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NOTES

Metabolic Fate of 2,3,5-Triiodobenzoic Acid in Laying Hens

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Abstract □ The whole body retention, distribution, and metabolism of carboxyl-^{14}C-labeled 2,3,5-triiodobenzoic acid (TIBA*) were studied in five laying hens. A single oral dose of TIBA* showed a 22-hr. biological half-life. No organ concentration of TIBA* was noted. TIBA* and seven labeled metabolites were found in excreta collected 6 to 12 hr. after the dose, with TIBA* representing the major end product. TIBA* and four labeled metabolites were detected in excreta collected during the 78 to 90-hr. interval; the metabolites occurred in greater proportion than did TIBA* in this sample.

Keyphrases □ 2,3,5-Triiodobenzoic acid (TIBA), carboxyl-^{14}C-labeled—metabolic fate □ Distribution, metabolism, whole body retention—TIBA, carboxyl-^{14}C-labeled □ Metabolites—TIBA, carboxyl-^{14}C-labeled □ Thick-layer chromatography—separation, identification

Greer and Anderson (1) reported that treatment of soybean plants with 2,3,5-triiodobenzoic acid (TIBA) at the beginning of flowering resulted in an increased seed yield due to two major types of effects. Their studies showed that TIBA caused the plants to change from vegetative to reproductive development more rapidly and also caused morphological changes which permitted more efficient utilization of sunlight by the plants. Since a residue of TIBA remains in soybeans grown from treated plants (2), the potential environmental health hazard of TIBA should be investigated prior to the general usage of the compound in agriculture. For this reason, a study of the retention, distribution, and metabolism of TIBA in laying hens was of interest.

Ice *et al.* (3, 4) employed TIBA labeled with ^{131}I in Position 2 (TI*BA) for metabolism studies in rats and lactating animals. In both studies, a significant thyroid concentration of ^{131}I was noted, and metabolism by deiodination was indicated. In rats and in lactating animals, the whole body retention of TI*BA was characterized by a two-component system.

Ware and Barker (5) administered carboxyl-^{14}C-labeled TIBA (TIBA*) orally to rats and found TIBA* and/or its labeled metabolites in all organs analyzed. Excretion of the compound was primarily through the urine, in which both TIBA and 2,5-diiodobenzoic acid were detected.

The metabolism of TIBA by chickens was investigated by Barker *et al.* (6) who employed TIBA labeled with ^{125}I in Positions 3 and 5. They found that 90% of the orally administered radioactivity was excreted within 48 hr. and that TIBA and 2,5-diiodobenzoic acid were present in the excreta. The same investigators dosed chickens and pigs with unlabeled TIBA and detected, by using gas chromatography, the presence of TIBA, 2,5-diiodobenzoic acid, and 3,5-diiodobenzoic acid in the chicken brains and in the thyroids of both chickens and pigs.

EXPERIMENTAL

Administration of TIBA*—Carboxyl-^{14}C-labeled TIBA (TIBA*) was available from the work reported by Spitznagle (2) and was purified immediately prior to use in this study by a method similar to that reported by Jarboe (7). An ethanolic solution of the impure TIBA* was applied to thick-layer plates (1.0 mm.) of purified silica gel[1] which were then developed three times each (12 cm. per development) in petroleum ether (30 to 60° fraction)–propionic acid (10:1 v/v) (8). The silica containing the pure TIBA*, located on the chromatograms by autoradiography, was removed from the plates and extracted with anhydrous ethyl ether, using a continuous extraction apparatus. The ether was allowed to evaporate at room

[1] Adsorbosil-1 with 10% binder, Applied Science Laboratories, State College, Pa.

Table I—The Retention of Orally Administered TIBA* and/or Its Radioactive Metabolites by Laying Hens

Time, hr.	% Excretion[a]	Standard Error	% Retention[b]
6	17.3	1.9	82.7
12	20.1	3.0	62.6
18	9.3	2.1	53.3
24	14.4	0.8	38.9
30	7.5	1.0	31.4
42	11.3	2.1	20.1
54	6.5	2.7	13.6
66	2.5	0.7	11.1
78	3.2	0.9	7.9
90	1.4	0.3	6.5
96	1.8	1.2	4.7

[a] Quantity excreted since previous sample collection; mean of five animals. [b] Calculated from the accumulated mean excretion data.

Table II—The Distribution of TIBA* and/or Its Radioactive Metabolites 96 hr. after Oral Administration to Laying Hens

Tissue	% × 10^{-4} of Dose/g. of Tissue[a]	Standard Error
Brain	2.7	0.4
Breast muscle	6.0	1.3
Fat	2.8	0.3
Gizzard	7.8	2.6
Heart	11.9	3.7
Kidney	36.3	11.8
Liver	9.8	2.8
Thigh muscle	6.6	2.0
Thyroid	7.4	0.9

[a] Mean of five hens.

temperature, giving crystals of purified TIBA*. The radiochemical purity of the purified TIBA* was greater than 99%, as determined by thin-layer chromatography, autoradiography, and ^{14}C assay of the chromatogram.

Five laying single comb white leghorn hens were given, by oral intubation, a single dose of a 2.23% solution of TIBA* in ethanol and glycerin (1:1 v/v). This dose contained 7.7 mg. of TIBA* and 57.1 μc of ^{14}C per kilogram of body weight.

Sample Collection and Analysis—The animals were housed individually in metabolism cages and were supplied a breeder laying mash and tap water *ad libitum*. Total excreta samples were collected from each hen at 6, 12, 18, 24, 30, 42, 54, 66, 78, 90, and 96 hr. Eggs were collected immediately after they were laid and were hard-cooked prior to analysis. The animals were sacrificed by decapitation 96 hr. after the dosage, and samples of the brain, breast muscle, fat, gizzard, heart, kidney, liver, thigh muscle, and the thyroids were obtained. Representative aliquots of the excreta, egg albumin, egg yolk, and tissue samples were digested with a 1 *M* methanolic solution of hyamine hydroxide[2] and mixed with 15 ml. of a liquid scintillator containing: PPO, 10.0 g.; naphthalene, 80.0 g.; *p*-xylene, 143 ml.; *p*-dioxane, 429 ml.; and a sufficient quantity of 2-ethoxyethanol to make 1 l. The samples were then assayed for ^{14}C in an internal sample liquid scintillation spectrometer. Internal standardization with benzoic acid-^{14}C was employed to correct for quenching. Sample-counting efficiencies of approximately 50% were obtained.

Metabolism—Twelve-gram aliquots of the 6 to 12-hr. and 78 to 90-hr. excreta samples from the one hen whose egg-laying pattern was most regular were extracted separately with anhydrous ether using a continuous extraction apparatus. After a 48-hr. extraction period, 60% of the ^{14}C present in the samples was extracted. Following this extraction, portions of the extracted samples were then hydrolyzed separately with 85% *o*-phosphoric acid and 2 *N* nitric acid. Less than 2% of the remaining ^{14}C could be extracted from the acid-treated samples with ether.

[2] A methanolic solution of hyamine hydroxide prepared according to Bruno (9) from Hyamine 10-X crystals, Rohm & Haas, Philadelphia, Pa.

Table III—The Relative Occurrence of TIBA* and Its Radioactive Metabolites in Ether-Extracted Excreta Samples[a]

Location of Radioactive Zone (R_f)	Identity[b]	Relative % Occurrence 6 to 12-hr. Extract	78 to 90-hr. Extract
0.00		5.1	8.9
0.08–0.10		1.4	0.6
0.19		1.0	—
0.31–0.35	TIBA	52.6	10.2
0.42–0.48	2,3-DIBA	1.7	3.2
0.56		0.3	—
0.71–0.75	2,5-DIBA	29.6	69.4
0.89	3,5-DIBA	4.3	—

[a] Determined by thick-layer chromatography on purified silica gel plates (1 mm.) developed in petroleum ether (30 to 60° fraction)–propionic acid (10 to 1 v/v). [b] Based on cochromatography with reference standards.

The ether extracts were concentrated and applied to thick-layer (1.0 mm.) plates of purified silica gel. The plates were developed three times each (12 cm. per development) in petroleum ether (30 to 60° fraction)–propionic acid (10:1 v/v) (9), air-dried, and autoradiographed. Reference standards of unlabeled TIBA and six suspected metabolites were chromatographed simultaneously on the same plates and were then visualized by spraying with a 0.1% solution of bromocresol green in acetone.

The radioactive zones located by autoradiography were scraped individually from the chromatograms and added to 18 ml. of a scintillator gel,[3] mixed thoroughly, and assayed for ^{14}C to determine their relative occurrence.

RESULTS AND DISCUSSION

The retention of TIBA* and/or its labeled metabolites at various intervals following oral administration of TIBA* is given in Table I. Mathematical analysis of these data suggests that TIBA* and/or its labeled metabolites have a single component retention system with a biological half-life of approximately 22 hr. This is in contrast to the two-component systems observed in studies using ^{131}I-labeled TIBA (3, 4). At the end of the 96-hr. study, 95.3% of the administered ^{14}C was recovered in the excreta.

Very small quantities of TIBA* and/or its labeled metabolites were detected in the tissues, as shown in Table II. At the end of the 96-hr. study, the maximum ^{14}C level detected in any tissue corresponded to less than 0.004% of the administered dose per gram of tissue. Fairly wide variations of tissue ^{14}C levels were noted among the five animals, as seen by the magnitude of the mean standard errors reported in Table II. This may be due in part to the very low level of activity present in all tissues and to biological variation among the hens. No organ concentration of ^{14}C was noted. This result is significant because of its contrast to studies in rats, cows, and goats which showed that the thyroid concentrated ^{131}I-labeled TIBA and/or its labeled metabolites, most probably in the form of $^{131}I^-$ ion (3, 4).

The level of ^{14}C present in all eggs collected during the 96-hr. study was very low. The maximum amount of ^{14}C detected in any yolk or albumin fraction corresponded to less than 0.008% of the administered dose per gram of egg fraction.

The TIBA* and its labeled metabolites present in the 6 to 12-hr. excreta extract were separated by thick-layer chromatography into eight fractions corresponding to four unknowns, TIBA, 2,3-diiodobenzoic acid (2,3-DIBA), 2,5-diiodobenzoic acid (2,5-DIBA), and 3,5-diiodobenzoic acid (3,5-DIBA). The ether extract of the 78 to 90-hr. excreta sample contained two unknowns, TIBA, 2,3-DIBA, and 2,5-DIBA, as determined by thick-layer chromatography. The relative occurrence of TIBA* and its labeled metabolites in the excreta extracts is given in Table III. These results show that the major labeled compound detected in the 6 to 12-hr. excreta sample was TIBA, which accounted for 52.6% of the ether extracted radioactivity. Metabolites present in smaller quantities included

[3] A 4% w/w solution of Cab-O-Sil Thixotropic Gel Powder, Packard Instrument Co., Downers Grove, Ill., and the liquid scintillator described above.

2,5-DIBA, 3,5-DIBA, and 2,3-DIBA. TIBA was present in the 78 to 90-hr. sample in a smaller proportion than in the 6 to 12-hr. sample, and accounted for only 10.2% of the extracted ^{14}C. However, the metabolites occurred in larger proportions in the later sample. The results of the metabolite study indicate that TIBA is metabolized to a significant extent by deiodination.

SUMMARY

In the authors' opinion, TIBA has a low probability of becoming an environmental health hazard for a number of reasons. Other investigators have reported the following: TIBA is applied to soybeans in very small quantities and only a minute fraction of the amount applied can be detected in the harvested beans; the toxicity of TIBA is relatively low in humans; and the major portion of TIBA ingested orally by rats, cows, goats, and chickens is excreted rapidly. The work reported here also shows rapid excretion of TIBA in chickens, and no egg or organ concentration of the compound.

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N-acyl Derivatives of Bis-(4-aminophenyl) Disulfide and its Thiolsulfinate

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Abstract □ Bis-(4-aminophenyl)-sulfone and several *N,N'*-diacyl derivatives have shown appreciable activity as antimalarials. With the assumption that a molecule more readily cleaved to a *p*-aminophenyl sulfur or oxidized sulfur anion, as a potential anti-PAB substance, might be a more effective antimalarial, a series of *N,N'*-diacyl derivatives of bis-(4-aminophenyl) disulfide was synthesized. Oxidation with peroxide followed by acylation gave the corresponding thiolsulfinates. Antimalarial activity was found for bis-(4-*p*-acetamidobenzenesulfonamidophenyl) disulfide and *N,N'*-bis-(α-aminoacyl) derivatives of bis-(4-aminophenyl)-sulfone.

Keyphrases □ Bis-(4-aminophenyl) disulfide and thiosulfinate—*N*-acyl derivatives synthesis □ Antimalarial activity—bis-(4-aminophenyl) disulfide derivatives □ IR spectrophotometry—structure □ UV spectrophotometry—structure

Diaminodiphenylsulfone (DDS) (1) and its *N,N'*-diacetyl derivative (2) have both shown appreciable antimalarial activity, particularly against strains of *P. falciparum* resistant to chloroquine and other widely used antimalarials. Evidence has been found to suggest that these compounds are effective by interfering with the utilization of PABA by the parasites (3). If this is the case, then compounds more readily cleaved *in vivo* to a *p*-aminophenyl sulfur or oxidized sulfur moiety might interfere more effectively with PABA utilization. Accordingly, a series of *N,N'*-diacyl derivatives of bis-(4-aminophenyl) disulfide and *S*-oxidized derivatives has been prepared for antimalarial evaluation. Other disulfides, including 5,5'-diacetamido-8,8'-diquinolyl disulfide, have shown antimalarial activity (4, 5).

Bis-(4-aminophenyl) disulfide was obtained by the procedure of Price and Stacy (6), in which sodium 4-aminophenylmercaptide was oxidized by 30% hydrogen peroxide to the disulfide. The product showed the expected IR absorption bands for an aromatic amine, in addition to two sharp bands at 1175 and 1065 $cm.^{-1}$. Bredereck (7) has attributed the presence of these bands in aromatic disulfides to the disulfide linkage and a 1,4-disubstituted aromatic ring, respectively. However, aliphatic disulfides have shown similar peaks in the 1050–1250 $cm.^{-1}$ region which were believed due to CH wag on the carbon adjacent to sulfur (8). UV absorption showed a peak at 256 mμ, characteristic of disulfides (9), with a shoulder at 290–295 mμ.

Peroxide oxidation of aliphatic disulfides has led to formation of thiolsulfinates, thiosulfonates, and α-disulfones, depending on reaction conditions (10). Also, percamphoric acid oxidation of alkyl or aryl disulfides gave mixtures of disulfides, thiolsulfinates, and thiolsulfonates (11). With bis-(4-aminophenyl) disulfide, peroxide oxidation gave only the thiolsulfinate, even on long standing. Heating resulted in decomposition. IR absorption of the product showed a peak at 1050 $cm.^{-1}$, in addition to those present for the disulfide, which is characteristic of thiolsulfinates (8, 12). UV