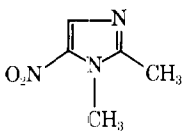
A Method for Analysis of Swine Tissue for the Primary Metabolite of Dimetridazole at the 2-ppb Level

Elliott M. Craine,* Michael J. Parnell,[1] and Loyal R. Stone

A method is described which quantitatively measures 2-hydroxymethyl-1-methyl-5-nitroimidazole, the primary metabolite of dimetridazole, at levels as low as 2 ppb in the tissues of pigs. The method involves extraction, concentration, and cleanup followed by measurement by differential pulse polarography in a borate buffer. From a public safety standpoint muscle and kidney are considered to be the critical tissues. In muscle and kidney average recoveries of 67 and 55% were obtained. The residue equivalent for control samples is 0.2 ppb or less for all tissues. A group of 15 pigs was medicated with dimetridazole in their drinking water for 5 days so that they received 10 mg/kg of body weight per day. At intervals after withdrawal of the medication groups of three pigs were slaughtered and analyzed for nitroimidazole residues by the method described. At zero withdrawal, residues in muscle and kidney which averaged 300 and 235 ppb, respectively, were the highest. Within 3 days after withdrawal of medication measurable residues were not present.

Dimetridazole or 1,2-dimethyl-5-nitroimidazole (1) has been used for many years to control histomoniasis in turkeys (Lucas, 1962). Recently it has been shown to be active in arresting the infection known as swine hemorrhagic dysentery (Anderson, 1973; Bech and Hyldgard-Jensen, 1972; Bechet *et al.*, 1970; Cotterau, 1971; Griffin, 1972; Miller and Fox, 1973).



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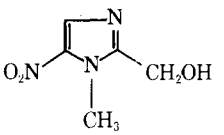

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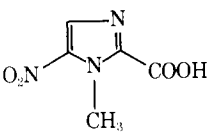

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Early metabolic studies with dimetridazole-^{14}C (Law *et al.*, 1963) showed that in turkeys the compound was oxidized to 2-hydroxymethyl-1-methyl-5-nitroimidazole (2), hereafter designated the alcohol metabolite. The latter was further oxidized to 1-methyl-5-nitroimidazole-2-carboxylic acid (3), hereafter designated the acid metabolite. Recently, a similar nitroimidazole, ronidazole, was shown to undergo rapid and complete biodegradation in turkeys (Rosenblum *et al.*, 1972). Unsworth and Maycey (1974) found that dimetridazole-^{14}C also undergoes extensive degradation when given to swine. However, low levels of nitroimidazole residues were present in tissues of pigs after medication with dimetridazole-^{14}C. Six hours after medication the highest levels were in muscle and kidney tissue. The major component of the residue was identified as the alcohol metabolite (2), the primary metabolite of dimetridazole. Lesser amounts of the parent compound and the acid metabolite (3) were found in the tissues. Seventeen hours after medication only the alcohol metabolite was detected in tissues and measurable levels of residue were present only in muscle. These results lead to the conclusion that from a public safety standpoint a method is required to measure the metabolite, 2-hydroxymethyl-1-methyl-5-nitroimidazole, in the two critical tissues of the pig, muscle and kidney.

Hess & Clark, Ashland, Ohio 44805.
[1] May & Baker Ltd., Dagenham, Essex, England.

Polarography has been used to measure dimetridazole in tissues (Kane, 1961; Parnell, 1973; Allen and McLoughlin, 1972) and in feeds (Cooper and Hoodless, 1967; Daftsios, 1964; Kane, 1961; Stevens, 1969). Parnell (1973) has developed a method, based on conventional dc polarography, for determination of dimetridazole in pig tissue down to 0.1 ppm. The present report describes a new procedure developed with the objective of measuring 2-hydroxymethyl-1-methyl-5-nitroimidazole in pig tissue. The method measures 2 ng/g (2 ppb) of nitroimidazole residue using a 100-g sample of tissue. The tissues are extracted with ethyl acetate after homogenization in the presence of sodium chloride and dibasic potassium phosphate. The extract is purified by three liquid–liquid transfers and chromatography on silica gel. The final liquid transfer brings the compounds into a hydrochloric acid solution. The chloride ions are removed and replaced with borate ions by way of an anion exchange resin. Solid borax is added giving a borate buffer concentrate at pH 8 which is then suitable for a differential pulse polarographic measurement. An internal standard of the alcohol metabolite is added to quantitate the measurement and thus final residue values relate to that compound.

DEVELOPMENT OF METHOD

Previous residue methods associated with the use of dimetridazole have been based on measurement of the parent compound itself (Allen and McLoughlin, 1972; Kane, 1961; Parnell, 1973). As noted above, metabolism studies with dimetridazole-^{14}C indicated that it was necessary to have a method to measure the alcohol metabolite (2) of dimetridazole for residue studies in swine. A measurement of other nitroimidazoles would be useful but the metabolite was the target compound. The metabolism work also demonstrated that muscle and kidney were the critical tissues and emphasis was placed on obtaining a reliable method with good recoveries of the metabolite from those tissues.

Although gas chromatography using an electron capture detector provides a sensitive method for determination of nitroimidazoles, the alcohol metabolite was found to be unstable at temperatures which would give reasonable retention times. The metabolite exhibits a well-defined polarographic response and therefore polarography was selected as the means of measurement. Differential pulse polarography allowed improved sensitivity so that measurement at levels as low as 2 ppb was accomplished.

During the last decade lack of an economical, reliable instrument has hindered the use of differential pulse polarography (Christie *et al.*, 1973). The situation has im-

proved with the recent introduction of the Princeton Applied Research (PAR) Model 174 polarographic analyzer which was used in this study. Christie *et al.* (1973) evaluated the Model 174 and cautioned on proper usage of the instrument. In agreement with their recommendations, the parameters of scan rate, drop time, and pulse amplitude were kept constant for the analysis described. The settings used were selected to obtain the maximum response; for example, maximum pulse amplitude for the instrument (100 mV) was used.

The supporting electrolyte used in polarographic methods for dimetridazole in tissues was a mixture of potassium chloride and potassium hydroxide. However, nitroimidazoles gave a stronger current response in the presence of borate ions. At the end of the concentration and cleanup steps the chloride ions present were replaced with borate ions to take advantage of the stronger signal. This was accomplished with a strong anion exchanger in the borate form. The boric acid solution arising was saturated with borax to give a borate buffer at pH 8. The procedures described eliminated interferences producing polarographic peaks in the region of −0.45 to −0.85 V and provided a base line satisfactory for analysis.

Components from the tissues suppress the polarographic signal of dimetridazole and the alcohol metabolite about 30%. Since the suppression varies, the amounts of nitroimidazole present are measured quantitatively by comparison to an added internal standard. The alcohol metabolite was used as the standard and all residue values are reported with reference to that compound.

EXPERIMENTAL SECTION

Apparatus. *Homogenizer.* A Silverson Mixer Emulsifier, standard laboratory model with a 0.75-in. tubular assembly (Global Process Equipment Inc., Hauppauge, N. Y.), was used.

Polarograph. A Princeton Applied Research (PAR) polarographic analyzer Model 174 was used in conjunction with a PAR Model 172 drop timer and dropping mercury electrode (dme). A section of 0.5-in. copper braided shield was placed around the dme to screen out electrical interferences. The shield was grounded to the timer head. PAR polarographic cells, Model 9300, with PAR outgassing tubes, Model 9330, were used. Polarograms were recorded on an Aminco X–Y recorder used with a voltage divider to attenuate the signal to the *Y* axis by a factor of 200.

Ag|AgCl Electrode. A solid silver wire (22 gauge) was coiled around a glass rod (0.25 in. o.d.) to form a helix with seven turns (helix height is 1.5 cm). The helix was coated with AgCl by electrolysis in 1 *N* hydrochloric acid.

Outgassing Assemblies. Before polarographic analysis interfering oxygen was removed with oxygen-free nitrogen with an outgassing assembly. Prepurified nitrogen was passed through an oxygen scrubbing system which consisted of two 500-ml gas washing bottles with fritted disks for dispersion. The first bottle contained 300 ml of vanadous chloride solution and the second contained 300 ml of saturated borax solution.

Glassware. Where possible amber glassware was used. Only Teflon stopcocks were used. After cleaning, all glassware was routinely rinsed with distilled water, ultra-water, and reagent grade acetone.

Materials. The solvents ethyl acetate, methylene chloride, and hexane were all Mallinckrodt nanograde or Burdick and Jackson, distilled in glass. Borax (disodium tetraborate) was Analar grade of BDH Chemicals Ltd. All other salts, hydrochloric acid, and sodium hydroxide were reagent grade. The silica gel was Hi-Flosil (60–200 mesh) from Applied Science Laboratories. Mercury for the dme was triple distilled.

A purified distilled water, labeled as "ultra-water," was used. Raw water was processed through a commercial deionization system and then distilled through an efficient glass still such as the Bellco Loughborough Still. The distilled water was percolated successively through columns of granular coconut charcoal, a cation exchange resin in the hydrogen form (Dowex 50-X8), and an anion exchange resin in the hydroxyl form (Dowex 1-X8). Quality of the ultra-water was checked routinely by polarography of a sample saturated with borax.

Anion exchange resin was standard grade Dowex 1-X2 (50–100 mesh) from Bio-Rad cycled to the borate form. To condition the resin it was cycled to the hydroxide form with 1 *N* sodium hydroxide and then back to the chloride form with 1 *N* hydrochloric acid. The resin was then converted to the borate form by percolation of saturated borax solution through the resin bed followed by wash with water until the effluent pH was neutral.

Preparation of Columns. For the silica gel chromatography a small glass wool plug was placed in the bottom of a closed 9 mm i.d. × 300 mm long glass column. Methylene chloride (10 ml) was poured into the column followed by the addition of 2 g (±0.1 g) of Hi-Flosil. The mixture was stirred with a thin glass rod to settle the adsorbent and remove air bubbles. Columns were prepared immediately before use.

The column for the ion-exchange step supported the resin on a sintered glass disk with a low hold-up volume. The column was amber, 9 mm i.d. × 150 mm long with a Teflon stopcock and seal. Each column was calibrated for a 5-ml bed of resin. Dowex 1-X2 (borate form) was added in slurry with ultra-water. A small plug of glass wool was placed on top of the resin bed without compacting it. The column was drained to the level of the glass wool plug.

Standard Solutions and Fortified Samples. A series of aqueous standard solutions was used for internal standards in the polarographic measure as well as for recovery studies. Solutions must be stored in the dark, and were stable for several weeks at room temperature. For a stock solution 100 mg of 2-hydroxymethyl-1-methyl-5-nitroimidazole was dissolved in ultra-water and made to a volume of 100 ml. A series of dilutions of the stock solution was made with ultra-water to give concentrations of 1.0, 10.0, 50, and 500 μg/ml.

Background or interference due to reagents was determined by processing a reagent blank. Ethyl acetate (230 ml) was placed in a 500-ml separatory funnel with 10 ml of ultra-water. The funnel was stoppered and shaken for 30 sec. The ethyl acetate phase was processed through the entire procedure. Polarograms of the borate concentrates from reagent blanks should have little or no wave in the region of −0.7 V.

The procedure was evaluated by fortifying the ethyl acetate of reagent blanks with portions of aqueous standards equivalent to 2, 5, and 10 ppb (200, 500, or 1000 ng of alcohol metabolite) and processing each sample. Similarly, recovery in the method was evaluated by fortifying 100-g portions of diced tissues with known amounts of aqueous standard. Each sample was then processed through the entire procedure.

Tissue Homogenization and Extraction. The tissues used for analysis were frozen because the freezing process removes interfering components. A diagram of the process is shown in Figure 1. The frozen tissue was sliced into pieces on a clean chopping board using a heavy 1.5-in. wood chisel. The tissue was subdivided into 1-cm cube size with a dissecting knife. A 100-g sample of diced tissue was weighed into a 250-ml centrifuge bottle. Ethyl acetate (90 ml; 60 ml for fat sample), 20 g of dibasic potassium phosphate, and 20 g of sodium chloride were added to the diced tissue sample.

The mixture was homogenized with a Silverson blender for 2 min or until the contents of the bottle appeared to be homogeneous. The sample was centrifuged for 10 min at about 900*g* and the ethyl acetate layer was decanted into a 500-ml storage flask. Ethyl acetate (60 ml) was

added to the residual in the centrifuge bottle. The compacted tissue was broken up with a stainless steel spatula, stirred vigorously for 2 min, and centrifuged for 10 min. The ethyl acetate layer was decanted. The second extraction was repeated two times and all extracts were combined. The combined extracts totaled about 230 ml for muscle and kidney tissue, 250 ml for skin, or 320 ml for fat. The increased volume of the extract from skin and fat is due to the increased amounts of solids which are soluble in ethyl acetate.

Cleanup and Concentration. The total ethyl acetate extract was transferred to a 250-ml separatory funnel (500-ml funnels were used for extracts from fat samples). To the extract was added 5 ml of 1 *N* HCl; the funnel was stoppered and shaken by hand for 30 sec. The phases were allowed to separate and then the acid layer was drained into a 50-ml glass-stoppered centrifuge tube. The extraction and separation were repeated with three additional 5-ml portions of 1 *N* HCl, combining the acid extracts.

Dibasic potassium phosphate (10 g) was dissolved in the acid extract. The tube was cooled in a water bath at 20°; then an additional 10 g of dibasic potassium phosphate was dissolved in the solution. The tube was cooled to room temperature.

Methylene chloride (10 ml) was added to the centrifuge tube, which was stoppered and shaken for 30 sec, and centrifuged for 1 min. The top layer was withdrawn to a 50-ml storage flask. The extraction was repeated four times using 10-ml portions of methylene chloride. The extracts were combined and 2 g of anhydrous granular sodium sulfate was added to dry the methylene chloride.

The entire methylene chloride extract was passed through a column of silica gel (Hi-Flosil). Methylene chloride (10 ml) was percolated through the column as a wash. Air was forced through the column by means of a rubber bulb to remove solvent from the column, the effluent was discarded, and 20 ml of water-saturated ethyl acetate was passed through the column collecting the eluate in a 60-ml separatory funnel.

The ethyl acetate eluate was extracted in the funnel with four successive 1.5-ml portions of 0.5 *N* HCl, shaking the funnel 20 sec for each extraction. The acid extracts were combined in a test tube and extracted with 5 ml of hexane. The hexane layer was removed and discarded.

The acid extract (5 ml) was pipetted onto an ion exchange column. The flow rate was adjusted to 1 drop in 5–10 sec; 5 ml of effluent was collected and discarded. Ultra-water was added to the column to maintain the flow rate at 1 drop in 5–10 sec, and 9 ml of effluent was collected. Samples may be held overnight at this point if stored in the dark.

Borax (1 g) was added to the retained effluent. The contents were mixed to saturate the solution with borax. The borate concentrate was analyzed by differential pulse polarography.

Polarographic Analysis. Differential pulse polarograms were run on 5.0-ml samples using a three-electrode system with borate buffer (pH 8) as the supporting electrolyte. The top of the polarographic cell has five openings; two openings were closed with stoppers. The outgassing tube was placed in the third opening. The stopcock of the outgassing tube controls the flow of gas so that it bubbles into the liquid of the cell or blankets the surface of the cell liquid. In the fourth opening a stopper was inserted to hold a platinum wire (counter electrode) which contacted the solution in the cell but not the mercury pool. The dme capillary was positioned into the helix of the Ag|AgCl electrode. The silver wire was taped to the capillary to hold the helix firmly in position so that it did not come below the end of the capillary. The combined dme capillary (working electrode) and the Ag|AgCl electrode (reference electrode) were positioned through the fifth opening (center) of the cell top. The capillary was not fixed rigidly as this would prevent dislodging the mercury drops.

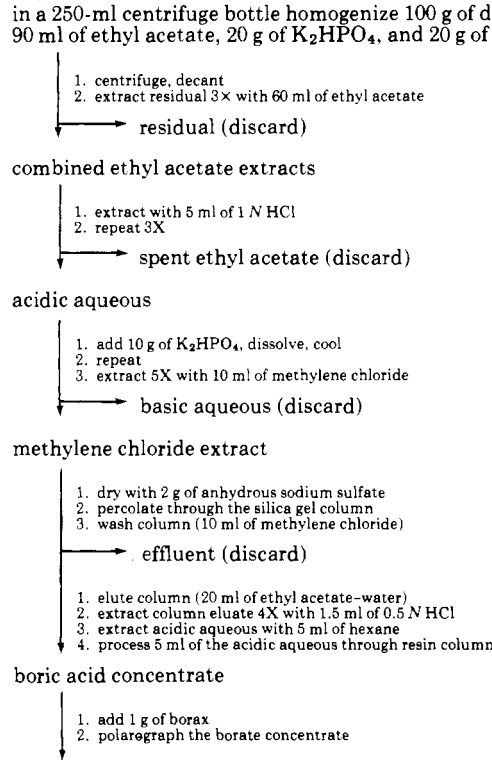

Figure 1. Diagram of extraction and cleanup.

Before a polarographic run the instrument was adjusted to the following settings: potential scan rate, 2 mV/sec; potential scan range, 0.75 V; modulation amplitude, 100 mV; operating mode, differential pulse; initial potential, −0.3 V; drop time, 2 sec. The current range setting depends on the level of residue. When the residue level is 20 ppb or lower, a current range of 0.5 μA is used. To position the scan properly on the chart it was necessary in our laboratory to set the output offset at −60. With our recorder full-scale deflection was set at 16 units or 16 cm. Thus 0.1 unit (1 mm) on the chart paper or 0.003 μA was readable. The relation between concentration and peak current is linear until the concentration is greater than 1 μg/ml. Measurement at levels above 20 ppb is valid, although it is necessary, of course, to increase the current range. At a current range of 1.0 μA the output offset was reduced to −30 and at 2.0 μA or higher no output offset was required.

For polarographic analysis an excess of solid borax was added to saturate the solution to be analyzed. The solution (5.0 ml) was placed into a clean dry polarographic cell base. The liquid was outgassed with oxygen-free nitrogen at a flow of 0.5 ft^3/hr for 10 min without mercury in the cell. The electrodes and capillary were washed with ultra-water. Excess water was removed gently from the electrodes and capillary with a clean disposable paper wiper. *Caution:* do not touch the end of the capillary. The cell was positioned firmly so that the capillary and electrodes are submerged. The liquid was outgassed with oxygen-free nitrogen for 5 min. The gas inlet was adjusted to blanket the surface of the liquid with oxygen-free nitrogen and the capillary gently tapped to dislodge bubbles of gas from the tip and activate the drop timer.

The selector switch was moved to the external cell position. The rise in current was observed on the current output meter and then the output offset switch moved to the negative circuitry; scan was from −0.3 to −0.9 V. If the concentration of residue was 20 ppb or greater the polaro-

Table I. Recovery of Alcohol Metabolite from Reagent Blanks Fortified with Aqueous Aliquots of Standard Solution of Metabolite

Sample	Alcohol metabolite added, ppb	Peak current (i_p)		
		Sample, μA	Sample plus standard,[a] μA	Recovery, %
1	0	0.003		
2	2	0.081	0.550	93.5
3	2	0.075	0.562	83.1
4	5	0.175	0.650	79.6
5	5	0.188	0.656	86.4
6	10	0.353	0.838	78.7
7	10	0.366	0.850	81.5

[a] The peak current after addition of internal standard raising the concentration by 100 ng/ml.

graphic wave exceeded the full scale current. If this occurred we returned to the initial potential and changed the current range and the output offset until an adequate polarogram was obtained.

An internal standard was used to quantitate the analysis. The base of the cell was removed and 50 μl of standard solution of the alcohol metabolite was added. The cell was rotated at a 45° angle to mix all liquid contents thoroughly. The cell was reassembled, the solution outgassed for 5 min, and a second polarographic scan was run. Current range and output offset had to be adjusted.

Peak current (i_p) at peak potential from both polarograms was calculated as was the increment of current due to the addition of the standard and the value of i_p/unit of alcohol metabolite. Using the value i_p/unit of alcohol metabolite the concentration in the borate concentrate was calculated and related to the original tissue sample.

EVALUATION OF RESULTS

Under the conditions of the polarographic analysis the nitroimidazoles produced a polarographic wave with a peak potential at about −0.7 V. Tissue blanks which are borate concentrates from control tissue samples gave polarograms with a continuously descending line with either no wave at −0.70 V or a slight wave amounting to 0.003 μA. Figure 2 is a typical muscle blank; in this case there is no wave at −0.70 V. Figure 3 is a scan of the same solution after addition of alcohol metabolite to make the concentration 30 ng/ml.

A linear relation exists between the concentration of a nitroimidazole in a borate concentrate and peak current in polarograms unless the concentration is >1 μg/ml. The linear response occurs in borate concentrates from tissue samples as well as reagent blanks. To show the linearity, a group of samples of control muscle tissue was processed through the procedure to borate concentrates which were then pooled. Aliquots (50 μl) of standard aqueous alcohol metabolite solution were added to 5.0-ml portions of the pool which were then polarographed.

A standard curve was prepared with fortified (spiked) reagent blanks. After ethyl acetate and water were mixed and separated, aqueous standards (0.2, 0.5, or 1.0 ml) of metabolite were added to the ethyl acetate portions. The samples were then processed through the entire procedure. An average recovery of 84% was obtained for six samples (Table I). A regression analysis of the data showed a linear relation between metabolite concentration in parts per billion (x) and peak current in microamperes (y) expressed by the equation $y = 0.0037 + 0.0356x$. Several samples of aqueous standards of dimetridazole (5 and 10 ppb) were processed through the procedure in the absence of tissue. Recoveries based on the alcohol metabolite as the internal standard averaged 35%.

To evaluate the method samples of control tissues were

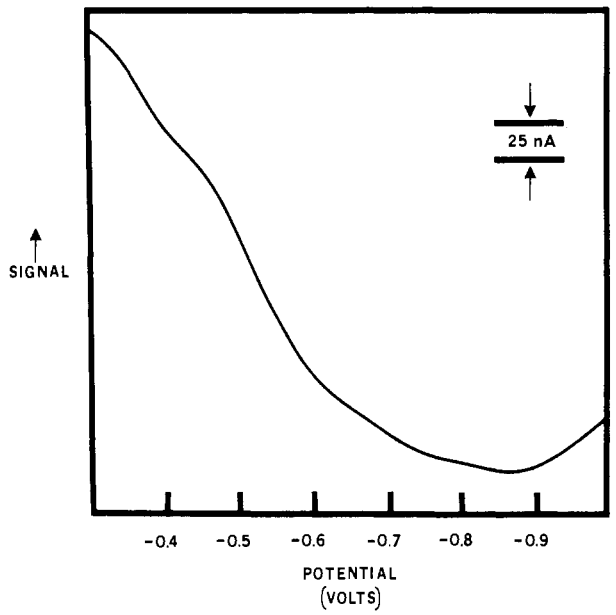

Figure 2. A polarogram of a typical muscle blank.

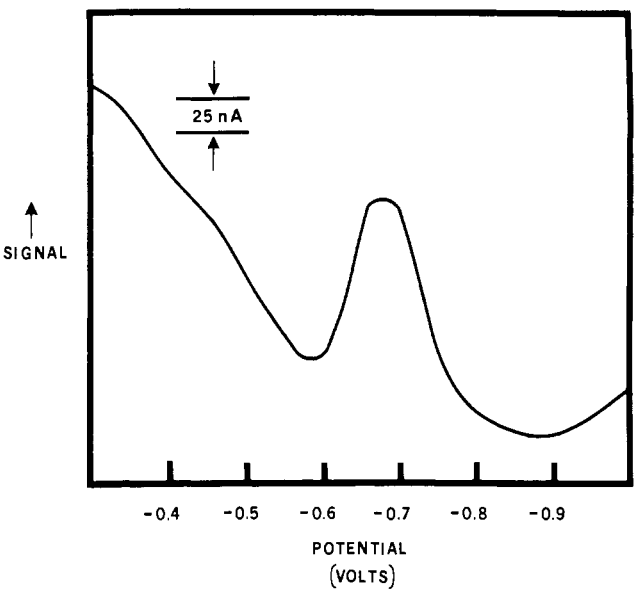

Figure 3. A polarogram of a muscle blank after the addition of alcohol metabolite to make the concentration 30 ng/ml. The response is about the amount obtained from a sample containing 5 ppb of residue.

fortified (spiked) with aliquots (0.2, 0.5, or 1.0 ml) of aqueous standard solution of metabolite before the homogenization step at levels equivalent to 2, 5, and 10 ppb and were analyzed. Values obtained from a series of pig tissue samples are presented in Table II. For the 21 muscle samples the average recovery was 67%. The method measured 2 ppb with an average peak current of 0.046 μA while tissue blanks (zero ppb) gave peak currents of 0.003 μA or less. For the kidney samples the average recovery was 55%. The method measured 2 ppb with an average peak current of 0.034 μA while tissue blanks (0 ppb) showed no wave so that peak current was 0.000 μA. A regression analysis of the data for the fortified muscle samples gave a linear relation between metabolite added in parts per billion (x) and peak current in microamperes (y), expressed by the equation $y = (-0.0048) + 0.0255x$. A regression analysis was carried out on the data of the kidney samples. Again the amount of metabolite added and peak current were related linearly as expressed by the equation $y = (-0.0031) + 0.0199x$. Samples of skin, fat,

Table II. Recovery of Alcohol Metabolite Added to Control Swine Tissue Samples

Tissue	No. of samples	DMZ-OH added, ppb	Peak current (i_p) Range, μA	Peak current (i_p) Av, μA	Av peak current after addition of internal standard, μA	Av recovery, %
Muscle	6	0	0.000–0.003	0.001		
Muscle	7	2	0.034–0.053	0.046	0.420	65
Muscle	7	5	0.094–0.138	0.116	0.489	67
Muscle	7	10	0.228–0.272	0.254	0.636	70
Kidney	5	0	0.000–0.000	0.000		
Kidney	5	2	0.028–0.041	0.034	0.391	52
Kidney	3	5	0.088–0.103	0.094	0.450	57
Kidney	4	10	0.194–0.200	0.197	0.569	57
Skin	2	0	0.000–0.000	0.000		
Skin	2	2	0.050–0.053	0.052	0.462	68
Fat	2	0	0.000–0.003	0.002		
Fat	2	2	0.056–0.056	0.056	0.462	74
Liver	2	0	0.000–0.000	0.000		
Liver	2	2	0.016–0.028	0.022	0.350	33

Table III. Analysis for Residues of Individual Samples of Tissues from Swine Medicated with Dimetridazole in the Drinking Water for 5 Days (Values Are Not Corrected for Recovery)

Pig no.[a]	Withdrawal time, days	Residue level Muscle, ppb	Kidney, ppb	Liver, ppb	Skin, ppb	Fat, ppb
168S	0	470	398	<2	194	50
170S	0	137	85	<2	102	13
175B	0	296	223	<2	74	11
181B	3	<2	<2	*b*	*b*	*b*
154S	3	<2	<2	*b*	*b*	*b*
162B	3	<2	<2	*b*	*b*	*b*
200B	5	<2	<2	*b*	*b*	*b*
215S	5	<2	<2	*b*	*b*	*b*
216S	5	<2	<2	*b*	*b*	*b*
180S	6	<2	<2	<2	<2	<2
177S	6	<2	<2	<2	<2	<2
179B	6	<2	<2	<2	<2	<2
218B	7	<2	<2	*b*	*b*	*b*
173B	7	<2	<2	*b*	*b*	*b*
169S	7	<2	<2	*b*	*b*	*b*

[a] S, sow; B, barrow. [b] Not analyzed.

and liver gave low tissue blanks with peak currents of 0.003 μA or less. Recoveries from fat and skin at 2 ppb were similar to muscle values. Recoveries from liver were unaccountably low. Unsworth and Maycey (1974) found a rapid degradation of dimetridazole in homogenates of swine liver which may account for some of the losses. The average recovery for all samples except liver was 63.5% with a standard deviation of 9.6%.

The method is capable of quantitatively measuring alcohol metabolite residues in swine tissues down to a level of 2 ppb. Levels below 2 ppb can be detected but not quantitatively measured. Thus, levels below 2 ppb should be reported as such.

TISSUE RESIDUE STUDY

A residue experiment was carried out with pigs medicated orally with dimetridazole through drinking water. Yorkshire swine were given dimetridazole in the drinking water at a level of at least 200 mg/l. for 5 days prior to withdrawal or slaughter. The pigs averaged 155 days of age and 184 lb at the start of medication. The average amount of drug given was 10 mg/kg of body weight per day which is within the efficacious dose range. The pigs were withdrawn from medication and assigned to groups of three at random except that there were no more than two pigs of one sex in any group. After the pigs were killed the critical tissues, muscle and kidney, of each pig were analyzed for residue. Selected samples of fat, skin, and liver were analyzed. Residue analyses are summarized in Table III.

At zero withdrawal all tissues except liver contained measurable amounts of nitroimidazole residues. As expected, muscle and kidney contained the highest amounts: an average of 300 and 235 ppb, respectively. Skin and fat residues averaged 123 and 25 ppb, respectively. At 3-day withdrawal, residues were not detected in muscle or kidney. At 5-day withdrawal, there were no detectable residues in five of six samples analyzed. The muscle of pig 215S had a detectable residue but it was <2 ppb. Residues were not detected in muscle, kidney, skin, fat or liver after 6-day withdrawal or samples of muscle and kidney after 7-day withdrawal. All values reported are not corrected for recovery.

DISCUSSION

The levels of residue in individual tissues confirm the observations of Unsworth and Maycey (1974) that the critical tissues in the use of dimetridazole are muscle and kidney, both for residue studies and regulatory purposes. The absence of residue in the liver indicates a rapid metabolic degradation of dimetridazole and its metabolites.

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