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EXCRETION, DISTRIBUTION AND METABOLIC FATE OF  $^3\text{H}$ - $\alpha$ -CHACONINE.

W. P. Norred, K. Nishie and S. F. Osman<sup>1</sup>

Richard B. Russell Agricultural Research Center  
United States Department of Agriculture  
Agricultural Research Service  
Athens, Georgia 30604

and

<sup>1</sup>Eastern Regional Research Center  
United States Department of Agriculture  
Agricultural Research Service  
Wyndmoor, Pennsylvania 19118

ABSTRACT

$^3\text{H}$ - $\alpha$ -Chaconine was poorly absorbed from the gastrointestinal tract and rapidly excreted in feces when administered orally to male rats. Intraperitoneal administration of low doses (5 to 10 mg/kg) resulted in urinary and fecal excretion of metabolites, and probably involved biliary excretion. High, toxic, intraperitoneal doses (15 to 25 mg/kg) depressed fecal and urinary elimination, and resulted in accumulation of tritium in various tissues. The major metabolite appeared to be the aglycone, solanidine.  $\alpha$ -Chaconine is very similar to  $\alpha$ -solanine in its elimination by and distribution in tissues of rats.



to the nitrogen atom by New England Nuclear Corp.<sup>2</sup> Thin layer chromatography (TLC) on silica gel G (0.25 mm thick) with 1% NH<sub>4</sub>OH: ethanol: chloroform (10:40:27) as developing solvent, a modification of the solvent used by Boll (1962), and detection with a windowless radiochromatogram scanner (Searle Actigraph III) revealed a major radioactive peak with an R<sub>f</sub> value (0.72) corresponding to that of authentic α-chaconine. A minor peak, comprising 15% of the radioactivity, with an R<sub>f</sub> of 0.89 was also detected. The <sup>3</sup>H-α-chaconine was subsequently purified by preparative TLC on 0.5 mm silica gel G plates and eluted from the plate with hot ethanol.

Male Sprague-Dawley rats (ARS Sprague-Dawley, Madison, Wisconsin) weighing 200-300 g were used in all experiments. For excretion and tissue distribution studies, α-chaconine was dissolved in propylene glycol: dimethyl sulfoxide (7:3), and <sup>3</sup>H-α-chaconine added to provide a dose of 3 x 10<sup>8</sup> dpm/kg. Rats were dosed orally (p.o) or intraperitoneally (i.p.) and housed in plastic metabolism cages with free access to food and water. At the termination of the experiment, the rats were anesthetized with ether, and blood was collected in a heparinized syringe by cardiac puncture. Rats were killed by overdose of ether, organs removed, rinsed in distilled water, weighed and homogenized in 9 parts of water with a Polytron PT 10 homogenizer (Brinkmann Instruments). Feces were homogenized in a similar manner. Urine samples (1 ml) were mixed in scintillation vials with 4 ml water and 10 ml Insta-Gel (Packard Instruments Co.) Blood samples (0.1 ml) feces or organ homogenates (0.2 ml) were solubilized

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<sup>2</sup>Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

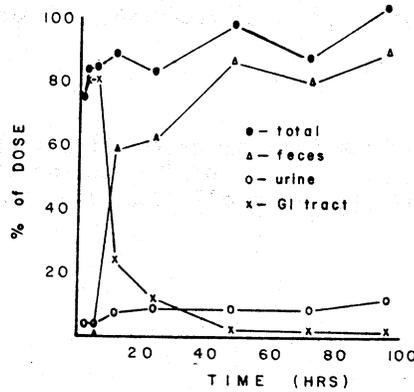
with 2 ml Soluene 100: isopropanol (1:1) (Packard Instrument Co.) and mixed with 15 ml Insta-Gel: 0.5N (9:1). Blood samples were bleached with 0.5 ml of 30% hydrogen peroxide prior to addition of Insta-Gel. Determinations of radioactivity were made in duplicate for all samples. Scintillation vials were stored in the dark at 10°C for at least 24 hr prior to counting in a Packard Tri-Carb Model 3330 liquid scintillation spectrometer. Counting efficiency was determined by the external standard channel ratio technique with quench curves prepared from tissue homogenates containing known amounts of tritiated water and varying quantities of chloroform as quenching agent.

For metabolic studies, rats were administered 5 mg/kg  $^3\text{H}$ - $\alpha$ -chaconine ( $2.3 \times 10^9$  dmp/kg) orally or parenterally. Urine and fecal samples were collected 24 hr later, feces homogenized in 4 parts water, and the samples adjusted to pH 11 with 10 N sodium hydroxide and extracted 3 times with ether. The ether extracts were dried with anhydrous sodium sulfate, evaporated to dryness under a stream of  $\text{N}_2$ , and the residues dissolved in a minimum of ether (about 0.5). Aliquots (5  $\mu\text{l}$ ) of the ether extracts were spotted on 0.25 mm thick silica gel G plates and developed with 1%  $\text{NH}_4\text{OH}$ : ethanol: chloroform (10:40:27) or the top phase of ethyl acetate: pyridine: water (30:10:30) Boll (1962). The plates were analyzed for tritium by radiochromatogram scanner. Also, bands of silica gel (0.5 mm) were scrapped from the plates directly into scintillation vials containing 4 ml  $\text{H}_2\text{O}$  and 10 ml Insta-Gel and radioactivity determined.

Oral administration of  $^3\text{H}$ - $\alpha$ -chaconine resulted in fecal elimination of 60% of the dose within 12 hr and 80% of the dose within 48 hr (Fig. 2).

Urinary excretion of tritium was 5% of the dose 3 to 6 hr after administration, and reached a plateau of 10% of the dose between 12 and 24 hr.

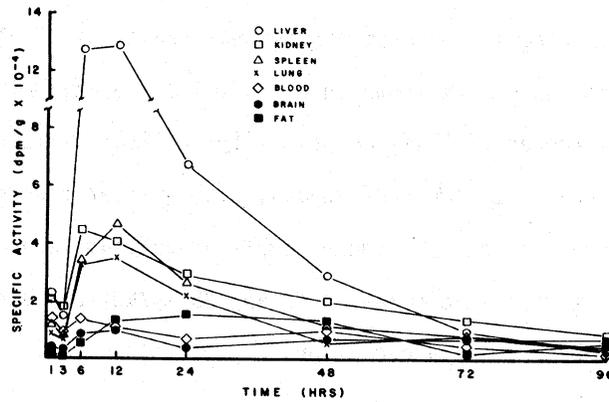
Fig. 2. Recovery of tritium after administration of a single dose of  $^3\text{H}$ - $\alpha$ -chaconine (5 mg/p.o.). Each point represents average determinations from 2 rats.



Twenty-four hours after treatment only 10% of the dose was detected in the gastrointestinal tract; after 48 hr, only 3% was detected.

Levels of radioactivity determined in selected tissues were maximum 6 to 12 hours after oral administration (Fig. 3). Liver contained the highest concentration of tritium, and kidney, spleen, and lung had

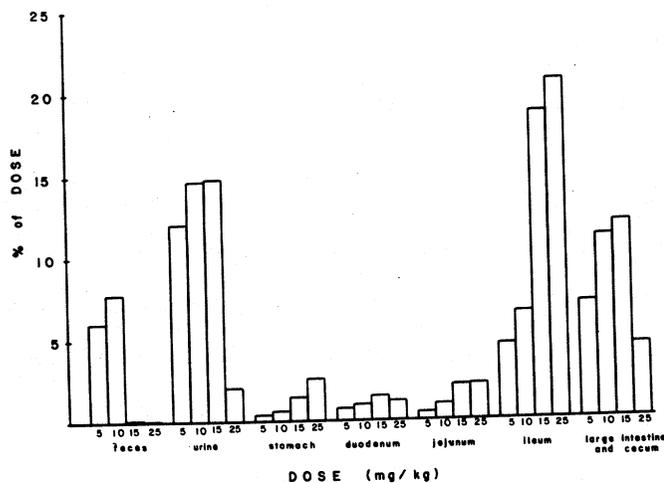
Fig. 3. Tritium distribution in tissues at various intervals after a single oral dose  $^3\text{H}$ - $\alpha$ -chaconine (5 mg/kg). Each value is average of determinations from 2 rats.



intermediate levels. Levels of radioactivity were low in blood, brain, abdominal fat, and in the following tissues which are not presented in Fig. 3 for reasons of clarity: adrenals, testis, pancreas, muscle, thymus, thyroid and heart.

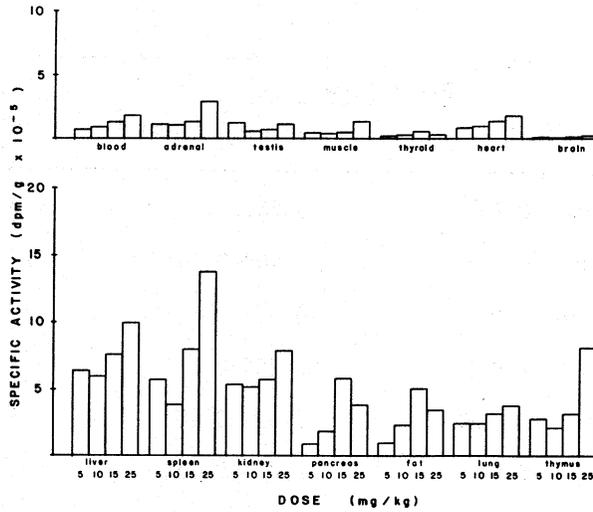
After intraperitoneal administration of  $^3\text{H}$ - $\alpha$ -chaconine, radioactivity was excreted primarily in urine (Fig. 4).

Fig. 4. Excretion and recovery of tritium from the gastrointestinal tract 24 hr after a single i.p. injection of  $^3\text{H}$ - $\alpha$ -chaconine. Values are averages of determinations from 2 rats at each dose.



Fecal elimination of tritium was apparent with doses of 5 and 10 mg/kg. However, excretion by this route was nil at higher doses, and the percentage of the dose remaining in the gastrointestinal tract, particularly the ileum, increased with dose. As shown in Fig. 5, intraperitoneal doses of  $^3\text{H}$ - $\alpha$ -chaconine tended to localize primarily in liver, spleen, kidney, pancreas, abdominal fat, lung and thymus, with smaller concentrations occurring in other tissues. Higher levels of tritium were found in these tissues at the toxic doses of 15 and 25 mg/kg, corresponding to impaired fecal and/or urinary excretion.

Fig. 5. Tissue concentration of tritium 24 hr after a single i.p. injection of  $^3\text{H}$ - $\alpha$ -chaconine. Values are average of determinations from 2 rats at each dose.



Tritium content was higher in every tissue studied after intraperitoneal administration than after oral treatment, as shown by i.p.:p.o. ratios greater than 1.0 in Table 1. The ratio was particularly high for kidney, which had a concentration in i.p. treated animals about 28 times that found in kidneys of p.o. dosed rats.

Table 1. Comparison of Tissue Distribution of Tritium 24 Hours After Oral or Intraperitoneal Administration of  $^3\text{H}$ - $\alpha$ -Chaconine (5 mg/kg)

Tissue	Percentage Dose <sup>a</sup>		Ratio ip:po
	Oral	Intraperitoneal	
Liver	1.294 $\pm$ 0.297	8.061 $\pm$ 0.770	6.2
Kidney	0.053 $\pm$ 0.013	1.469 $\pm$ 0.097	27.7
Spleen	0.036 $\pm$ 0.002	0.471 $\pm$ 0.078	13.1
Pancreas	0.031 $\pm$ 0.003	0.206 $\pm$ 0.041	6.6
Adrenal	0.004 $\pm$ 0.0002	0.022 $\pm$ 0.002	5.5
Testis	0.047 $\pm$ 0.013	0.474 $\pm$ 0.165	10.1
Thymus	0.013 $\pm$ 0.001	0.259 $\pm$ 0.035	19.9
Heart	0.018 $\pm$ 0.0015	0.108 $\pm$ 0.009	6.0
Lung	0.088 $\pm$ 0.032	0.568 $\pm$ 0.077	6.5
Thyroid	0.0005 $\pm$ 0.00015	0.002 $\pm$ 0.0006	4.0
Brain	0.015 $\pm$ 0.006	0.046 $\pm$ 0.010	3.1
Blood <sup>b</sup>	0.172 $\pm$ 0.039	1.362 $\pm$ 0.391	7.9

<sup>a</sup>Each value is the average of duplicate determinations from 2 rats  $\pm$ S.E.

<sup>b</sup>Based on total blood volume of 64.1 ml/kg body weight (Altman and Dittmer, 1964).

Metabolism of  $^3\text{H}$ - $\alpha$ -chaconine

When  $^3\text{H}$ - $\alpha$ -chaconine was administered orally, 63.4% of the tritium was extractable from fecal homogenates into ether, but only 15.7% of the activity in urine could be extracted. After i.p. administration, less activity could be extracted from feces (51.8%), but only slightly more was extractable from urine (18.6%). Thin layer chromatography of the ether extracts revealed a major constituent in both urine and feces of both p.o.-and i.p.-treated rats with an  $R_f$  value the same as the aglycone of  $\alpha$ -chaconine, solanidine. Feces of p.o.-dosed rats also contained a radioactive component having the same  $R_f$  as unchanged  $\alpha$ -chaconine, which represented about 25% of the total radioactivity of the feces. Development of the plates with a different solvent, the top layer of ethyl acetate: pyridine: water (30:10:30) as described by Boll (1962), showed that the fecal and urine extracts contained 2 or more compounds more polar than solanidine, but less polar than  $\alpha$ -chaconine. These compounds were minor components, representing only 1-5% of the total activity, and were most apparent in urine of intraperitoneally-dosed rats.

DISCUSSION

The poor gastrointestinal absorption and rapid fecal excretion of  $\alpha$ -chaconine observed in this study indicates that  $\alpha$ -chaconine, like  $\alpha$ -solanine (Nishie et al., 1971), has low oral toxicity. When administered intraperitoneally, however,  $\alpha$ -chaconine is acutely toxic (Nishie et al., 1975) and the compound and/or its metabolites begin to accumulate in tissues at doses (15 to 25 mg/kg) which interfere with urinary and fecal elimination. Excretion of tritium in feces and accumulation in the GI

tract of lower doses after i.p. administration indicates biliary excretion of  $^3\text{H}$ - $\alpha$ -chaconine. Fecal excretion appears to be about 50% as effective as urinary excretion (Fig. 4), and probably accounts for the rapid elimination of tritium from blood and other tissues.

Distribution and metabolism of  $\alpha$ -chaconine are similar to that of  $\alpha$ -solanine reported previously (Nishie et al., 1971). We had felt that differences in the sugar moiety of the two compounds might influence GI absorption. This does not appear to be the case. The testing of other glycoalkaloids found in potatoes for degree of GI absorption and toxicity is necessary in order that the safety of certain potato hybrids may be evaluated.

#### ACKNOWLEDGEMENT

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