(15) Higuchi, T., Rao, A. N., and Busse, L. W., *J. Am. Pharm. Assoc., Sci. Ed.*, 43, 344(1954).
(16) Patel, N. R., and Hopponen, R. E., *J. Pharm. Sci.*, 55, 1065(1966).
(17) Nogami, H., Hasegawa, J., and Miyamoto, M., *Chem. Pharm. Bull.*, 15, 279(1967).

Keyphrases

Cornstarch grains
Tablet disintegration–starch grain damage
Crystalline substance–starch grain damage
Compression force–starch grain damage
Starch swelling–tablet disintegration

Metabolic Fate of Orally Administered 2,3,5-Triiodobenzoic Acid in Lactating Animals

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The whole body retention, excretion, distribution, thyroid uptake, and metabolism of 2(^{131}I),3,5-triiodobenzoic acid (TI*BA) were studied in goats and a cow. A single oral dose of TIBA exhibited a two-component whole body radioactivity retention curve and was excreted primarily in the urine. TIBA plus nine metabolites, four of which were identified, were found in the urine. The major metabolite was 2,5-diiodobenzoic acid (2,5-DIBA). Trace amounts of 2,3-diiodobenzoic acid (2,3-DIBA), orthoiodobenzoic acid (OIBA), and iodide ion were found. TIBA was metabolized by deiodination. Iodide ion was concentrated in the thyroid and excreted by way of milk and urine.

In soybeans, 2,3,5-triiodobenzoic acid (TIBA) affects plant morphology and flowering response (1, 2). When properly used, soybean production is increased through a better utilization of photosynthate, lodging is decreased, and a more compact plant results (3). Spitznagle (4) has reported a residue in soybeans treated with TIBA. Soybean products are used for animal and human food consumption and the question arises as to the environmental health safety of TIBA. The metabolic fate of TIBA in lactating animals is important to a thorough understanding of the potential hazards.

Ice *et al.* (5) reviewed TIBA and using TIBA labeled with ^{131}I in the two position, orally administered to rats, observed two whole body retention components—one with a biological half-life of 11.8–17.9 hr. and the second of 395–403 hr. Of the administered dose, 70–78% was excreted in the urine, while 3–4% was excreted in the feces over 4 days. Three metabolites plus TIBA and iodide ion were located in the urine. Whole body retention of radioactivity was evident. Distribution studies in rats indicated a marked thyroid uptake of radioactivity.

Ebert and Ware (3) and Barker *et al.* (6) using carboxyl labeled TIBA-^{14}C orally administered to rats, found TIBA and/or its metabolites in all organs analyzed. Seventy-five percent of radioactivity was excreted *via* the urine and 29% *via* the feces. Chromatographic studies indicated two to six metabolites plus TIBA in the urine.

Gutenmann *et al.* (7) found no TIBA in the milk or feces of the dairy cow during a period of 4 days. In urine, 13.5% of the dose excreted was TIBA. A second compound in the urine was identified as 2,5-DIBA and accounted for 53.5% of the TIBA fed.

EXPERIMENTAL

Instrumental Methods—Whole body radioactivity was measured in goats using large volume liquid scintillation counting techniques. The basic mechanisms and characteristics of the 2 π liquid scintillation detector have been previously reported (8). The detector has since been modified by the addition of an upper detecting tank, which allows 4 π geometry. Dosages were adjusted to provide negligible coincidence loss.

A 3-in. diameter standard NaI(Tl) crystal, centrally located in a steel vault, was used for counting liter samples of excreta and tissue. A 3-in. diameter NaI(Tl) well crystal was used to determine the radioactivity in 3-ml. samples of tissue, to count eluent fractions from chromatography columns, to

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quantitate metabolites scraped from thick-layer chromatograms, and to count milk samples. Thyroid uptake was determined using a standard 2-in. diameter NaI(Tl) crystal in a collimator, DS-5.[1] Each crystal was connected to appropriate electronics and used in a differential mode. All radioactivity determinations were corrected for background, counter efficiency, and decay as required.

Gel filtration chromatography columns were made by placing a slurry of prewashed 100 Gm. (dry weight) Sephadex G-15[2] into a glass column 122 cm. long and 2.2 cm. i.d. A pledget of glass wool was used at the base and top of the dextran gel bed. Characteristics of the column were: bed length 32 cm.; void volume 135 ml.; and flow rate 0.4 ml./min.; deionized water was the eluent. Five-milliliter fractions were collected in a fractionator[3] with volumetric attachment. Sample size placed on the column was 5 ml.

The thick-layer (1.0-mm.) chromatographic and autoradiographic procedures used were similar to those reported earlier (5). The adsorbent was changed to 85 Gm. of MN-Kieselgel G-HR/UV[4] mixed with 137 ml. of deionized water to form a slurry capable of coating 5 plates each 20 × 20 cm. The solvent systems used were: (*a*) petroleum ether–propionic acid, (10:1, v/v) (3); (*b*) *n*-butanol–acetic acid–water, (4:1:1, v/v) (5); (*c*) ethyl acetate–methanol–2 *N* ammonium hydroxide, (100:40:60, v/v, upper phase) (9); (*d*) ethyl acetate–methanol–0.2 *N* acetic acid (100:40:60, v/v upper phase) (9). Unlabeled standard compounds were detected visually under ultraviolet light (254 mμ).

Radioactive TIBA was synthesized and purified as previously reported (5) immediately prior to experimental use. Radiochemical purity was established by thin-layer chromatography in each solvent system followed by autoradiography.

A precison level of experimental work was established so that an error greater than 5% (at the 95% confidence level) would not be accepted. The mean standard error and the Pearson product-moment correlation coefficient were calculated according to the method outlined by Downie (10).

Whole Body Retention—Five adult female milk goats in the 8–9 month of lactation were used. The average daily milk volume was 300 ml. per goat. The goats weighed 39.5–60.4 Kg. and were maintained on a daily lactation ration consisting of 1.9 Kg. of commercial goat chow and legume hay *ad libitum*.

Prior to dosing, a whole body radioactivity measurement was made on each goat to determine the quantity of natural radioactivity appearing in the differential counting window used. This base level of natural radioactivity was subtracted from whole body determinations made after dosing.

Four of the goats were each given a single dose of 0.1 mg. TI*BA, representing 0.3 μCi. The doses were prepared by pipeting 10 μl. of an alcoholic solution of TI*BA into a No. 000 gelatin capsule and allowing the alcohol to evaporate. Each capsule administered with a balling gun to avoid mastication. Thirty minutes were allowed to elapse before the first whole body radioactivity determination was made. For each determination, the individual goat was immobilized in a steel container (11) without tranquilizers or anesthesia. Twelve 1-min. radioactivity determinations were made on each goat at each time interval. To prevent counting the capsule as a point source, the first net count rate 30 min. after dosing was assigned 100% retention. To measure any possible cross external contamination from excreta, a fifth goat was used as a control. This animal received similar treatment as the four other goats with the exception that a placebo was administered.

Excretion—Five adult goats in the third month of lactation weighing 47.8–62.3 Kg. were acclimatized to metabolism cages (12) for 2 days prior to experimentation. Each goat was catheterized with a Bardex Foley catheter with Bard inflation valve[5] (size 16 French, 5-ml. balloon) and the urinary bladder drained. The goat was milked dry and then given a dose of 5.0 mg. TI*BA representing 5.4 μCi. After dosing, all urine, feces, and milk were recovered at 6 hr., and then at 12-hr. intervals thereafter up to 114 hr. All excreta were weighed and samples 1 L. size taken by weight for radioactivity determinations.

The procedure was repeated using a 3-year old Holstein cow weighing 477 Kg. The cow had calved for the first time 5 months earlier. During the study, the cow was maintained on a daily lactation ration of 18.2 Kg. corn silage, 11.4 Kg. of a 16% protein concentrate, and 2.3 Kg. hay. The single dose to the cow was 1.197 Gm. of TI*BA representing 55.92 mCi. The dose, in a No. 12 veterinary capsule, was administered to the cow with a balling gun. Average daily milk volume was 17.5 Kg. After dosing, all excreta were recovered and weighed at 6 hr. and then at 12-hr. intervals up to 162 hr. One-liter samples by weight were taken for radioactivity determination. Total residue determinations were made on the feces according to "Standard Methods" (13).

Thyroid Uptake—During the excretion study a concomitant determination of thyroid uptake was made on the same five lactating goats dosed with TI*BA. The collimated NaI(Tl) crystal was centered 10 cm. from the thyroid gland of each goat and two radioactivity measurements were made every 12 hr. through 210 hr. after administration of TI*BA. The procedure was repeated on the flank of each goat.

Distribution—Four adult lactating goats weighing 51.4–75.0 Kg. were used. Each goat was milked dry, catheterized, and the urinary bladder drained as in the excretion study. The catheter was then sealed. Each goat was given a single dose of 150 mg. of TI*BA containing 5.0 mCi. Eight hours after dosing, the catheter was reopened, the bladder drained, and the goat milked dry. The goat was stunned by a sharp blow on the head and sacrificed by bleeding from a severed jugular vein. A 1-L. blood sample was collected. The goat was dissected, with the kidneys, thyroid, liver, spleen, brain, heart, and gastrointestinal tract being removed. Lean and fat tissue were obtained from the hind quarter and inner surface of the loin, respectively.

[1] Nuclear-Chicago, Des Plaines, Ill.
[2] Pharmacia Fine Chemicals Inc., Piscataway, N. J.
[3] Gilson Medical Electronics, Middleton, Wis.
[4] Macherey, Nagel and Company, Duren, Germany.
[5] Davol Rubber Company, Providence, R. I.

The gastrointestinal (G.I.) tract was emptied of its contents, weighed, and mixed uniformly. One-liter samples of fat, liver, lean, blood, and G.I. tract were taken by wet weight. The heart, spleen, kidney, and brain were weighed and frozen. Two samples of frozen tissue were removed, weighed, and placed in counting vials. The thyroid gland was weighed, minced, and a 3-Gm. sample taken. Radioactivity measurements were made on all samples and compared to a standard of similar geometry with known specific activity. The percent of the administered dose of TIBA and its metabolites was determined for each organ. Also calculated was the percent of dose per kilogram of organ tissue. A normalized concentration was determined by setting the percent per kilogram of blood equal to 100.0 and correcting all other concentrations to this normalized value.

Metabolism—Accumulated 8-hr. urine, milk, and the thyroid gland from the goats and accumulated 6–18 hr. urine and milk from the cow were analyzed for metabolites. Gel filtration columns and thick (1.0-mm.) layer chromatograms were used. Five-milliliter samples of urine, treated[6-8] urine, skim milk, treated skim milk,[9] and digested thyroid tissue[10] were separated on gel filtration columns.

The identification of TIBA and iodide ion in the column eluent was accomplished by adding TI*BA and iodide-^{131}I ion as internal standards to the urine and milk samples placed on the column. The eluent fractions under each peak and duplicated fractions that had been acidified were forced air evaporated to 0.5 ml. and spotted on thick-layer chromatograms in aliquots of 50 μl. Also chromatographed were treated[6-8] urine samples, skim milk pancreatin,[9] digested thyroid tissue,[10] standard solutions of suspected metabolites, whole urine, and skim milk. A chromatogram was developed in each solvent system. Each chromatogram was autoradiographed 16 days, and the position of metabolites compared to appropriate standards.

Milk Analyses—The skim portion of the milk was obtained by centrifuging 10 ml. of whole milk for 10 min. at 4500 r.p.m. The cream layer was penetrated with a 5-in., 20-gauge needle attached to a syringe and the skim milk portion removed. The partition of radioactivity in cream and skim milk was determined by comparing the relative concentrations of radioactivity in whole milk and skim milk.

Protein bound iodide in milk was determined by adding 5 ml. of 20% trichloroacetic acid (TCA) to 5 ml. of whole milk, stirring, and centrifuging 10 min. at 4500 r.p.m. The nonprotein bound fraction (supernatant) was removed. The protein fraction was treated similarly two more times with 5 ml. of TCA. The nonprotein fractions were combined and the quantity of nonprotein bound radioactivity was compared with the radioactivity of whole milk.

[6] Acidified urine. Urine was acidified to pH 3 using 1 *N* HCl, shaken, and allowed to stand 24 hr.

[7] Alkaline urine. Urine was made alkaline to pH 11 using 1 *N* NaOH, shaken, and allowed to stand 24 hr.

[8] Urine–beta glucuronidase. Ten milliliters of urine and acetate buffer solution pH 4.5 were mixed with 100,000 u. (454 mg.) beta-glucuronidase (Type B-1, Bovine Liver, Sigma Chemical Co., St. Louis, Mo.). The solution was stirred, shaken, and incubated at 37° for 48 hr. The resultant suspension was centrifuged 10 min. at 4500 r.p.m. and 5 ml. of supernatant taken as the sample.

[9] Skim milk–pancreatin. An aliquot of whole milk was added to pancreatin (20 mg./ml.), shaken, and incubated at 37° for 48 hr. The resultant suspension was centrifuged 10 min. at 4500 r.p.m. and a 5-ml. supernatant was taken as the sample.

[10] Digested thyroid tissue. To a cold finger condenser was added 2 Gm. of minced thyroid tissue, 1 ml. of 0.1% thiourea solution, and 10 ml. of 1 *N* NaOH. The condenser was air evacuated, sealed, and heated on a steam bath 4 hr. The digested tissue was centrifuged and 5 ml. of supernatant used for the sample.

RESULTS AND DISCUSSION

The results of the whole body retention study are shown in Table I. The data indicated at least 2 biological half-life components, represented by the equation

$$Y = 19.9e^{(-0.0164)(X \text{ hr.})} + 71.8e^{(-0.0512)(X \text{ hr.})},$$

where Y is the percent retained at X hours. A long biological half-life component of 423 hr. was indicated and had a correlation coefficient of $r = 0.87$. A short biological half-life component of 13.5 hr. was found and had a correlation coefficient of $r = 0.99$. The derived whole body retention equation assumes milk production to be invariant at 300 ml. daily.

The results of the excretion study are shown in Table II. During the acclimatization period prior to experimentation, total excretion records were maintained. Total excreta before and during the study were compared and no significant difference was seen in the quantity of milk, urine, or feces produced due to the TIBA treatment.

Urinary excretion was the primary route of excretion accounting for 76.1% of the administered radioactivity in goats after 114 hr. Milk and fecal excretion accounted for 12 and 2.7%, respectively. Using the derived whole-body retention equation, the accountable dose was 107.4% of the administered dose.

The whole body retention equation was derived from goats with an average daily milk production of 300 ml. In the excretion study, however, the average daily milk production per goat was markedly higher with 1930 ml. With increased milk production during the excretion study, there was increased excretion by the mammary gland at the expense of whole body retention. Weaver (14) has shown that iodide ion excretion in milk varies directly as does the quantity of milk produced. In the cow, 57.5% of the administered dose was excreted in the urine, 12.2% in the milk, and 11.4% in the feces.

Thyroid and flank uptake studies of TIBA

TABLE I—PERCENT WHOLE BODY RETENTION OF ORALLY ADMINISTERED TI*BA IN LACTATING GOATS

Time, hr.	Mean Retention,[a] %	Mean S. E.
0[b]	100.0[c]	0.0
8	76.3	12.5
16	47.8	9.9
24	40.5	8.0
32	30.3	7.2
40	27.2	8.1
52	22.9	7.2
64	20.6	6.9
76	19.6	6.4
88	18.0	6.5
112	16.8	6.0
136	15.7	6.0
160	15.4	5.7
184	14.8	5.4

[a] Percent of administered dose calculated as equivalent TIBA. [b] Thirty min. after dosing. [c] Arbitrarily assigned 100.0%.

TABLE II—PERCENT EXCRETION OF TIBA AND ITS METABOLITES FOLLOWING ORAL ADMINISTRATION OF TI*BA IN LACTATING ANIMALS

	5 Goats[a]						Cow[b]			
	Urine		Milk		Feces					
Time, hr.	Mean %	Mean S. E.	Mean %	Mean S. E.	Mean %	Mean S. E.	Urine %	Milk %	Feces %	Fecal Solids %
6	29.2	5.2	2.0	1.0	...[c]	...[c]	8.2	0.6	...[c]	16.4
18	33.1	0.6	3.1	1.3	0.4	0.1	25.7	2.6	0.8	20.0
30	8.2	1.3	2.6	1.0	0.6	0.1	9.2	2.1	3.1	19.6
42	2.9	0.9	1.6	0.4	0.6	0.2	7.4	1.8	2.2	22.3
54	1.0	0.3	1.1	0.4	0.3	0.2	2.5	1.4	1.7	20.3
66	0.7	0.4	0.6	0.1	0.3	0.1	1.5	1.1	1.3	20.2
78	0.3	0.2	0.4	0.1	0.2	0.1	1.0	0.8	0.7	26.2
90	0.3	0.2	0.3	0.1	0.1	0.1	0.7	0.6	0.6	18.9
102	0.2	0.1	0.2	0.1	0.1	...[c]	0.3	0.3	0.4	19.0
114	0.2	0.2	0.1	0.1	0.1	...[c]	0.3	0.3	0.2	19.6
126	...	...	...	...	...	...	0.2	0.2	0.1	20.8
138	...	...	...	...	...	...	0.2	0.2	0.1	22.3
150	...	...	...	...	...	...	0.2	0.1	0.1	24.1
162	...	...	...	...	...	...	0.1	0.1	0.1	23.4
Total	76.1		12.0		2.7		57.5	12.2	11.4	

[a] Weighed 47.8–62.3-Kg. dose was 5 mg. TI*BA representing 5.4 μCi. [b] Weighed 477 Kg. dose was 1.197 Gm. TI*BA representing 55.2 mCi. [c] Less than 0.05%.

and/or its metabolites were done concomitantly on the five goats used in the excretion study. Thyroid uptake occurred for 5 days after a single dose before declining in radioactive content. Comparison of the flank uptake curve with the thyroid uptake curve, indicated a definite thyroid involvement in TI*BA metabolism.

The distribution of radioactivity in each goat after 8 hr. is shown in Table III. Of the administered dose, 41% remained in the G.I. tract after 8 hr. TIBA and/or its metabolites were distributed throughout all tissue samples analyzed. The kidney was indicated as the primary organ excreting absorbed TIBA. The thyroid contained 0.19%, of the administered dose and had a relative concentration of radioactivity 28 times the blood concentration after 8 hr. In the organs examined, the lowest level of radioactivity was found in the brain, which accounted for less than 0.01% of the dose administered.

Column chromatographic separation of whole urine produced a pattern typified by Fig. 1. Internal standards of iodide (^{131}I) ion and TI*BA were eluted with peaks 2 and 8, respectively. Column chromatography of acidified urine produced the iodide peak and an enlarged peak 9 at the expense of peaks 3 to 8. The effect may be due to the release of iodinated benzoic acids from conjugation and a change of the iodinated benzoic acids in the ionized form to the molecular form. The urinary metabolites were quantitated by summing the radioactivity associated in each peak and determining the relative percent of each peak contributing to the total radioactivity. The results are shown in Table IV.

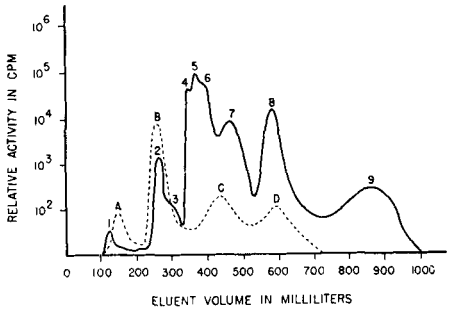

*Fig. 1—Typical column chromatogram of urine (_____) and skim milk (.....) radioactivity after oral TI*BA administration to goats, using a Sephadex G-15 column and deionized water as the eluent.*

TABLE III—TISSUE DISTRIBUTION OF TI*BA AND ITS METABOLITES[a] IN FOUR LACTATING GOATS

	% Dose in Organ		Relative Concentration[b]	
Organ	Mean	S. E.	Mean	S. E.
Kidney	0.72	0.16	270.2	48.5
Heart	0.11	0.02	34.0	5.2
Thyroid	0.19	0.03	2,835.0	1,357.0
Blood	...[c]	...[c]	100.0[d]	0.0
Spleen	0.02	0.00	16.2	2.3
G. I. tract	41.35	8.43	405.2	168.7
Liver	0.46	0.14	23.8	1.9
Fat	...[c]	...[c]	7.0	1.8
Lean	...[c]	...[c]	9.5	2.2
Brain	0.01	0.00	2.9	0.3

[a] Animals were sacrificed 8 hr. after oral administration of TI*BA. [b] Calculated using the equation: [(% dose in organ/Gm. × 10^3)(100)/(% dose in blood/Gm. × 10^3)]/4 = Relative Concn. [c] Not calculated, as the total organ weights were unknown. [d] Arbitrarily set equal to 100.0.

TABLE IV—RELATIVE PERCENT OF TI*BA AND ITS METABOLITES FOUND IN THE URINE AND SKIM MILK OF LACTATING GOATS[a] AND A COW[a] AFTER ORAL ADMINISTRATION OF TI*BA

Peak No.	Eluent Vol., ml.	Goat No. 11	Goat No. 12	Goat No. 13	Goat Mean	Cow
	Relative % Metabolites in Urine[b]					
1,2,3	0–320	2.4	1.7	0.8	1.6	0.3
4,5,6,7	321–525	39.9	33.5	49.0	40.8	70.0
8,9	526–995	57.7	64.8	50.2	57.6	29.7
	Relative % Metabolites in Skim Milk[b]					
A	0–250	6.6	7.6	5.0	6.4	0.4
B	251–320	76.2	92.4	87.8	85.5	97.8
C	321–525	...	0.0	3.8	...[d]	0.7
D	526–995	17.2[c]	0.0	3.3	...[d]	1.1

[a] Accumulated 8 hr. urine or skim milk sample from goats and accumulated 6–18 hr. urine or skim milk from a cow. [b] Column chromatographic separation using Sephadex G-15 with deionized water as the eluent. [c] Represents combined peaks C and D. [d] Data unavailable.

Components of the urine eluent, acidified urine eluent, and urine eluent peaks acidified after passing through the column were identified using thick-layer chromatography. The eluent of peak 9 from

acidified urine indicated a mixture, primarily of 2,5-DIBA and TIBA with traces of 2,3-DIBA and OIBA. The concentrated eluents of whole urine fractionated into peaks 4, 5, and 6, consisted of predominately 2,5-DIBA with trace quantities of 2,3-DIBA. Peak 7 was seen only as a trace in two of the examined goats but was as shown in the third goat and the cow. When eluent from peak 7 was acidified, it was converted to TIBA.

Column chromatographic separation of skim milk metabolites produced the typical pattern illustrated in Fig. 1. Internal standards of iodide (^{131}I) ion and TI*BA were eluted with peaks B and D, respectively. Peak A may be explained as a physical absorption of radioactivity on some high molecular weight compound in milk as it was eluted with the white color of milk near the void volume. Peak A was seen in all skim milk samples including controls, but was not seen in the pancreatin-treated skim milk. The identity of peak C is unknown. The skim milk metabolites were quantitated by summing the radioactivity associated with each eluted peak. The results are shown in Table IV. While goat 12 showed only iodide ion, peak B, all other goats and the cow examined produced a predominate iodide ion peak and traces of two other radioactive components, peaks C and D.

Digested thyroid tissue from goats was also chromatographed on gel filtration column into two major peaks, one of which appeared at the void volume indicating a high molecular weight compound being passed directly through the column. This peak tailed, giving a positively skewed curve which carried into a second peak consisting of iodide ion. The large amount of peak overlapping prevented any quantitative evaluation of the results. The first peak probably represents the iodine-containing compounds found in the thyroid that result from iodide ion concentration by the thyroid gland.

Thick-layer chromatography in solvent system *a*, separated whole urine into three fractions; origin, TIBA area (R_f 0.37), and 2,5-DIBA area (R_f 0.53). Whole urine eluent from the column chromatogram, representing peaks 4, 5, and 6, was acidified and concentrated. Quantitative evaluation indicated 2,5-DIBA (R_f 0.53) to be the major metabolite. Traces of the metabolite 2,3-DIBA (R_f 0.29) were evident. In goat 13 and the cow a trace metabolite at R_f 0.15 was also seen. Concentrated peak 9 eluent from the column chromatogram of acid hydrolyzed urine consisted of 2,5-DIBA, TIBA, and the metabolite OIBA (R_f 0.41). The three major areas on the chromatogram were quantitated by scraping from the plates and counting the radioactivity associated with each area. The results are shown in Table V. No migration of the metabolites in skim milk or in digested thyroid occurred in this solvent system.

TABLE V—RELATIVE PERCENT OF TIBA AND ITS METABOLITES FOUND IN THE URINE OF LACTATING GOATS AND A COW AFTER ORAL ADMINISTRATION OF TI*BA[a]

Location		Relative % Metabolites in Urine				
		Goat[b] No.				
R_f	Area	11	12	13	14	Cow[b]
0.00–0.29	Base	24.24	23.16	89.98	34.67	28.26
0.30–0.44	TIBA	49.58	59.59	8.63	46.94	27.16
0.45–0.59	2,5-DIBA	26.17	17.25	1.39	18.39	44.58

[a] Thick-layer chromatograms on MN-Kiesel gel and a solvent of petroleum ether (30–60°); propionic acid (10:1 v/v) (16). [b] Accumulated 8-hr. urine sample in goats and accumulated 6–18-hr. urine sample in a cow.

Thick-layer chromatography in solvent system *b* separated urine metabolites into seven positions. Iodide ion (R_f 0.47) and 2,5-DIBA (R_f 0.86) were found along with TIBA (R_f 0.83). The trace at R_f 0.80 was seen in concentrated eluents of peak 9 from the column and gave an R_r of 1.0 with OIBA standard. Three unknown metabolites appeared at R_f 0.54, 0.64, and 0.70.

Chromatograms of skim milk in solvent system *b* showed a major spot corresponding to iodide ion plus a trace of radioactivity at the origin. Pancreatin-treated skim milk contained the iodide ion without the residue at the origin. Digested thyroid supernatant remained at the origin in this solvent system except for a trace at R_f 0.54 and the iodide ion at R_f 0.47. Extraction of digested thyroid with acidified *n*-butanol indicated the presence of iodide ion (R_f 0.47), a metabolite at R_f 0.54, and traces at R_f 0.80 and 0.89.

Thick-layer chromatography using solvent system *c* separated urine metabolites into TIBA (R_f 0.73), 2,5-DIBA (R_f 0.76), iodide ion (R_f 0.30), and six additional metabolites. A ninth metabolite, OIBA (R_f 0.79), was obtained from the concentrated column eluent of peak 9. The six unknown metabolites were located at R_f 0.39, 0.42, 0.44, 0.49, 0.60, and 0.69.

Chromatograms of skim milk in solvent system *c* indicated a predominate iodide ion and trace radioactivity at the origin. Pancreatin-treated skim milk removed the residue at the origin and increased the concentration of the iodide ion. Two other unidentified trace metabolites were seen in the milk of both the cow and the goats (with the exception of goat 12) at R_f 0.44 and 0.76. Digested thyroid produced only iodide ion and a residue at the origin. Digested thyroid tissue extracted with acidified *n*-butanol produced additional metabolites at R_f 0.39, 0.44, 0.73, and 0.76.

Thick-layer chromatography of urine using solvent system *d* indicated TIBA (R_f 0.54), 2,5-DIBA (R_f 0.52), iodide ion (R_f 0.42), three major metabolites at R_f 0.50, 0.46, and 0.38, and three minor metabolites at R_f 0.95, 0.60, and 0.28. The metabolite at R_f 0.95 was only seen in goat 13. A spot at R_f 0.60 was found in the urine of all animals examined and corresponded to an impurity found in the crude synthesis product of TIBA-^{14}C and TI*BA.

Skim milk from the treated animals was separated by solvent system *d* into a major metabolite of iodide ion (R_f 0.42) and a nonmigrating residue. The residue at the origin was not seen in pancreatin-treated skim milk. Except for goat 12, traces of two other metabolites in skim milk at R_f 0.54 and 0.38 were found. Chromatograms of the supernatant of the thyroid digest of goats left a residue at the origin, the iodide ion (R_f 0.42) and traces at R_f 0.52 and 0.54. The spots at R_f 0.52 and 0.54 were also evident in the acidified *n*-butanol extracts of the digested thyroid tissue.

The mean recovered volume of skim milk from whole milk for the four goats was 89.8% (±3.9) and contained 91.0% (±2.3) of the radioactivity of whole milk. Accordingly, the radioactivity was seen to be evenly concentrated in the skim milk and cream portions of goat's milk. A similar relationship was evident in the cow's milk. Protein-bound

radioactivity in whole milk was 19.4% (±10.2) in the goats and 6.2% in the cow of the total radioactivity in whole milk.

SUMMARY AND CONCLUSIONS

1. Whole body retention studies of TI*BA in four adult lactating goats showed a two-component system—one with a biological half-life of 13.5 hr. and the second of 423 hr.

2. Excretion studies in five adult lactating goats and one cow showed that the primary route of excretion was *via* the kidney. For the goats, 76, 12, and 3%, respectively, of the administered dose was excreted in the urine, milk, and feces. Corresponding values for the cow were: 57.5, 12.2, and 11.4%.

3. Distribution studies, 8 hr. after oral administration of TI*BA to goats, indicated TIBA and/or its metabolites were located in the kidney, heart, thyroid, blood, spleen, G.I. tract, liver, fat, lean, and brain. The G.I. tract contained 41% of the administered dose at 8 hr. The average relative concentration of radioactivity in the thyroid was 28 times the average blood concentration. Thyroid uptake occurred for 5 days after oral TIBA administration.

4. Metabolite studies of the urine indicated TIBA and nine metabolites. Four of the metabolites were identified as 2,5-DIBA, 2,3-DIBA, OIBA, and iodide ion. In the 6–18 hr. urine sample of the cow, TIBA accounted for 28% of the radioactivity while the predominate metabolite, 2,5-DIBA, accounted for 45% of the radioactivity. In goats, 21% of the radioactivity in the accumulated 0–8 hr. urine sample was attributable to 2,5-DIBA while 55% was TIBA. The remaining (24%, goat and 27%, cow) metabolites in the urine are explained as representing iodide ion and conjugated forms of OIBA, 2,3-DIBA, 2,5-DIBA, and TIBA.

5. Milk analyses indicated iodide ion as the predominant (82.8–100.0%) metabolite. Eighty-one percent of the radioactivity within the milk was nonprotein bound in the goat while 94% was nonprotein bound in the cow. The two unknowns in skim milk ranged from 0% up to 17.2% of the radioactivity in the milk of goats and was 1.8% of the cow's milk.

REFERENCES

(1) Zimmerman, P. W., and Hitchcock, A. E., *Contrib. Boyce Thompson Inst.*, **12**, 321(1942).
(2) Galston, A. W., *Am. J. Botany*, **34**, 356(1947).
(3) Ebert, A. G., and Ware, J. H., International Minerals and Chemical Corp., Skokie, Ill., 1965, personal communication.
(4) Spitznagle, L. A., Master of Science Thesis, Purdue University, Lafayette, Ind., 1966.
(5) Ice, R. D., Breckinridge, C. E., Jr., and Christian, J. E., *J. Pharm. Sci.*, **55**, 497(1966).
(6) Barker, W. M., Thompson, D. J., and Ware, J. H., International Minerals and Chemical Corp., Skokie, Ill., 1967, personal communication.
(7) Gutenmann, W. H., Bache, C. A., and Lisk, D. J., Presented at TIBA Workshop, International Minerals and Chemical Corp., Skokie, Ill., 1966.
(8) Christian, J. E., Kessler, W. V., and Ziemer, P. L., *Int. J. Appl. Rad. Isotopes*, **13**, 557(1962).
(9) West, C. D., Wayne, A. W., and Chavre, V. J., *Anal. Biochem.*, **12**, 41(1965).
(10) Downie, N. M., and Heath, R. W., "Basic Statistical Methods," Harper and Row, New York, N. Y., 1959, pp. 85, 117.
(11) Shaw, S. M., Kessler, W. V., and Christian, J. E., *J. Pharm. Sci.*, **54**, 1208(1965).
(12) Hansard, S. L., Comar, C. L., and Plumlee, M. P., *Nucleonics*, **9**, 13, 38(1951).
(13) "Standard Methods for the Examination of Water and Wastewater," 12th ed., American Public Health Assoc., Inc., New York, N. Y., 1965, p. 534.
(14) Weaver, J. C., Kamm, M. L., and Dobson, R. L., *J. Am. Med. Assoc.*, **173**, 872(1960).

Keyphrases

2,3,5 Triiodobenzoic acid (TIBA)
Metabolic fate, TIBA-lactating cow, goats
Thyroid uptake—TIBA
Excretion, TIBA-urine, milk, feces
Scintillation counting-whole body
Column chromatography—TIBA identification
Thick-layer chromatography—component identification

Complexing Behavior of Starches with Certain Pharmaceuticals

By ZEYAD MANSOUR and EARL P. GUTH

A study has been made of the complexing behavior of a number of starches and starch fractions with benzoic acid, *p*-hydroxybenzoic acid, sorbic acid, and other selected molecules in aqueous solutions at 30°. By means of the solubility method of analysis it was found that the low molecular weight polymer, amylose, is the main complexing component of starch. The starches showed different affinities for the same drug according to their content of amylose. A correlation of these complexes to those of "starch-alcohols" and "starch-iodine" was made.

STARCH has a multitude of applications in many areas of our life; *e.g.*, foods, drug therapy, and many industries. It has a long history of usefulness in medicine and pharmacy. Its use, however, has been entirely empirical. It is described as a protective, an adsorbent, and as a diluent in texts on pharmacology, with no explanation of the mode of action. Starch appears

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