of 2,6-diiodohydroquinone is currently under consideration.

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