ABSORPTION, DISTRIBUTION, AND EXCRETION OF IMIDOCARB DIPROPIONATE (3,3'-bis-(2-imidazolin-2-yl) carbanilide) IN SHEEP

A Dissertation

by

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ABSTRACT

ABSORPTION, DISTRIBUTION, AND EXCRETION OF IMIDOCARB DIPROPIONATE (3,3'-bis-(2-imidazolin-2-yl) carbanilide) IN SHEEP. (August 1974)

Yusuf Otaru Aliu, D.V.M., Cornell University.

Spectrophotometric and thin-layer chromatographic methods for quantitative and qualitative determination of imidocarb in biologic specimens are described. Imidocarb was extracted under basic conditions from plasma, urine, milk, bile and homogenized tissue samples into organic solvents. Following extraction and concentration in 0.82 N HCl, the drug can be qualitatively identified by thin-layer chromatography and spectrophotometry. The detection limit for estimation of pure imidocarb in aqueous solution by spectrophotometry is equivalent to a concentration of 1.0 µg/ml in plasma and other body fluids and 5.0 µg/Gm in tissues. With thin-layer chromatography, the minimum detection level is 0.21 µg.

Following intravenous injection of imidocarb dipropionate (2.0 mg/kg of body weight) into sheep, the high initial plasma concentration of 10.8 µg/ml fell

very rapidly to 1.9 µg/ml in 1 hour, and was less than 1 µg/ml in 4 hours post injection. When 4.5 mg/kg of body weight of imidocarb was injected intramuscularly into sheep, peak plasma concentrations of 7.9 µg/ml were attained within 4 hours. This was followed by a rapid decline within the next 2 hours to 4.6 µg/ml, and then by a very slow decline of several weeks duration. Trace amounts were still present in the plasma 4 weeks after treatment. The drug was bound to plasma proteins to the extent of 21-53%, and the apparent volume of distribution was slightly higher than the total body water.

Imidocarb was widely distributed in the tissues of sheep. Concentrations in the tissues studied were considered high and detectable amounts were present 4 weeks after administration. Twenty-four hours after administration, the highest concentrations occurred in the kidneys, liver and brain. Using ^{14}C-labelled imidocarb to study its distribution in the brain, significant radioactivity was detected in all regions with highest concentrations occurring in the pituitary gland, the pineal body and the olfactory bulb. The concentrations of radioactivity measured in plasma (0.013 µg/ml) and sheep red blood cells (0.016 µg/ml) indicated an equal

partitioning between plasma and red blood cells.

No metabolic or biotransformation products could be detected by the methods of assay. Approximately 11-17% of the administered dose was excreted in the urine within 24 hours; thereafter, the excretion rate was very low and detectable amounts were present for 4 weeks. The rate of renal clearance of imidocarb was found to be considerably below glomerular filtration rate indicative of tubular reabsorption.

The relatively high concentrations of the drug found in the bile suggest that biliary excretion of imidocarb is an important route of excretion. High concentrations were also found in the milk of lactating ewes. When the milk was fed to nursing lambs, no drug could be detected in their plasma.

Imidocarb did not affect oxidative phosphorylation of isolated rat liver mitochondria.

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Dedicated to the fond

memory of my late father

OJO ALIU

for his deep understanding of change.

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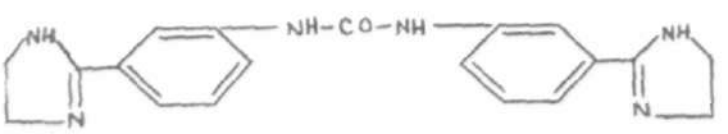
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CHAPTER I

INTRODUCTION

The introduction in 1939 of aromatic diamidines which display broad antiprotozoal, antibacterial, and antifungal properties inaugurated a new era of advance in the therapy of several diseases (53). Investigations were also stimulated by an interest in the hypoglycemic effects (6, 26) of these compounds leading to the development of a series of highly valuable diamidine drugs.

A number of diamidines of the carbanilide series has been shown by Schmidt and coworkers to have babesicidal activity in mice. Of these compounds, imidocarb[a] (3,3'-bis-(2-imidazolin-2-yl) carbanilide, or 1,3-bis-(2-imidazolin-2-yl) phenyl urea) was found to be most effective (8, 75). Its structural formula is:



The citations in this dissertation follow the style of the Journal of the American Veterinary Medical Association.

a. Burroughs-Wellcome and Co., Ltd., Raleigh, N.C.

Recently interest in imidocarb has centered around its apparent effectiveness in the treatment and prophylaxis of babesiosis (7, 8, 10, 11, 12, 28, 50, 72, 75, 81, 92) and anaplasmosis (7, 51, 70, 71).

Pulmonary edema, renal damage and hepato-toxicity, caused by imidocarb have been reported recently by Adams in cattle (1), and by Corrier in goats (16). The cholinergic manifestations of acute imidocarb intoxication have been described by Todorovic, et al (81). Pharmacokinetic and metabolic studies of imidocarb have been hampered by the lack of a sufficiently simple and sensitive method of detection in the tissues of animals receiving therapeutic doses. Since it is effective against Babesia and Anaplasma, more information is needed as to its distribution and fate in the body of domestic animals.

The objective of this research was twofold: (i) to develop simple, efficient extraction and detection procedures whereby imidocarb could be measured in low concentrations in biologic tissues and fluids; (ii) to determine its absorption, distribution, and excretion in sheep.

CHAPTER II

REVIEW OF LITERATURE

Assay of Diamidines

Prior chemical procedures for aromatic amidines utilized either colorimetric determinations or measurement of fluorescent end products of reactions between specific compounds and the amidines. Ekeley and Ronzio in 1935 (22-24) first studied, from the standpoint of preparative organic chemistry, a reaction between the aromatic amidines and glyoxal. They described a series of yellow-colored derivatives which were obtained when aromatic amidines were heated with glyoxal in alkaline solution or condensed further with aldehydes. The reaction was complex, and the results were uncertain and dependent on the conditions employed.

In 1944, Fuller (30) developed a procedure based upon the reaction between aromatic amidines and glyoxal. By heating amidines with a very small amount of glyoxal at pH 9 in the presence of a borate buffer, reliability was improved, and the end product could be measured colorimetrically. The method was reported suitable for estimating amidines in blood, urine, and feces (25). It was applicable for 50 different unsubstituted

aromatic amidines, but not for those containing methyl groups attached to the amino-nitrogen atom. Furthermore the reaction did not occur with guanidines, biguanides, amines, or aliphatic amidines and was dependent on the amount of glyoxal used.

Devine in 1944 (19) reported a procedure for the estimation of amidines using the brown color produced when an amidine is heated with a large excess of glyoxal in strong caustic soda. This method, however, had several disadvantages in that the glyoxal reagent is unstable and must be made up daily; the conditions of heating (30 seconds in a boiling water bath) were difficult to reproduce exactly and the color developed was unstable. Hampton (37) found it to be applicable for stilbamidine in pure solution, but not for stilbamidine in the presence of urine or plasma.

The colorimetric methods of Fuller and Devine were sensitive only to 10 µg/ml in pure solution, which is not sufficiently sensitive for concentrations achieved during therapy. Later, however, Fuller (29, 30) reported that reaction products from some aromatic amidines fluoresced in ultra-violet light at dilutions as low as 0.01 µg/ml. Compounds of the diamidine series which are sparingly soluble, showed no color change with

variations in pH and did not fluoresce. Devine assayed stilbamidine spectrophotometrically and found the sensitivity of this method to be of the same order as that for colorimetric methods.

In 1946, Jackson and associates (44), by application of a reaction originally studied by Ekeley and Ronzio (22-24), developed methods for aromatic amidines by measuring the fluorescence of the reaction product (a glyoxalidone) between an aromatic amidine, glyoxal, and benzaldehyde in alkaline aqueous solution. They worked with stilbamidine, propamidine, pentamidine, phenamidine, and p-carbethoxybenzamidine. The two procedures described for extracting these diamidines from plasma and urine were time consuming. The first involved preparation of protein-free centrifugates with dialyzed iron; but it was limited to higher concentrations of the drug. The second involved extraction of the aromatic diamidine with butyl alcohol. It allowed for analysis of smaller quantities (0.5 µg) of the compound, but required considerably more time. For general applicability to amidines, the methods of Jackson et al had certain advantages over the procedures described by Fuller (29, 30) and Devine (19). They were less dependent upon the concentrations of reagents used

and provided a more nearly linear relationship between the intensity of fluorescence and the concentration of the amidine employed. Samples of urine, however, frequently contained extraneous compounds which inhibited the reaction. In addition the distribution coefficient of the glyoxaline derivative between butanol and an alkaline aqueous solution of urine differed somewhat from the coefficient of distribution between butanol and a pure alkaline aqueous phase at the same pH. For these reasons, spiking of urine samples was necessary to improve the efficiency of estimation of the diamidine in urine.

The specificity of the reaction between aromatic amidines, glyoxal and benzaldehyde used by Jackson and associates (48), has been further investigated by Waalkes and De Vita (85) who developed a fluorometric method whereby pentamidine could be measured in body fluids and tissues. Aromatic amidines are readily and quantitatively extracted from basic aqueous solutions by specific organic solvents (14, 29, 30). In the determination of pentamidine, Waalkes and colleague found the best reagents to be immiscible alcohols and organic acetates. N-Butanol was the most useful for plasma. Both n-butanol and ethyl acetate were

satisfactory for pentamidine in urine. The fluorescence of the final end product was enhanced by the presence of 1-octanol and ethyl acetate. Due to small amounts of water present, the final 1-octanol solutions were always slightly cloudy. The addition of a small volume of 2-propanol gave completely clear solutions essential for fluorometric analysis. The method could detect amounts of pentamidine as small as 0.2 µg/ml of plasma or per gram of tissue. This was sufficiently sensitive for the plasma, urine and tissue determinations in man following therapeutic daily doses of pentamidine isethionate.

Waalkes and De Vita also used thin-layer chromatography to identify pentamidine in urine and tissue extracts; but it was a less sensitive method than the fluorometric procedure. The solvent used was a mixture of butanol, acetic acid, and water (6:2:2 by volume).

Stilbamidine gives a brilliant blue fluorescence in ultraviolet light. This fluorescent property was employed by Henry and Grindley in 1942 (43) and by Hampton in 1947 (37) for the detection and estimation of stilbamidine in biological fluids. A standard drop of the unknown fluid was placed on filter paper and allowed to dry and its fluorescence was compared with that of

similar drops of known concentrations of the drug. This method was sensitive to concentrations as low as 0.25 µg/ml and it has been employed by Fulton and Grant (33) to compare the uptake, localization, and effect of stilbamidine on normal and resistant strains of Trypanosoma rhodesiense.

Using the fluorescent property of stilbamidine, Henry et al (44) developed a method for extracting and estimating the drug in biological tissues. The drug was extracted by boiling the tissues in a water-bath for several hours with 10 per cent hydrochloric acid. The stilbamidine in the hydrochloric acid extract could then be readily estimated by comparing the fluorescence of a spot of the solution on filter paper with that of a series of standard spots. No indication was given as to the sensitivity or limitation of the test.

Stilbamidine labelled with radioactive carbon-14 was synthesized by Reid and Weaver in 1951 and administered into the tail vein of mice for studying the distribution and excretion of the compound (69). Tissues, feces and blood were collected and dried in vacuo. Each tissue or an aliquot sample was burned in a

combustion train to carbon dioxide, which in turn was converted to barium carbonate and its radioactivity measured by the method of Dauben et al (17).

Fulton and Mathew (34) also prepared carbon-14 labelled stilbamidine in order to detect its absorption by trypanosomes and tissues. They used the fluorescent properties of stilbamidine in conjunction with radioactive scanning and paper chromatographic techniques. These researchers were the first to use paper chromatography for the separation of diamidines and the amides derived from their metabolism. The solvent system used was a mixture of pyridine, isoamyl alcohol, and water (7:7:6 by volume). The chromatograph was run for 18 to 24 hours and the spots were detected on the dried paper by ultraviolet light. The chromatograms were also scanned for radioactivity. Fluorescent non-radioactive spots were frequently observed, but no definite indication of non-fluorescent radioactive spots was found. They concluded that the method would be applicable for most other diamidines and could be made very sensitive and accurate.

Radioisotope counting was also used by Launoy and others (52) in 1960 to study the distribution and elimination of carbon-14 labelled pentamidine in mice

and rats. In 1972, Virji and Laverty (84) studied the absorption and tissue distribution of tritium-labelled bunamidine hydrochloride, an amidine found effective against Echinococcus granulosus in dogs (35). The samples were analyzed for radioactivity using liquid scintillation spectrometry. A disadvantage to the use of radioisotopes is that it is not possible to distinguish the chemical nature of the labelled compound and, thus, the compound measured may be a metabolite rather than the parent drug.

Several biological assay procedures have been utilized for the determination of diamidines. The presence of trypanocidal diamidines in serum could be detected by adding sensitive trypanosomes to the fluid and incubating for 24 hours (40). Diminazene aceturate exerted a bacteriostatic action on Brucella and this action has been employed to estimate its concentration in the blood serum of treated dogs with a sensitivity of 0.1 to 1 µg/ml (4).

Clarke (14) has reported that amicarbalide, a babesicidal carbanilide very similar in structure to imidocarb, could be extracted by organic solvents from alkaline aqueous solutions. The extracted drug could then be quantitatively and qualitatively identified by

paper or thin-layer chromatography. With paper chromatography, a Whatman No. 1 paper was used after it was buffered by dipping in a 5% solution of sodium dihydrogen citrate and dried at 25°C for 1 hour. The solvent system consisted of 4.8 Gm of citric acid in a mixture of 130 ml of water and 870 ml of n-butanol. It was developed by ascending chromatography for 5 hours. The spot was located either by iodoplatinate or by examination under ultraviolet light at 254 nm. The R_f value of amicarbalide by this system was 0.03. Using thin-layer chromatography, the solvent system was a mixture of strong ammonia solution and methanol (1.5:100 v/v). Development was by ascending chromatography for 30 minutes. The chromogenic agent was acidified iodoplatinate; the R_f value was 0.05. Other diamidines (diminazene aceturate, phenamidine, stilbamidine) have also been identified by paper and thin-layer chromatography (14, 34).

Pellizari et al (66) utilized gas-liquid chromatography to detect imidocarb, but accurate measurement below 1 µg/ml was not possible. These investigators modified the procedure described by Dombrowski and Pratt (21) for fluorometric determination of the drug. The detection limit for estimation of imidocarb in aqueous solution by fluorimetry was 4 ng/ml. While the method

is very sensitive, it has many disadvantages. It is long, tedious and almost impractical for a large number of samples; many of the diazotization reagents (eg., DAP in acetate buffer, ammonium sulfamate, sodium nitrite) have to be prepared fresh daily.

Pellizari and coworkers also studied the use of thin-layer chromatography in determining the purity of imidocarb and its acid hydrolysis product. They used silica gel HF 254 (Merck) as the adsorbent and two different solvent systems [n-butanol/acetic acid/water (2:1:1 by volume), and methanol/diethylamine (9:1 by volume)] . The R_f value of imidocarb was found to be approximately 0.39 and 0.29, in the 2 solvent systems, respectively; for imidocarb-AHP it was 0.44 and 0.55, respectively. Visualization was by long and short wave ultraviolet light. No attempt was made to quantitate imidocarb by this method.

Both ultraviolet and infra-red absorption spectra have been used for qualitative and quantitative determination of diamidines (32 , 39 , 43 , 58). The first specific spectrophotometric method for the estimation of diamidines was developed by Fulton and Goodwin in 1945 (32). Their method was based on the fact that stil-bamidine, in aqueous solution, exhibits a strong

spectral absorption band with maximum at 329 nm. They recommended its use for the determination of those members of the diamidine series useful in clinical practice, because all of these drugs have a definite absorption band in the near ultra-violet region. Changes in the character of the absorption spectrum after exposure to light, whereby the ethylene linkage of stilbamidine became saturated, were observed. They hoped that metabolites of these drugs in the animal body could be similarly detected. The extraction procedure was simple and the method was capable of measuring the drug content of serum to 5 µg/ml. Amicarbalide in 0.1 N sulphuric acid has been found to have maximum absorption at 232 nm and an inflexion at 257 nm. In the infra-red region the principal peaks of amicarbalide occurred at 1195, 1590, or 1672 cm (14).

Several workers have reported on the use of microcolor tests for estimating diamidines (14, 83). A simple field method for the determination of pentamidine and dibromopropamidine in body fluids, which was based on the development of a yellow-orange color by diamidines with Fearon's penta-cyano-ammonio-ferrate reagent, was reported by Trought in 1949 (83). Clarke (14) has reported that amicarbalide gave a pale yellow/

red reaction to the Vitali's test with a sensitivity of 0.25 µg. Stilbamidine has been estimated by an ammonium molybdate test (14). In the presence of stilbamidine, a pale blue color was obtained with a sensitivity of 1 µg. Clarke (14) and Trought (83) concluded that many compounds gave various shades of yellow, orange and brown which are of little diagnostic value.

Microcrystal tests are among the oldest, the simplest and the most sensitive tests used in toxicology. Although unsuitable as a primary method of identification of an unknown compound, these tests have been used to confirm the provisional identification of some diamidines made from chromatographic or spectrophotometric evidence (31).

Pharmacology of Diamidines

Absorption

To cause a therapeutic effect, a drug must reach its site of action in effective concentrations and remain at these sites for a sufficient length of time. The tissue concentration of a drug depends upon several factors. The concentration of a drug in the blood is determined by the amount administered, rate of absorption, rate and extent of distribution, and rate

with which the drug and its metabolites are excreted. The absorption, distribution in the body, and excretion of a drug depend to a large extent on the lipoid nature of the particular cellular boundaries that the drug must penetrate, and the intrinsic lipophilic properties of the drug.

The diamidine compounds possess limited water solubility (79) and are not well absorbed when given by mouth (40). Beveridge (8) has reported that imidocarb was much less toxic to mice when administered orally than when given by injection. She gave no indication as to the amount or proportion of the drug absorbed by this route, except that it had a negligible effect against Babesia rodhaini. Virji and Laverty (84) fed tritium-labelled bunamidine to fasted and non-fasted rats and dogs. They found that there was a great deal of individual variation, both with respect to the amount absorbed as well as the time taken to attain a peak plasma level. They also found that the degree of absorption was not dependent upon feeding or starving, but that other physiological processes were involved.

When given by subcutaneous injection, all diamidines are moderately absorbed according to the absolute size of the dose. After subcutaneous injection

of stilbamidine into mice under ultraviolet illumination, a faint bluish glow could be seen at the site of injection 9 days later, indicating local fixation by the tissues (41). When diminazene aceturate at a dose rate of 7 mg/kg was injected intramuscularly into dogs, the serum concentrations were maximal in 3 hours at 3 µg/ml, and were less than 1 µg/ml at 16 hours (4). Launoy et al (52) have reported that pentamidine, when administered by intramuscular injection, conferred prolonged resistance to infection by Trypanosoma gambiense or T. rhodesiense due to a depot sort of prophylactic effect. This route was much safer than the intravenous route, but in both instances, the drug was removed very rapidly from the circulating blood and deposited in tissues.

After intravenous or intraperitoneal injection of all diamidines, the concentrations in the blood fall very rapidly. When 10 mg/kg of stilbamidine was injected intravenously into rabbits, the blood concentration was about 0.5 µg/ml at 2 hours and less than 0.05 µg/ml after 6 hours (39). Fulton and Goodwin (32) recorded the serum levels of stilbamidine in mice over a period of 120 minutes after a single intravenous injection. The highest drug concentration was found within a few

seconds of administration, and decreased rapidly during the first hour. After single intravenous injections of 0.4 mg of carbon-14 labelled stilbamidine isethionate into mice Reid and Weaver observed that in as little as two hours there was only a minute trace of radioactivity present in the plasma and the red blood corpuscles (69). Virji and Laverty (84) measured the plasma levels of tritium-labelled bunamidine hydrochloride after a rapid intravenous injection of 0.6 mg/kg to dogs. A peak plasma concentration was attained in 2 minutes; but at the end of 160 minutes, no significant plasma levels were noted. After a single intravenous injection of 0.5 mg/kg of imidocarb to a cow, a peak plasma level of 6.6 µg/ml was achieved within 2 minutes and declined rapidly to 0.5 µg/ml in 4 hours (63).

Distribution

Earlier work on the distribution of diamidines (15, 41, 58 , 65 , 73 , 77) has led to the conclusion that extensive retention and storage for prolonged periods must occur. The difficulties encountered in assaying the drug in body tissues has hampered determination of the sites and length of storage.

Henry and Grindley (43), using the fluorometric assay procedure, added stilbamidine to whole sheep blood

and found that most of the compound disappeared from the plasma. These investigators concluded that erythrocytes have a high adsorptive capacity for the drug, but in animals treated with stilbamidine they did not note binding of the drug by the erythrocytes. Fulton and Goodwin (32) repeated these experiments and were unable to confirm the findings of Henry and Grindley. Also in a later study by Henry and colleagues (44), it was observed that even after a series of injections, no detectable amount of stilbamidine was present in the plasma of sheep and none was present in the erythrocytes. The liver of a sheep that died three months after the termination of a course of injections, presumably from the delayed toxic effects of the drug, contained a high concentration of stilbamidine. Rats were also reported to accumulate stilbamidine extensively in their livers, but differed from the sheep in that much of the drug was eliminated from the liver. No organ, even the skin, was entirely free from stilbamidine in a rabbit killed 2 months after a course of injections of the drug.

Using ultraviolet illumination to detect the presence of stilbamidine in mice after subcutaneous injections, Hawking and Smiles (41) demonstrated that stilbamidine collected chiefly in the liver, kidneys,

possibly in the serous covering of the small intestine, the skin at the site of injection, and the paw pads.

Several investigators (15, 58, 73 , 77) have drawn attention to neuropathy occurring in patients some 2-5 months after treatment of visceral leishmaniasis (Kala-azar) with fresh solutions of stilbamidine. From a careful clinical analysis of the signs and symptoms, Napier and Sen Gupta, in 1942 (58), and Sen Gupta, in 1943 (77), reported that the lesion produced is located in the trigeminal nucleus, a property probably related to its ethylenic component. Collard and Hargreaves, in 1947 (15), and Sati, in 1948 (73), found that the condition had a much wider distribution. In some cases it involved the seventh and ninth cranial nerves, and in others the areas of distribution of the cervical and dorsal spinal nerves were affected as well. In dogs poisoned with stilbamidine, Oastler and Fidler (65) found definite lesions in the central nervous system, most commonly in the thalamus and caudate nucleus but also in the cerebral cortex, cerebellum, brain stem and spinal cord.

In 1951 Snapper and coworkers (78) conducted an extensive study of the distribution of stilbamidine and 2-hydroxystilbamidine in parenchymatous organs and

tumours. These investigators found that only 5-10% of the amount of either of the drug injected intravenously was excreted in the urine. A greater part was deposited in various organs, especially the liver, kidneys, adrenal glands and the sweat glands. The deposition seemed to differ somewhat in different species. In mice more drug was found in the kidneys than in the liver, and in man and rabbits, the opposite was true. Twenty-three months after injection of 2-hydroxystilbamidine, appreciable amounts could be recovered from the liver of a myeloma patient. In another case, marked traces were found in the liver three years and three months after the last injection.

Reid and Weaver (69) injected carbon-14 labelled stilbamidine into the tail vein of mice and measured the concentration of radioactivity in various tissues. They found that the highest concentrations appeared in the kidneys and liver, followed in order by the heart, lungs and intestines, with smaller concentrations in the other tissues. The distribution pattern was established within the first 2 hours and did not change substantially thereafter, although the total amounts in these tissues tended to decrease with time.

Fulton and Mathew (34) measured the distribution of

radioactivity in tissues of rats at various intervals after the administration of carbon-14 labelled stilbamidine. They also used paper chromatography to determine the chemical nature of the radioactive substances. After 2 hours, the liver contained 25% and the kidney 7.5% of the dose injected. The lung, spleen, and heart took up relatively little but retained radioactivity for long periods of time. The serum concentration of the drug was about 0.25 µg/ml 2 hours after administration, and thereafter the concentration fell quickly to zero. The erythrocytes were also found to have no appreciable amount of radioactivity after 4 hours. This confirmed the earlier findings by other workers that diamidines are not stored in the erythrocytes (32, 44, 69).

Launoy and others working with carbon-14 labelled pentamidine have reported that in mice and rats, the liver and the kidneys stored pentamidine for periods of months (52). Pentamidine crossed the placenta but did not appear in the milk of nursing mothers. The brain fixed only a small amount of the drug but stored it for several months. These investigators concluded that the fixation of pentamidine in tissues is the most important factor in its use as a prophylactic agent in

trypanosomiasis.

Nimmo-Smith et al (61, 63), have found that imidocarb dihydrochloride was retained for a long time in tissues following subcutaneous or intramuscular administration to cattle. The highest concentrations were found in the kidneys and liver and modest amounts were reported in the skeletal muscle and fat. The liver and kidneys from two fetuses taken from treated cows showed high concentrations of the drug. Two weeks after administration of imidocarb, the liver and kidneys of the dams contained 4.3 and 6.3 µg/Gm of tissue respectively, while the corresponding values for the fetuses were 3.8 and 3.9 µg/Gm. This report is in agreement with the findings of Launoy and others (52) that diamidines cross the placental barrier.

In 1970, Bills et al (9) studied the hepatic uptake of tritium-labelled bunamidine hydrochloride in dogs. They found that blood from the carotid artery contained less than half the amount of labelled material when ^{3}H-bunamidine had been given 3 minutes earlier into the splenic vein. This supported the idea that hepatic uptake is an important factor in determining the levels of circulating bunamidine. Virji and Laverty (84) also

working with tritium-labelled bunamidine found the highest concentrations of radioactivity in the liver and kidneys of rats and dogs, with moderate levels in the spleen and heart.

Metabolism

Early studies on the fate of stilbamidine showed that about 10-50% of the intravenously administered drug was excreted unchanged in the urine of both humans and laboratory animals (32, 49). Hawking and Smiles (41) have demonstrated from a qualitative examination of tissues under ultraviolet light that no fluorescence of the kidneys could be observed 4 days after injection of stilbamidine. However, at 4 days Reid and Weaver (69) found radioactivity in these organs sufficient to give visible fluorescence. The latter investigators concluded that it was, therefore, likely that stilbamidine was converted in one or more of the tissues of the body to a different chemical form, but that the oxidation of 14-C of stilbamidine by the body was negligible.

Henry et al (44) in their chromatographic studies of stilbamidine observed that there appeared to be no tendency for hydrolysis of the amidine group to the amide group to occur in the body. This observation was also confirmed by chemical analysis. This finding is

important since the first hydrolysis product is more toxic than the parent compound (42 , 43 , 49).

Fulton and Mathew (34) observed only minor proportions of the two amides, 4-amidino-4' carbanolystilbene and 4:4'-dicarbamoylstilbene, in the chromatographic behaviour of the urinary end products of stilbamidine. Extracts were made of the liver and kidneys of treated rats and chromatograms of the extracts were scanned for fluorescence and radioactivity. These investigators reported that even after long periods, the remaining radioactivity was largely due to unaltered drug and that only traces of the related amides were present.

Using two different samples of 14-carbon labelled pentamidine, one with radioactive carbon in the central straight chain pentane structure, and the other with radioactive carbon in the aromatic ring, Launoy and his associates studied the deposition and metabolism of pentamidine in mice and rats (52). Pentamidine was found to be excreted intact in the urine and the feces as there was no evidence of any metabolic-breakdown products. Waalkes and De Vita (85) have also confirmed by thin-layer chromatography that pentamidine is not metabolized to any appreciable extent by mice and

humans.

In her study of the toxicity and therapeutic activity of imidocarb in rats against Babesia rodhaini, Beveridge (8) observed that after oral administration of imidocarb to mice, the compound was much less toxic than when administered by other routes but is less effective against the parasite. Roby (70) has also studied the inhibitory effect of imidocarb on experimental anaplasmosis in splenectomized calves. He reported that the development of acute Anaplasma marginale infection in splenectomized calves was inhibited by subcutaneous injection of imidocarb in single doses of 2.5 or 10 mg/kg, but not by a single dose of 25 mg/kg given by mouth. Since blood and other tissue levels of the drug were not determined by any of these workers, they could only speculate as to the absorption and fate of the drug. It was suggested that the drug was either poorly absorbed, destroyed by the acid of the stomach or that it was metabolized by the gastrointestinal epithelium or by intestinal microorganisms. The first communication on the possible role of cells other than hepatic or renal on metabolism of foreign substance was Herter's and Wakeman's report in 1899 ([45]). In 1973, Hartiala ([38]) reviewed in great

length the metabolism of hormones, drugs and other substances by the gut and concluded that the specific role of the epithelial drug-conversion functions appeared to resemble closely those of the liver. In many cases these functions appeared to change the lipid-soluble, nonpolar compounds to less lipid-soluble, polar metabolites. This then serves the purpose of decreasing transfer into the body and facilitates elimination from the body. The alterations may be accompanied by a decrease in both biological activity and toxicity.

Excretion

Some of the diamidines, such as diminazene aceturate and amicarbalide are excreted fairly quickly but pentamidine, propamidine, stilbamidine, and imidocarb are retained in the body for long periods of time. For this reason an intramuscular injection of pentamidine in man will prevent infection with Trypanosoma gambiense for 6 months (40). In 1941, Hawking and Smiles (41) reported that stilbamidine was excreted in the urine principally during the first 7 hours, but that after 2 days the excretion was probably small. Blue fluorescence of the gall bladder indicative of the presence of the drug in the bile was not

observed. In 1951, Reid and coworker (69) injected radioactive stilbamidine into mice and found large amounts of radioactivity in both the urine and the feces. The total fecal excretion during the first 8 days was greater by 50% than the urinary excretion during the same period of time. The rate of urinary excretion reached a maximum during the first 24 hours (17% of the intravenously injected dose), while the fecal peak occurred during the second 24 hours (14% of injected dose). These workers found relatively high levels of radioactivity in the bile during the first 24 hours. Since the daily bile secretion of the mouse is about 2 ml and contains about 280 mg of solids, they deduced that a large part, possibly all, of the radioactivity of the feces was due to excretion in the bile. Henry and Grindley (43) reported that only 10% of a single dose of stilbamidine injected intravenously into human patients was excreted in the urine. The rate of excretion in the first few hours was comparatively high. This was followed by a rapid fall to a low excretion rate, which slowly diminished until after 2 1/2 days it became undetectable.

Kirk and Henry (49) have observed that during a course in which 9 injections of stilbamidine of 50 mg

each were given on alternate days to human patients, urinary excretion of the compound increased until finally about 80% of each dose was eliminated, the major part during the first 24 hours following each injection. Hampton, in 1947 (37), found a similar pattern of urinary excretion of 3 diamidines (stilbamidine, iodostilbamidine, and hydroxystilbamidine) in rabbits and rats after single and repeated injections. In 1958, Bauer (5) reported that diminazene aceturate was mostly metabolized or excreted in the urine during the first 24 hours after injection; hence in cows given 3.5 mg/kg intramuscularly its prophylactic action against trypanosomes did not persist for more than 2 days.

Fulton and Mathew (34) using radioactive tracer technique found that during the first 24 hours following intravenous administration of ^{14}C-stilbamidine, the radioactivity in urine and feces was 2.5 and 6.1% respectively of the injected dose. This is in agreement with the findings of Reid and Weaver (69) who used the same tracer technique. After a single oral administration of tritium-labelled bunamidine hydrochloride, the amount of radioactivity in the urine of normally-fed dogs within 7 hours was equivalent to 0.37% of the

administered dose. After starving for 48 hours, the amount excreted was equivalent to 0.32% within 8 hours (84). No radioactivity was detected in the urine after 24 hours. The low urinary excretion rate and the low plasma levels suggest rapid tissue uptake of the drug.

Resume

Imidocarb has been measured in aqueous solutions by fluorimetry, gas-liquid chromatography and by thin-layer chromatography (61, 62, 66). Nimmo-Smith and Savage (63) have used carbon-14 labelled imidocarb to study its distribution and persistence in the blood and tissues of cows.

Following intramuscular injection of ^{14}C-labelled imidocarb dipropionate into cows, it was reported to be rapidly absorbed. Significant residual radioactivity was detected in various tissues during the 6 month span of their expirement. The highest concentrations were found in the liver, kidneys, and adrenal gland. Up to 33% of the dose administered was excreted in the urine within 7 days; during the same period 10% of the dose was eliminated via the feces. Imidocarb was reported by these workers to appear promptly in the milk of lactating cows. Also significant radioactivity was detected in the tissues of fetuses taken from treated pregnant cows,

indicating that imidocarb crosses the placenta.

CHAPTER III

MATERIALS AND METHODS

Reagent-grade chemicals and solvents were used. All aqueous reagents were made with distilled water. Injectable solutions were made with sterile distilled water or sterile physiological saline solution. Imidocarb dipropionate[b] was supplied by the manufacturer. All dosages and concentrations are given as the free base and hereafter are so reported.

Assay Methods

Extraction procedure:

One milliliter of 4 N NaOH was added to an equal volume of plasma, urine, milk, or bile; to 1 ml of whole blood or washed red blood cells was added 1 ml of water to prevent emulsion formation, and then 2 ml of NaOH. The resulting solution was mixed vigorously on a Vortex mixer for 30 seconds. Since the extraction procedure depends on an alkaline pH to precipitate imidocarb from solution, the pH of the resulting solution was adjusted to approximately 13.80 as determined with a pH meter.

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b. Veterinary Trial Material No 1663, Burroughs Wellcome Co., Research Triangle Park, Raleigh, N.C.

Ten milliliters of isoamyl alcohol-hexane mixture (2:3 v/v) were added, and the tube was shaken vigorously for 2 to 3 minutes and centrifuged at 546 x g for 10 minutes. The supernatant organic-phase was then transferred to a glass-stoppered centrifuge tube containing 5 ml of 0.82 N HCl. The tube was mixed for 2 to 3 minutes on a Vortex mixer and the two phases separated by centrifuging for 10 minutes at 546 x g. The organic layer was removed by aspiration with a fine tipped disposable Pasteur pipet and discarded. About 3 ml of the aqueous phase was transferred to a 1-cm quartz cuvette with a Pasteur pipet and scanned with a spectrophotometer[c].

For tissue level determination, one gram of tissue was homogenized in 5 ml of 0.82 N HCl using an all glass tissue homogenizer or a Potter-Elvehjem glass homogenizer with an electrically driven, teflon coated, pestle. The homogenate was centrifuged at 546 x g for 15 minutes and a volume of the supernatant phase equivalent to 0.25 gm of tissue was used for extraction. An equal volume of 4 N NaOH was added to adjust the pH to approximately 13.80.

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c. Beckman--Model DBG or ACTA II, Beckman Instruments, Fullerton, Ca.

The remainder of the procedure was the same as for plasma, and other body fluids.

Detection by spectrophotometry:

Absorption spectra of pure imidocarb and extracted drug were scanned with double bean ultraviolet spectrophotometers equipped with Beckman 10" recorders over the range of 200-340 nm. For measurements at a single wavelength, the maximum absorbance was at 238 nm with a molar extinction coefficient of 4.36×10^4 (Fig. 1, p45). Reagent blanks were used as reference solutions for pure imidocarb. Specific biologic blanks (i.e., tissues and fluids from untreated sheep) were also carried through the procedure and used as reference solutions for corresponding samples from imidocarb treated animals.

In order to determine the sensitivity of the spectrophotometric method of assay, two methods were used for preparing standards. In the first method, known amounts of imidocarb were added prior to homogenization of the tissues; whereas, in the second method, the drug was added to the supernatant phases obtained after centrifugation of the homogenates. All samples of the various body fluids and tissues assayed were read against their appropriate blanks.

The percentage recovery of imidocarb from biologic specimens assayed by the above method and the reproducibility of the method were determined. A range of concentrations of imidocarb dipropionate (1 to 75 µg/ml of plasma or per gram of tissue) were added to homogenized tissues and fluid samples collected from untreated sheep. Determinations were made on 1-ml amounts of body fluids or homogenate aliquots equivalent to 0.25 gm of tissue. The amount of imidocarb, in micrograms, in the sample analyzed was calculated from the equation:

$$\frac{Ua}{Sa} \times C$$

Where: Ua = absorbance of the unknown

Sa = absorbance of the standard

C = concentration of the standard in µg/ml or µg/Gm

The possible interference by metabolic or other naturally occurring products and other diamidines (e.g., diminazene aceturate) was examined. Imidocarb was extracted with a mixture of isoamyl alcohol and hexane from the body fluids and tissues of sheep receiving the drug. The organic phase was extracted with 0.82 N HCl

and the ultraviolet absorption spectrum of the aqueous solution determined. More definite proof of specificity was obtained by thin-layer chromatography (80). The acid solution was evaporated to dryness in a rotary evaporator[d]. The residue was redissolved in a few drops of 0.82 N HCl and spotted on a thin-layer plate.

Detection by thin-layer chromatography:

Reagent and tissue blanks, and standard imidocarb solutions carried through the extraction procedure were spotted on thin-layer plates with the unknown samples. Glass plates, 20 x 20 cm, were coated with a slurry consisting of 30 Gm of silica gel G (Merck) in 60 ml water to give a layer 0.25 mm thick; the plates were first air dried overnight and then activated in an oven at 110°C for 1 hour. They were stored in a desiccator containing calcium chloride. Ten to thirty microliters of the sample extracted into 0.82 N HCl were spotted on the plate with a microsyringe or with a 1 µl capillary tube. The upper phase of the two phase system resulting from a mixture of n-butanol, water and glacial acetic acid (50:50:12 v/v) was used as the solvent system. The solvent was changed after 2 successive developments.

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d. Büchi Rota-vapor, Glasapparatefabrik, Switzerland.

The developing chamber, a 21 x 21 x 10 cm glass covered container, was equilibrated by lining the inside walls with filter paper moistened with the solvent and allowed to stand for 1 hour. The plates were developed for 1 1/2 to 2 hours by ascending order.

After drying in air, the plates were examined under a short wavelength (254 nm) ultraviolet lamp[e] to check for fluorescent white spots and then sprayed with iodoplatinate reagent to determine if purple or blue spots developed.

Radio-isotope techniques:

Plasma, lysed red blood cells and brain tissue were collected from sheep treated with ^{14}C-labelled imidocarb dipropionate. Each sample was prepared for determination of radioactivity in two different ways. In the first method, duplicate 200 mg portions of the brain sample and 0.2 ml of plasma and red blood cells were air-dried and taken for combustion in a furnace[f] designed for the quantitative trapping of carbon dioxide containing all ^{14}C from the sample (36).

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e. Mineralight--Model R51, Ultraviolet Products, San Gabriel, Ca.

f. Packard Tri-Carb sample oxidizer--Model 305.

The carbon dioxide-absorbing mixture consisted of 1 volume of redistilled ethanolamine, 2 volumes of methanol and 2 volumes of toluene containing PPO (15 Gm/L) and POPOP (0.3 Gm/L).

In the second method, duplicate 100 mg portions of the brain samples were weighed into scintillation vials. After adding 1.0 ml of tissue solubilizer[g] the vials were incubated at room temperature for 24 hours. Then 10 ml of liquid scintillation fluid[h] were added. The plasma samples (0.1 ml) were counted directly after adding the scintillation fluid; 3 ml of hydrogen peroxide was used to decolorize the red blood cells (0.1 ml) before counting. Chemiluminescence was prevented by keeping the samples in the dark overnight. Measurements of radioactivity were made in a liquid scintillation spectrometer[i].

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g. Soluene 100, Packard

h. Aquasol, New England Nuclear universal liquid scintillation fluid.

i. Beckman--Model LS--235.

Corrections for quenching were made through the application of quench correction curve and by the addition of internal standard to each sample after obtaining the gross counts. Correction for recovery from the furnace was made by use of an external standard. These procedures permitted calculation of the absolute disintegration rate of each sample.

Treatment of Experimental Sheep and Collection of Samples

Adult sheep in good physical condition weighing approximately 45 kg were used. Single intravenous and intramuscular injections of imidocarb dipropionate were made, except for experiments on renal clearance. Blood samples were collected from the jugular vein and heparin was used as an anticoagulant. Plasma was obtained by centrifuging the blood samples at 546 x g for 25 minutes. Air embolism was used to kill the sheep and all tissue and fluid samples were stored at -53°C.

Plasma and milk levels:

Seven sheep were given single intramuscular injections of 4.5 mg/kg of imidocarb in aqueous solution. Blood samples were drawn from the jugular

vein of each sheep before drug administration and at various intervals thereafter for 4 weeks. Excretion of imidocarb in the milk of 4 lactating ewes was determined. Three ewes were treated intravenously (2 mg/kg of body weight) and one ewe received an intramuscular injection of imidocarb at a dose of 4.5 mg/kg of body weight. Each mammary gland was hand stripped at various intervals, and an aliquot milk sample taken for assay. Aliquots of the milk were fed to 3 suckling lambs in order to determine the possibility of transfer of imidocarb through the milk to suckling young. Blood was collected at 4, 6, and 24 hours from the lambs.

Plasma protein binding:

The extent to which imidocarb was bound to sheep plasma proteins was determined by equilibrium dialysis (2). Plasma samples obtained at various time intervals from 3 sheep treated intravenously with the drug (2 mg/kg of body weight) were dialyzed in cellophane dialysis bags against equal volumes of isotonic phosphate buffer of pH 7.42 at 37^{o}C for 24 hours with gentle agitation. At the end of the dialysis period, the dialysates and the dialysands were analyzed for imidocarb concentration by spectrophotometry. The percentage of the drug bound was calculated according to the equation:

Plasma, lysed red blood cells and brain were collected 24 hours later for measurement of radioactivity.

Renal excretion:

Three sheep were kept in individual metabolism cages, and their urine was collected free of feces into conical flasks. The urine samples were collected prior to an intramuscular injection of imidocarb (4.5 mg/kg) of body weight), and at various intervals for 2 days to determine the rate of excretion and the total amount of drug excreted. Thereafter, urine samples were collected daily for a week and at weekly intervals for 3 weeks to determine the period of excretion of imidocarb.

Renal clearance:

The conventional method was used to study the renal clearance of imidocarb. The drug was infused at constant rate into the jugular vein of 3 hydrated, unanaesthetized ewes and the clearance of imidocarb calculated by the conventional formula:

$$UV/P$$

Where: U = urine concentration of imidocarb

V = urine flow rate

P = venous plasma concentration of imidocarb.

The bladder was catheterized with a Bardex retention catheter (size 12). Moderate water diuresis was induced by the administration of at least 2 liters of water by stomach tube 1 1/2 to 2 hours before urine collection and by constant infusion of a 0.012% solution of imidocarb in physiological saline solution at the rate of 3 ml/min. Urine samples were collected for three 10-minute periods following a 24-hour initial intramuscular dose (2.5 mg/kg of body weight), and a 30-minute constant intravenous infusion (0.36 mg/min) period. Blood samples were taken during the last two minutes of each urine collection period. The blood was obtained from an indwelling catheter in the jugular vein with heparin as an anticoagulant and the plasma was separated by centrifugation.

The renal clearance of imidocarb was compared with the simultaneous determination of renal clearance of endogenous true creatinine (ETC) which provided an estimate of glomerular filtration rate (GFR). The Folin-Wu method (27) was used for the determination of the creatinine level in plasma and urine.

Effect of Imidocarb on Rat Liver Mitochondrial Respiration and Oxidative Phosphorylation

The effect of imidocarb on rat liver mitochondrial respiration was measured by the technique described by Chance and Williams (13), using a Clark-type oxygen electrode[j]. Mitochondria were separated from rat liver by differential centrifugation (76). Succinate and alpha-ketoglutarate were used as respiratory substrates. During State 2 respiration of rat liver mitochondria with alpha-ketoglutarate as substrate, malonate was used to block the oxidation of succinate. When the activity of succinic dehydrogenase was being measured, rotenone was used to inhibit NADH-linked respiration in the presence of succinate. Imidocarb was added to the reaction mixture during State 4 respiration in the presence of succinate or alpha-ketoglutarate. The reaction mixture (3 ml, 27°C) contained 0.25 M sucrose, 10 mM potassium phosphate (pH 7.4), 2 mM EDTA, 5 mM $MgCl_2$, and 0.5 ml mitochondrial preparation.

[j] Yellow Springs Instrument Company, Yellow Springs, Ohio.

CHAPTER IV

RESULTS

The ultraviolet absorbance of imidocarb is shown in Fig. 1 and the linear relationship between imidocarb concentrations and absorbance obtained by regression analysis are shown in Figs. 2 and 3. In all cases the linear correlation coefficients (r values) were greater than 0.95 which is highly significant ($P < .01$). From these curves it was estimated that imidocarb could be accurately measured in plasma and other body fluids at a level of 1 µg/ml (1 ppm) and in tissues at a level of 5 µg/ml (5 ppm). For the tissues represented, at least 3 replicates at 7 points were used to fit the curves. The homogeneity of the regression coefficient (b values) were tested by Student's t test to determine if they could be considered to be estimates of a common β. No significant difference was found at the 5% error level. By analysis of covariance it was determined that while the regression coefficients are homogeneous, the regression lines for the various tissues are not the same. The 2 methods of preparing tissue standards were compared by Student's t test and no significant difference was found between the two.

Results of the studies made to determine the

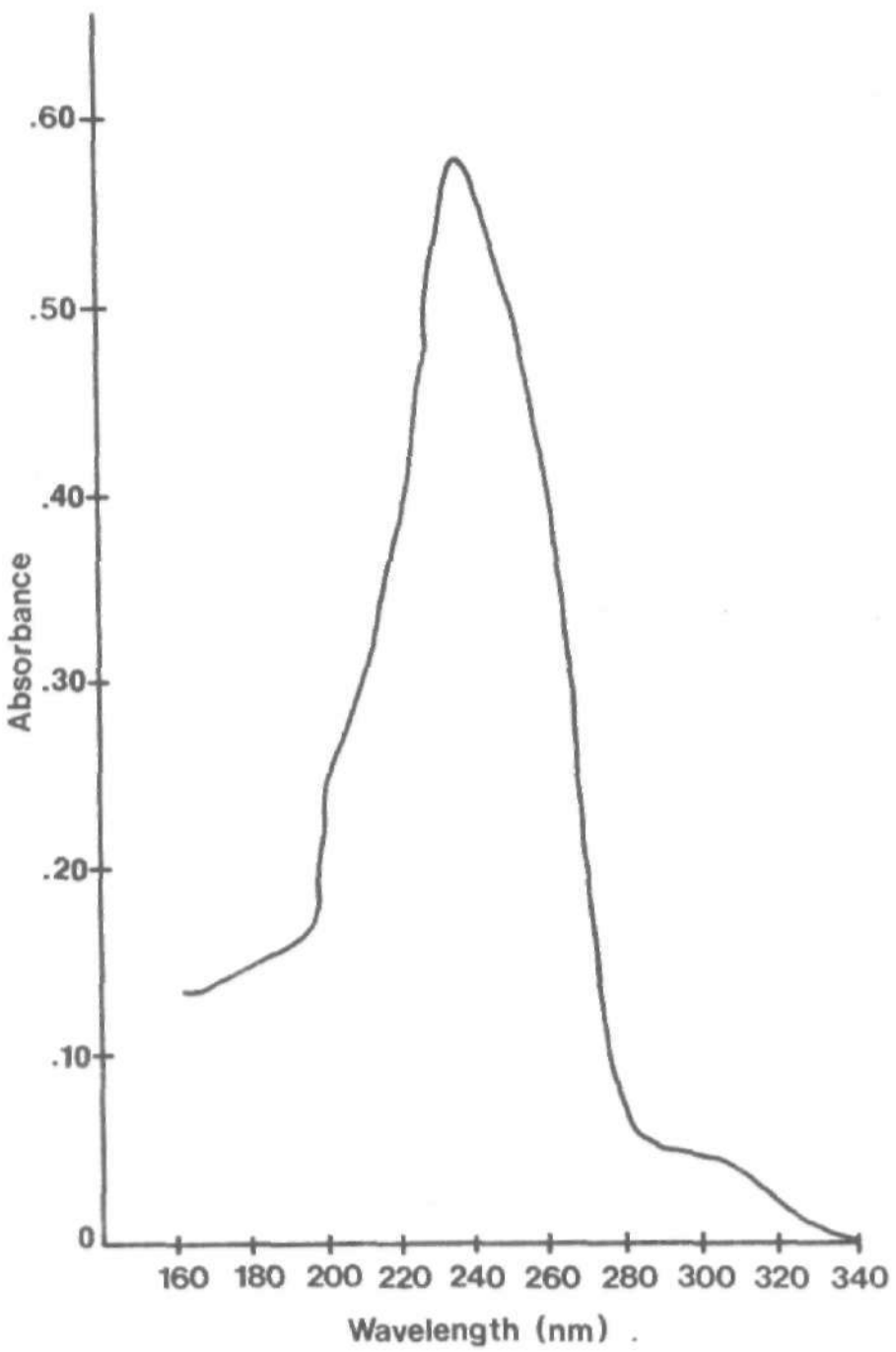


Fig.1 – Ultraviolet absorption spectrum of imidocarb in .82 N HCL at a concentration of 4.5 µg/ml.

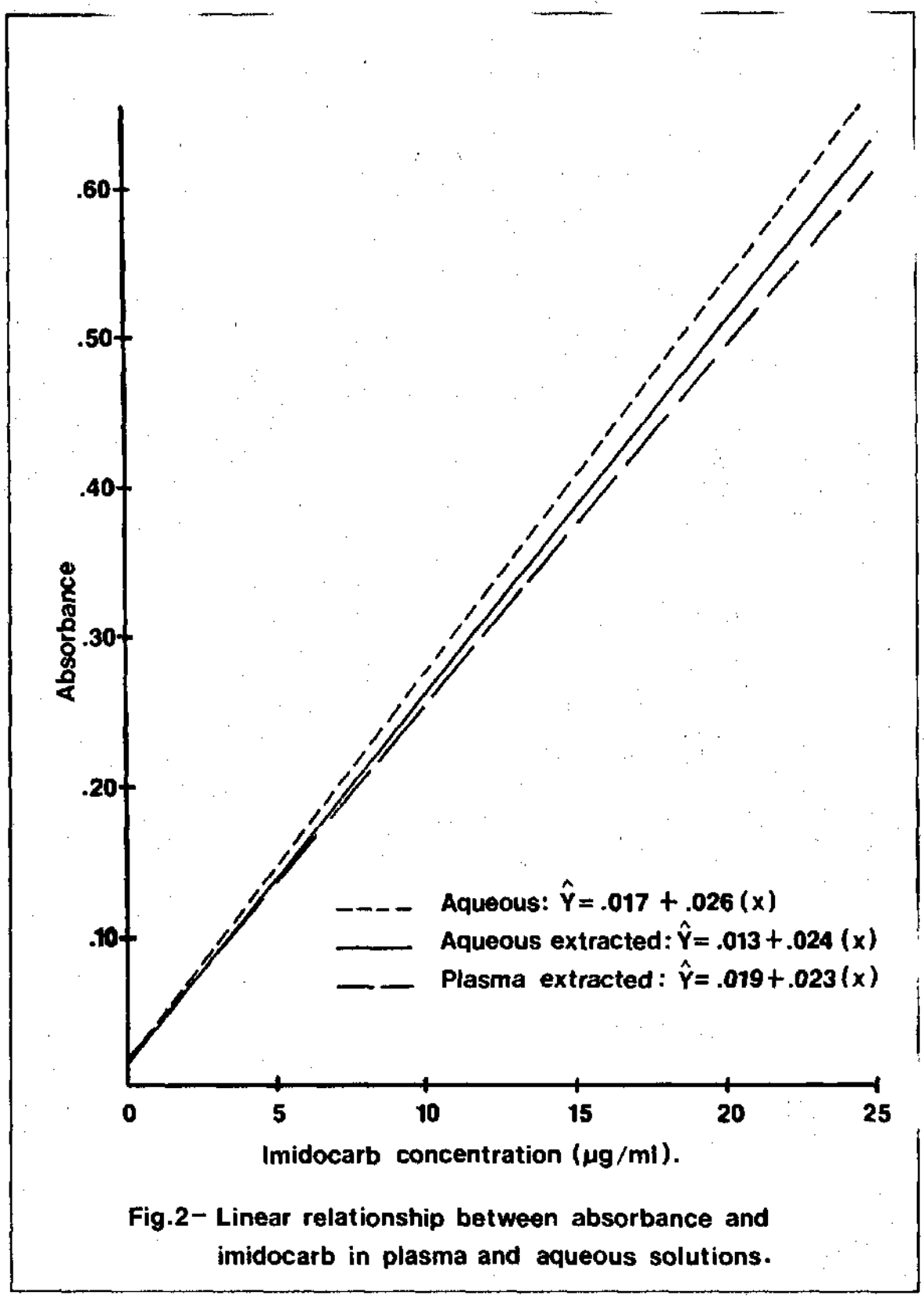


Fig.2– Linear relationship between absorbance and imidocarb in plasma and aqueous solutions.

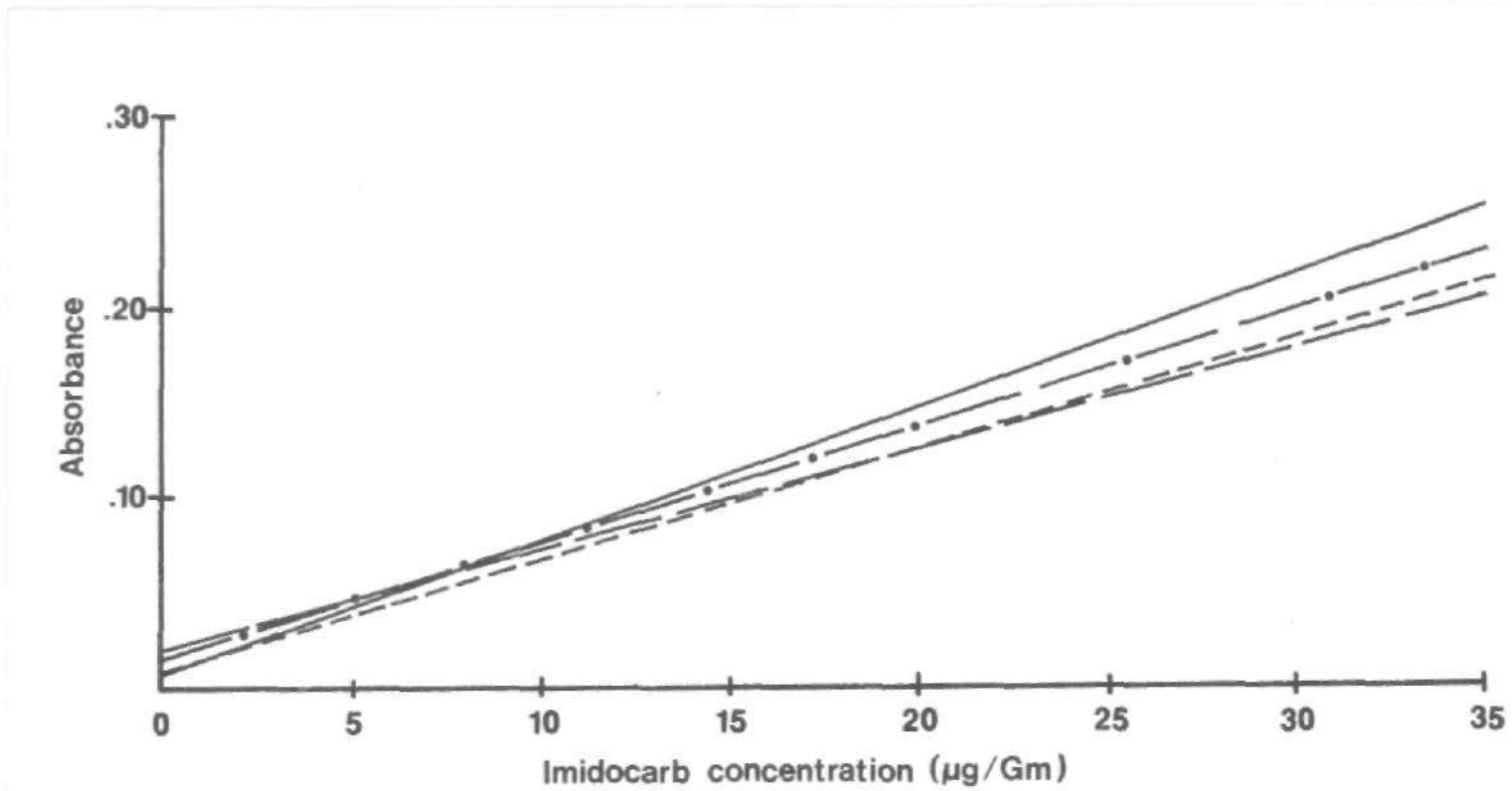


Fig. 3 – *Regression lines for imidocarb concentration in liver, fat and kidney as compared to aqueous standard.*

———— Aqueous: $\hat{Y} = .0067 + .0071\,(x)$
———•——— Kidney: $\hat{Y} = .0167 + .0057\,(x)$
– – – – – – Fat: $\hat{Y} = .0069 + .0060\,(x)$
—— —— Liver: $\hat{Y} = .0186 + .0052\,(x)$

recovery rates of added imidocarb and their reproducibility are summarized in Table 1. Recovery rates ranged from 70% for whole blood to 96.2% for urine. The standard deviations were low, ranging from 0.3% for bile to 5.6% for liver.

When carried through the assay procedures, the acid extract yielded chromatographically a white fluorescent spot under ultraviolet light with the same R_f as the standard imidocarb; control samples and reagent blanks produced no detectable fluorescence. When the thin-layer plate was sprayed with iodoplatinate location reagent, a single purple or purple with bluish center spot with an R_f of 0.39, identical to that of authentic imidocarb, was produced by each sample (Fig. 4). By this technique the smallest detectable amount was 0.21 µg. Control and reagent blanks were negative.

Plasma levels of imidocarb:

The mean plasma changes in imidocarb levels of 7 sheep following the intramuscular injection of 4.5 mg/kg of the drug are graphically illustrated in Figs. 5, 6 and 7. Fig. 5 gives the changes that occurred during the first 24 hours; whereas, Fig 6 gives those occurring over a 28-day period. The semi-log regression

TABLE 1. Recovery of imidocarb added to tissues and fluids of sheep.

Specimens	No of samples	Amount (µg/Gm or µg/ml) Added	Recovered	Per cent Recovered[k]
Plasma	21	1-25	.99 - 22.4	92.9 ± 3.8
Milk	3	25	23.7	94.7 ± 2.4
Urine	7	25	24.0	96.2 ± 1.6
Bile	3	25	23.3	93.3 ± 0.3
Liver	15	5-75	4.6 - 56.8	82.1 ± 5.6
Kidney	15	5-75	4.8 - 61.8	87.5 ± 4.6
Fat	15	5-75	4.4 - 63.6	85.5 ± 0.9
Brain	3	25	21.6	86.5 ± 2.5
Spleen	3	17.5	15.9	91.0 ± 2.2
Muscle	3	25	22.6	90.5 ± 3.0
Whole Blood	4	25	17.5	70.0 ± 1.0
Washed RBC	4	25	18.8	77.8 ± 1.8

k. mean ± standard deviation

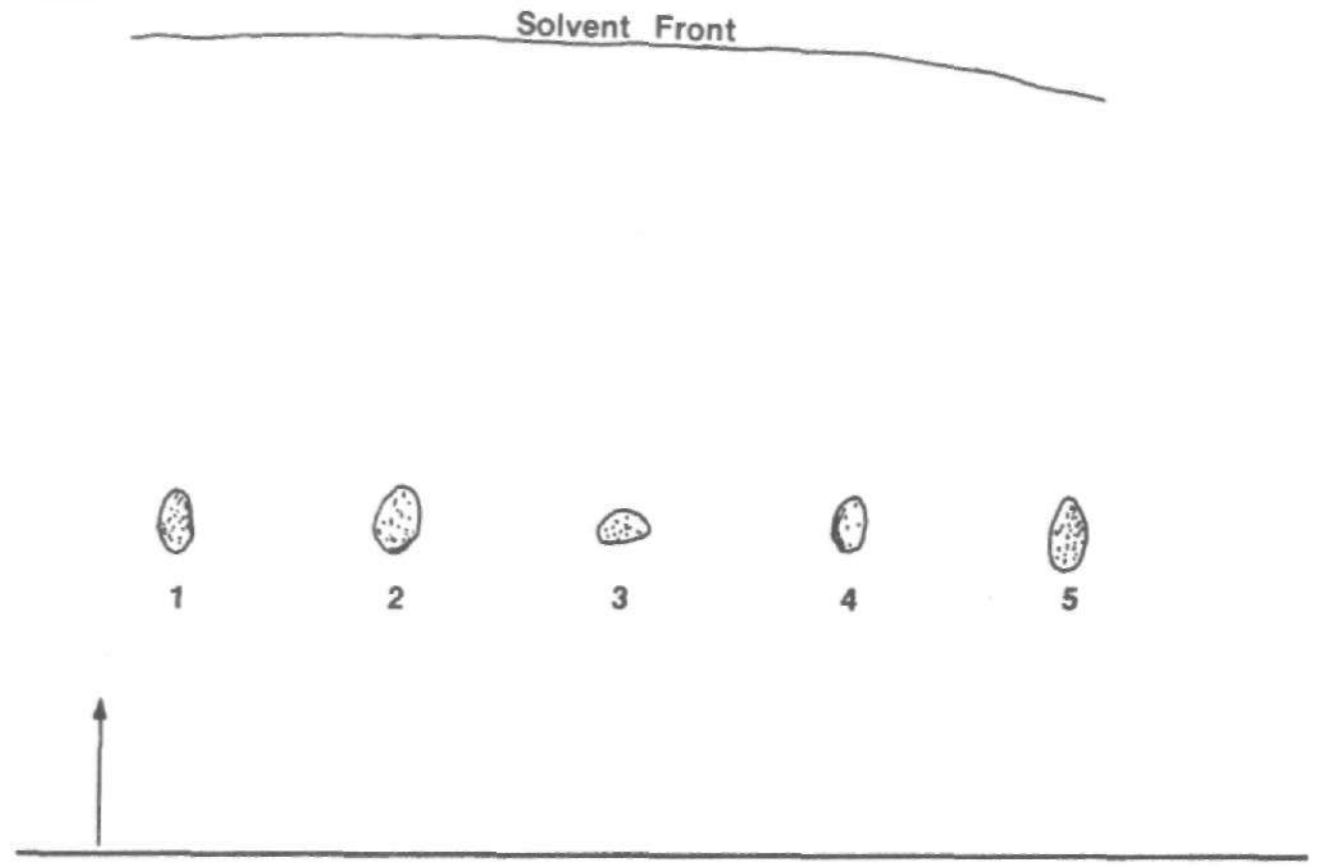


Fig.4- Ascending chromatogram of isoamyl alcohol-hexane extracts of liver (1), plasma (2), urine (4) and of unextracted urine (5) and standard imidocarb in 0.82 N HCL (3).

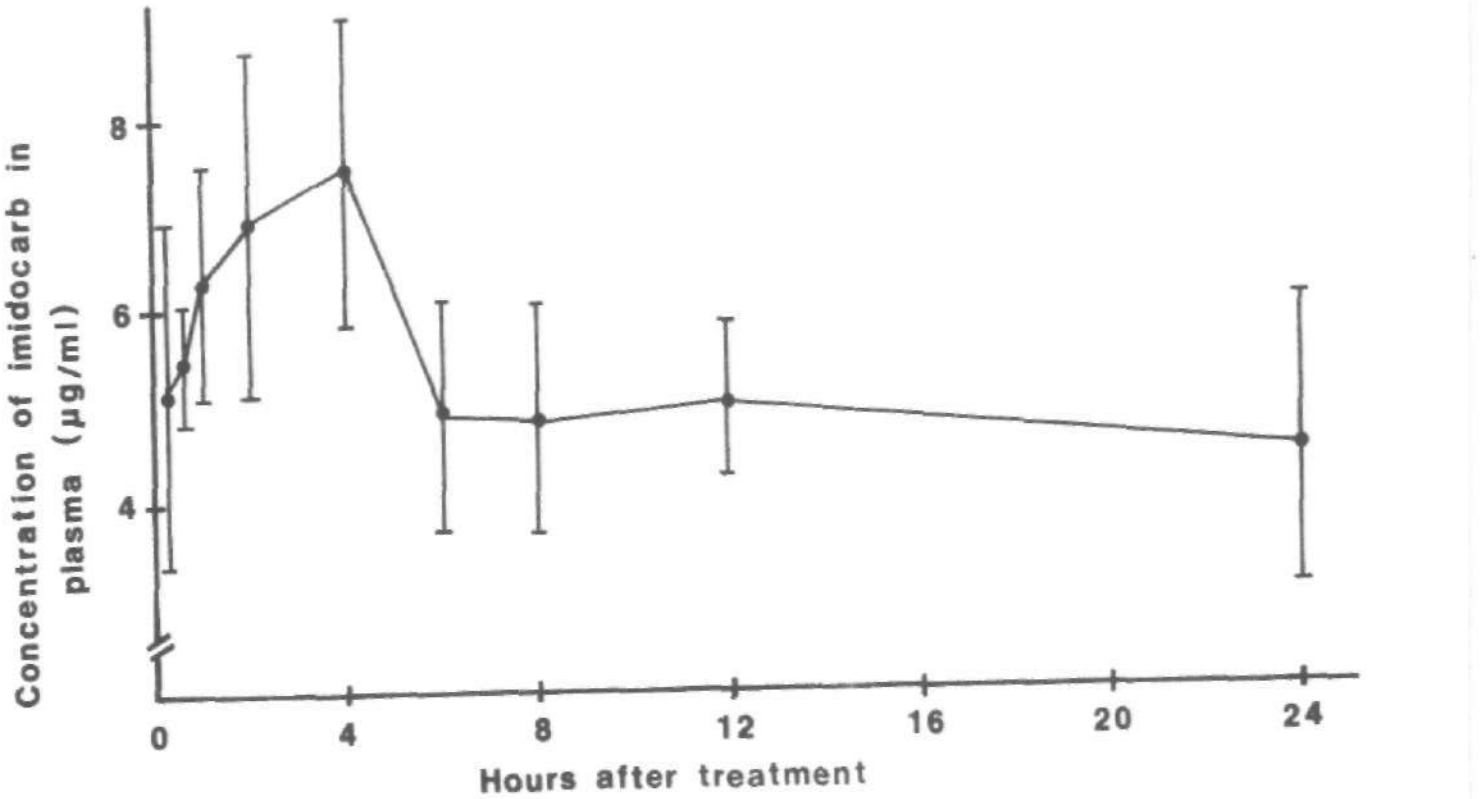


Fig.5 – Plasma levels of imidocarb (means ± 1 S.D.) in 7 sheep for the first 24 hours following the intramuscular injection of the drug (4.5 mg/kg of body weight).

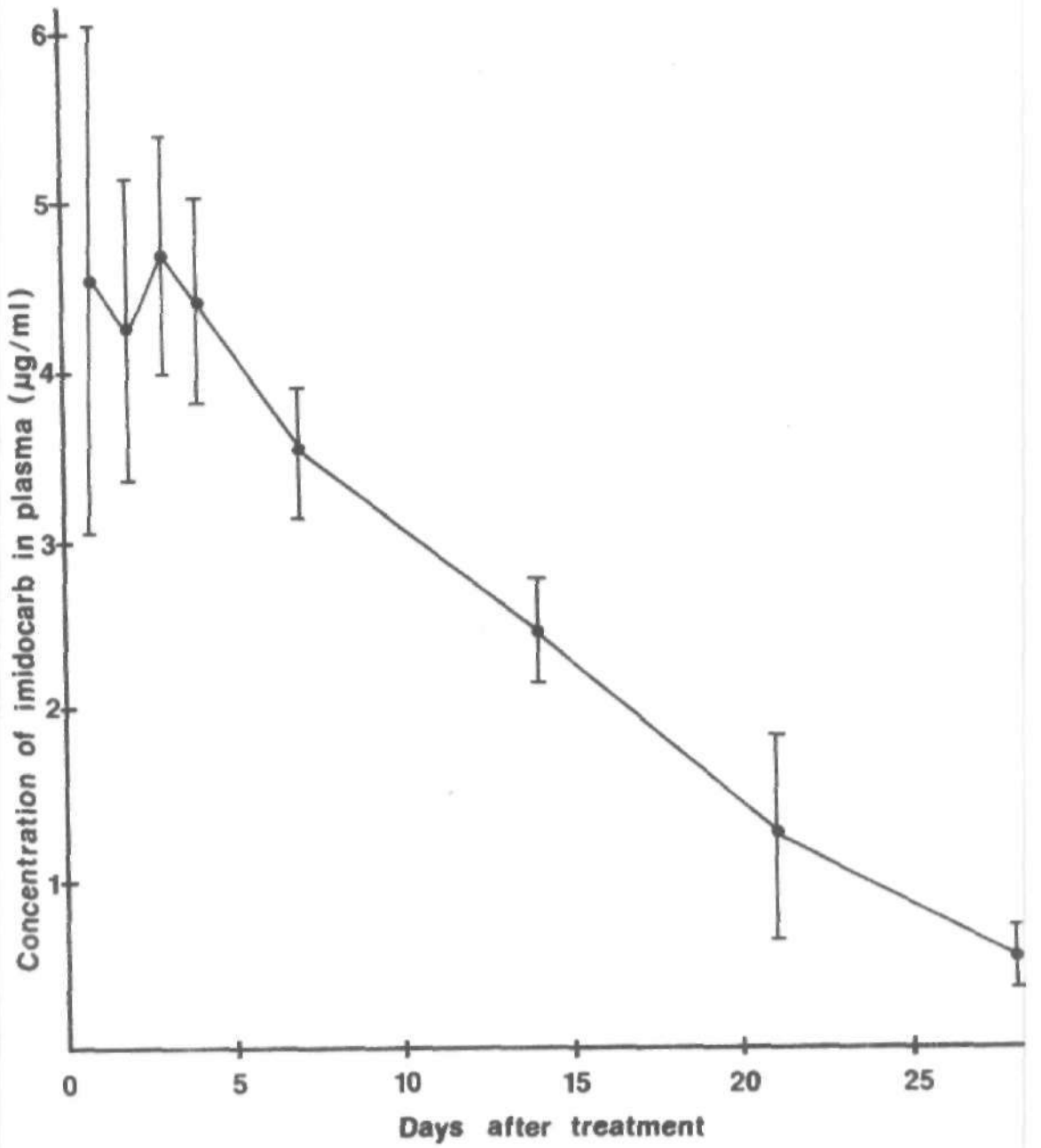


Fig. 6 – Plasma levels of imidocarb (means ± 1 S.D.) in 7 sheep for 28 days following the intramuscular injection of the drug (4.5 mg/kg of body weight).

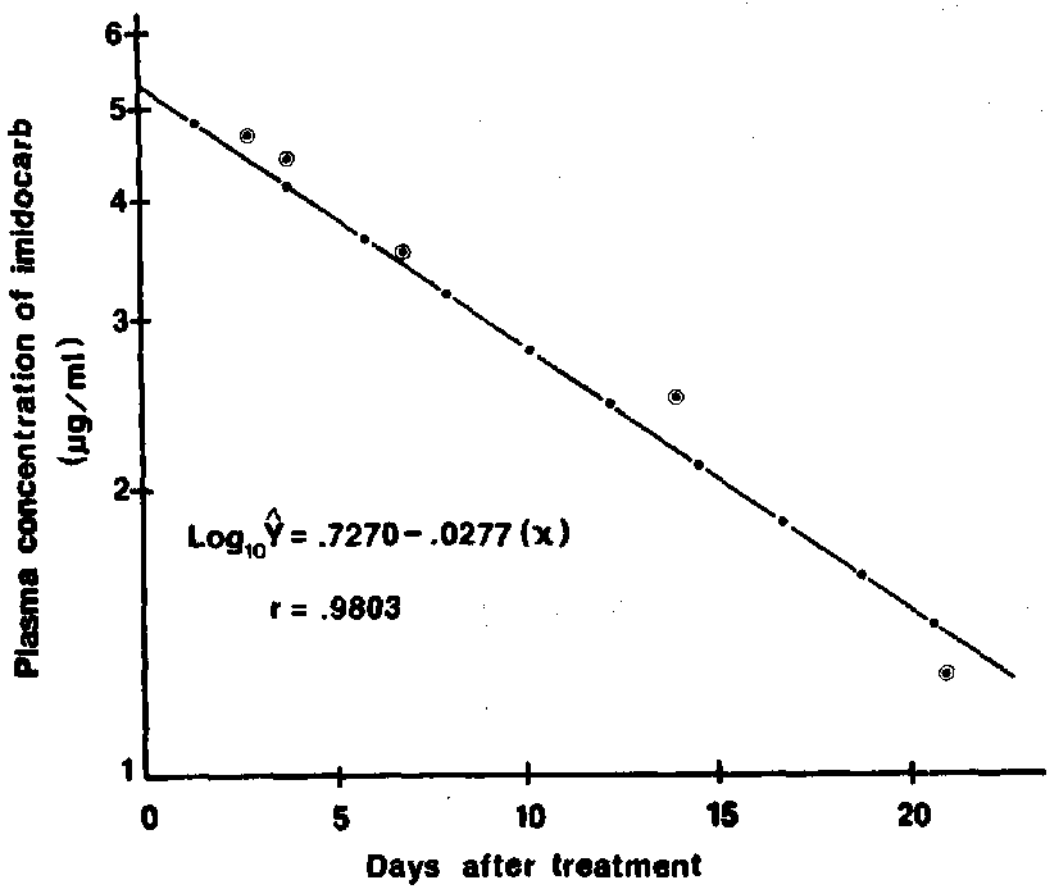
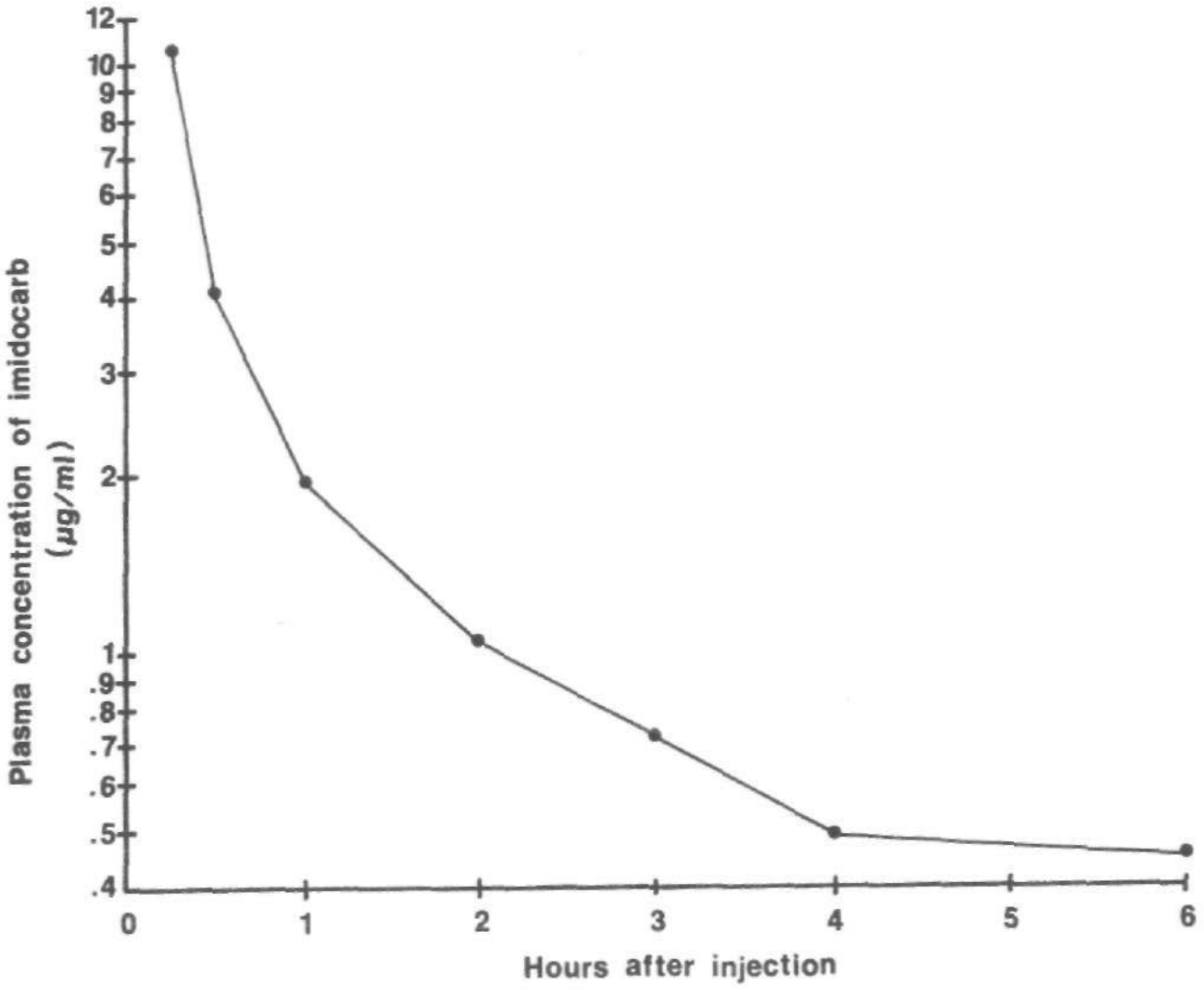


Fig.7 – Regression line of mean plasma concentration of imidocarb in 7 sheep for days 3–21 inclusive following the intramuscular injection of the drug (4.5 mg/kg of body weight). Semi–log plot.

Fig. 8--Decay of the mean plasma levels of imidocarb in 3 lactating ewes following the intravenous injection of the drug (2 mg/kg of body weight). Semi-log. plot.


Plasma concentration of imidocarb (µg/ml)
12
10
9
8
7
6
5
4
3
2
1
.9
.8
.7
.6
.5
.4
0
1
2
3
4
5
6
Hours after injection

hours for the next 5-hour period. Correspondingly, the rate constants of elimination from plasma were 1.74 $hour^{-1}$ and 0.43 $hour^{-1}$.

Plasma protein binding:

The binding of imidocarb by plasma proteins was determined by dialysis. In a preliminary study to determine the in vitro binding of imidocarb by sheep plasma proteins, maximum binding was found to occur at plasma concentrations of 7.4 to 18.9 µg/ml with 53 to 62% of the drug bound (Table 2). When imidocarb was given intravenously (2 mg/kg of body weight) with plasma concentrations varying from 1.3 to 6.1 µg/ml, binding ranged from 20.7 to 53.3% (Table 2). The linear relationship (correlation coefficient, $r = .9526$, $P < .01$) between in vivo plasma protein binding and drug concentrations is illustrated by double-reciprocal or Lineweaver-Burk plots (Figs. 9A and B). The y-intercept of Fig. 9A gives a theoretical maximum of 68.96% binding at a plasma protein concentration of 5.73 ± 0.85 mg%.

Tissue distribution:

Imidocarb was found to be rapidly and widely distributed in all tissues studied (Table 3). At the

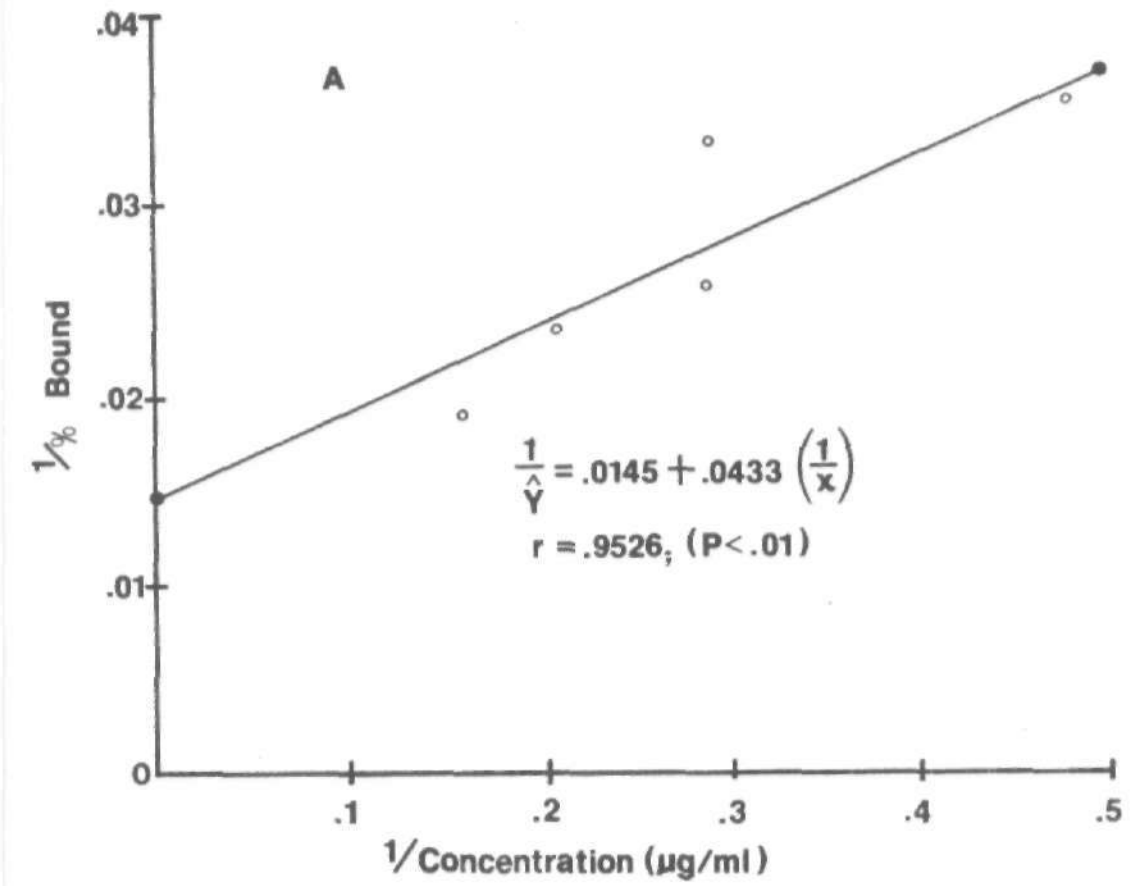
Table 2. *In vitro* and *in vivo* protein binding of imidocarb in sheep plasma.

Concentration (µg/ml)			
Total plasma	Plasma phase	Buffer phase	Per cent bound
	In vitro		
74.2	31.2	16.1	48.4
35.4	15.1	7.7	48.8
18.9	8.6	4.0	53.7
7.4	3.7	1.4	62.2
	In vivo		
6.1	4.7	2.2	53.3
4.8	3.4	2.0	41.3
3.4	2.3	1.4	39.3
3.4	2.0	1.4	30.0
2.1	1.4	1.0	28.6
1.9	1.2	0.9	27.7
1.3	0.8	0.6	20.7

Figs. 9A&B--Linear relationship between the total concentration of imidocarb in sheep plasma and its degree of protein binding. Average values for 3 sheep. Lineweaver-Burk plots:

A--Double-reciprocal plot.

B--Linear plot.


A
.04
.03
.02
.01
0
1/% Bound
$\frac{1}{\hat{Y}} = .0145 + .0433\left(\frac{1}{x}\right)$
r = .9526; (P<.01)
.1
.2
.3
.4
.5
1/Concentration (µg/ml)

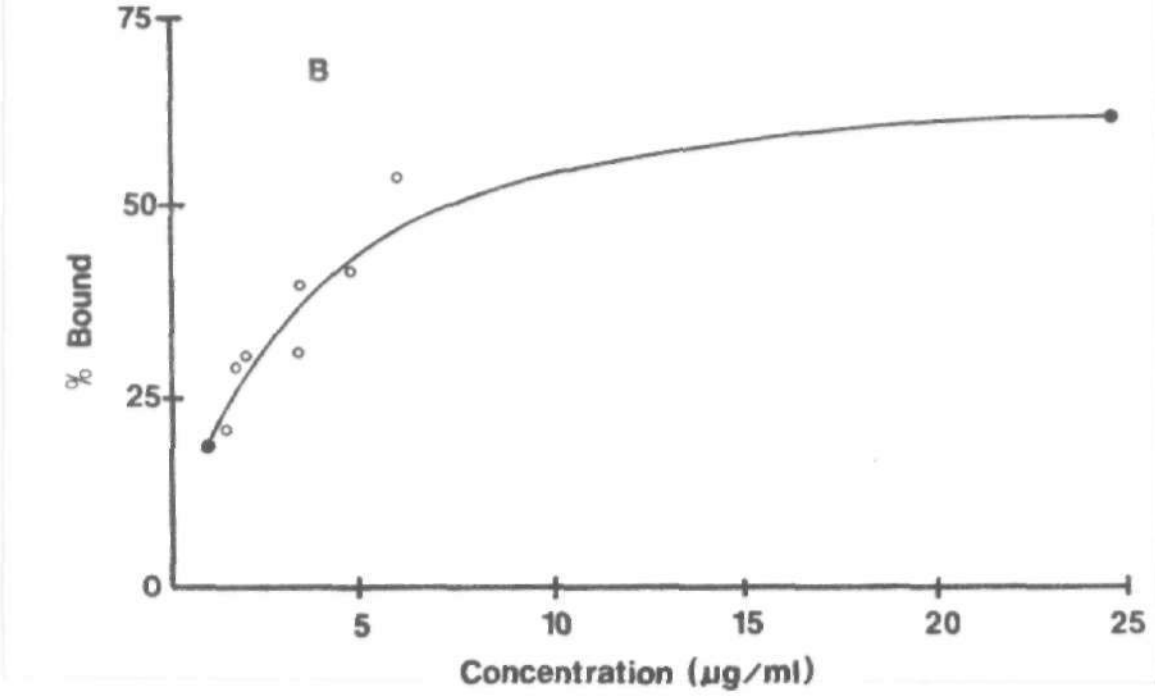

B
75
50
25
0
% Bound
5
10
15
20
25
Concentration (µg/ml)

Table 3. Distribution of imidocarb in various tissues of sheep following intramuscular administration of the drug (4.5 mg/kg of body weight.

	Concentration µg/Gm of tissue; µg/ml of fluid				Ratio
					24 hr
Tissue	4 hr	6 hr	24 hr[1]	32 day	32 day
Brain	<5	<5	43.9	32.9	1.3
Pancreas	ND	ND	25.0	31.4	0.8
Uterus	35.6	24.6	21.3	17.7	3.0
Heart	5.8	9.4	11.0	15.3	0.7
Spleen	<5	10.5	17.0	<5	>3
Adrenal gl.	8.7	10.1	12.6	14.0	0.9
Liver	9.2	21.5	36.0	12.8	2.8
Mammary gl.	8.0	16.4	8.5	12.3	0.7
Fat	20.9	15.2	6.5	11.8	0.6
Ovary	ND	ND	24.0	11.5	2.1
Muscle	<5	<5	11.1	9.4	1.2
Kidney	82.0	127.8	116.6	6.3	18.5
Lung	14.5	14.0	17.0	<5	>3
Bile	2.0	4.6	10.6	3.4	3.1
Milk	4.5	5.3	5.6	ND	___
Plasma	7.9	4.4	4.6	<1.0	>4.6

1. average tissue levels for 2 sheep, all other tissue levels are for 1 sheep each.

end of 24 hours, concentrations were particularly high in the kidneys (116.6 µg/Gm), brain (43.9 µg/Gm),and liver (36.0 µg/Gm). The kidney level had decreased appreciably after 32 days and the level in the brain had declined by only 25%. The concentrations in the other tissues stu studied were high at 24 hours, ranging from approximately 6.5 µg/Gm for the peripheral fat to 25.0 µg/Gm for the pancreas. These values did not change greatly at the end of the 32 day-period of study. Values ranged from 2.8 µg/Gm for the lungs to 31.4 µg/Gm for the pancreas. The level of imidocarb in the liver of a sheep dying 11 days following the intramuscular injection of the drug was 10.7 µg/Gm and the kidney concentration, 33.5 µg/Gm, versus a plasma level of 3.1 µg/ml. Extensive kidney and liver damage was found on necropsy.

Distribution of ^{14}C-labelled imidocarb:

The distribution of ^{14}C-labelled imidocarb in the pituitary gland, pineal body, within the blood, and within the brain and spinal cord is given in Table 4. The pituitary gland and pineal body contained exceptionally high amounts 0.52 and 0.17 µg/Gm, respectively. Imidocarb was found in all regions of the central nervous system examined. The lowest levels were found in the spinal cord and medulla (0.030 to 0.037 µg/Gm).

Table 4. Residual radioactivity of ^{14}C-labelled imidocarb expressed in µg/Gm or µg/ml of tissue in the CNS, pituitary gland, pineal body and blood of 2 sheep 24 hours after an intramuscular dose of 50 µg/kg of body weight.

Tissue	Sheep number	
	1	2
Spinal cord	.030	.035
Medulla	.037	.033
Cerebellum	.064	.061
Cerebral cortex	.058	.037
Olfactory bulb	.070	.090
Optic tract	.037	.033
Pituitary gland	.512	.529
Pineal body	.125	.221
Thalamus	.063	.031
Hippocampus	.070	.061
Rostral colliculus	.052	.056
Caudal colliculus	.061	.055
Lateral geniculate	.054	.034
Plasma	.016	.010
Red blood cells	.018	.013

With exception of thalamus (0.031 µg/Gm) and the olfactory bulb (0.090 µg/Gm) in sheep 2, all other values fell between 0.050 and 0.070 µg/Gm. In the blood, imidocarb was about equally distributed between the plasma and the red blood cells.

Urinary excretion:

The amount of imidocarb excreted at various intervals in the urine of 3 sheep for the first 48 hours subsequent to administration are given in Table 5, and the rates of urinary excretion for the same interval are shown in Fig. 10. For the first 24 hours urinary excretion rates were high and 14.49% of the administered dose was excreted. Excretion rates then dropped precipitously during the next 24 hours when only 1.14% was excreted. Low concentrations in the urine persisted for the remainder of the experimental period declining slowly with time (Fig. 11). During this period a strong linear correlation ($r = 0.8624$, $P < .01$) existed between plasma and urine levels.

Ultraviolet absorption spectra of extracts of the urine were examined and in no case revealed absorption peaks which might suggest the presence of biotransformation products of imidocarb. Small aliquots of the urine collected over 32 days and chromatographed

Table 5. Urinary excretion of imidocarb following intramuscular administration of the drug (4.5 mg/kg of body weight). Average values for 3 sheep.

Time hrs.	Urine conc. µg/ml	Urine vol. ml/min.	Excretion rate µg/ml/min.	Excreted Amount µg	Excreted % total dose	Excreted Accumulated %
0.5	75	.19	14.25	428	0.24	0.24
2	225	.07	15.75	1418	0.79	1.03
4	165	.09	14.85	1782	0.99	2.02
6	269	.17	45.73	5488	3.05	5.07
8	154	.20	30.80	3696	2.05	7.12
12	143	.10	14.30	3432	1.91	9.03
24	105	.13	13.65	9828	5.46	14.49
48	13	.11	1.43	2059	1.14	15.63

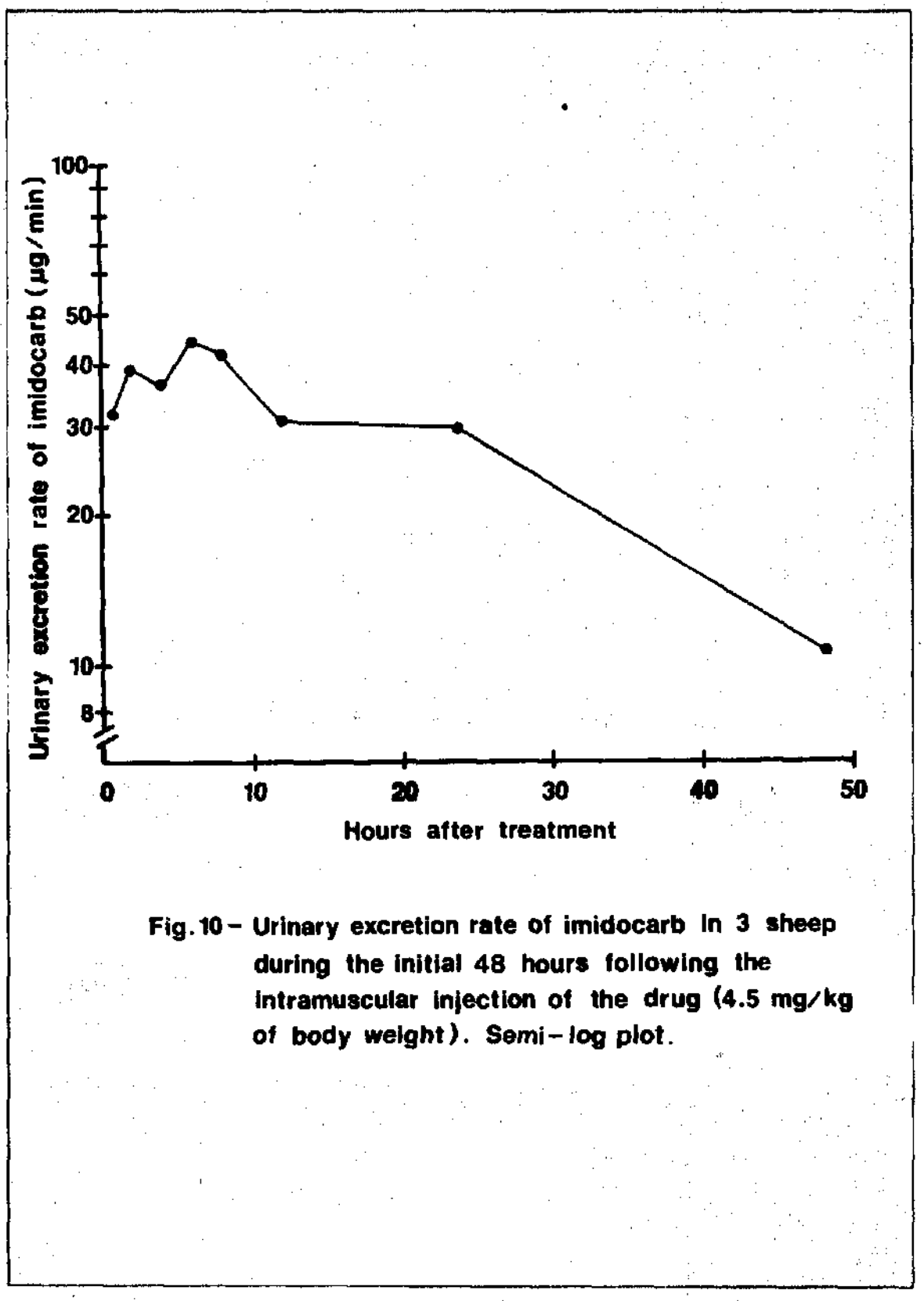
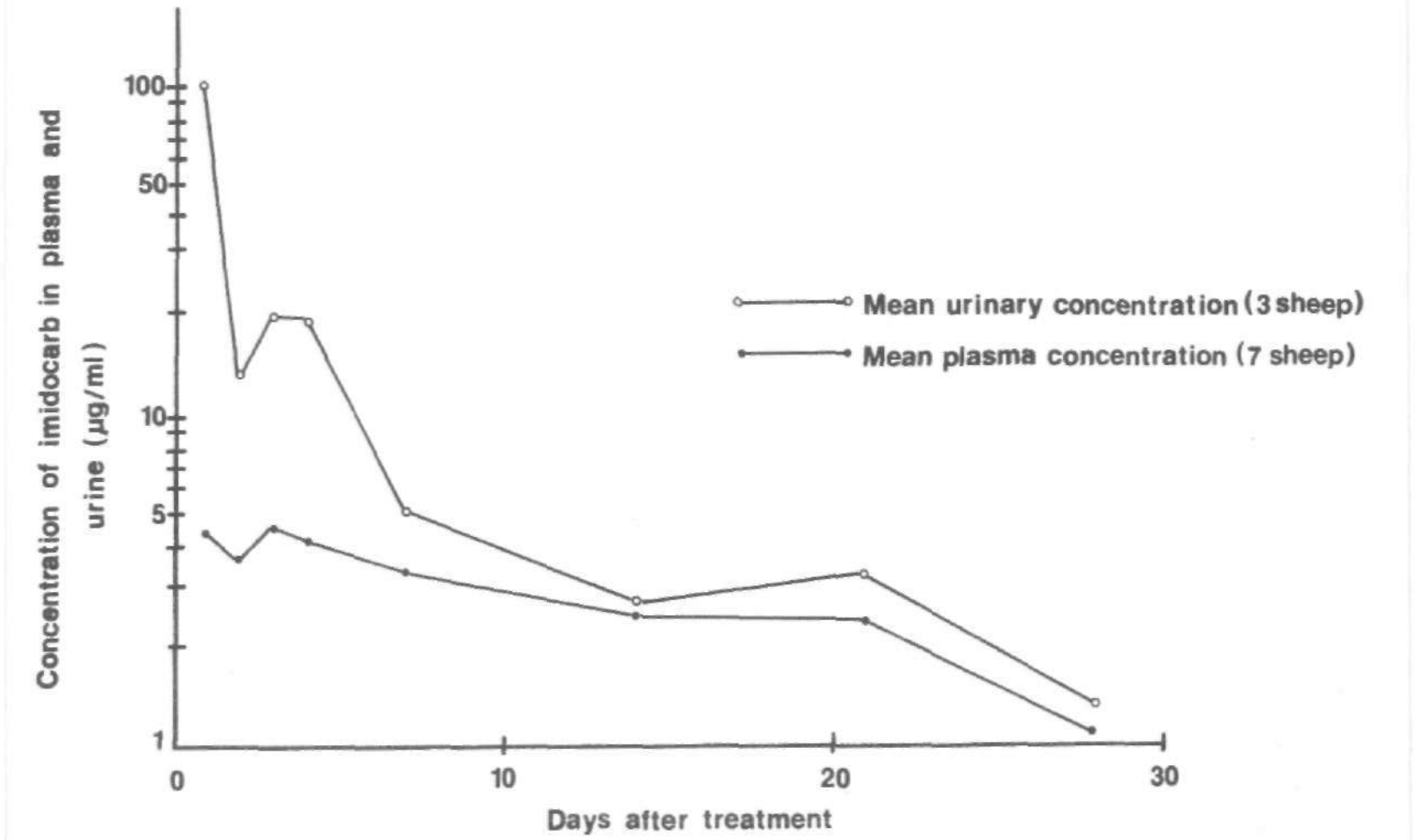


Fig. 10 – Urinary excretion rate of imidocarb in 3 sheep during the initial 48 hours following the intramuscular injection of the drug (4.5 mg/kg of body weight). Semi-log plot.


Concentration of imidocarb in plasma and urine (µg/ml)
100
50
10
5
1
0
10
20
30
Days after treatment
Mean urinary concentration (3 sheep)
Mean plasma concentration (7 sheep)

directly on thin-layer plates yielded only one spot which corresponded to the authentic imidocarb (Fig. 4). These results indicate that metabolites and conjugates of imidocarb are not excreted in any appreciable amounts in the urine. In addition, chromatograms of extracts made from liver, kidney, and other tissues showed that the drug existed in these tissues in the unaltered form.

Renal clearance:

The renal clearance of imidocarb did not vary greatly among the 3 ewes studied. Each experiment involved triplicate successive 10-minute clearance periods. Average values for each experiment are present in Tables 6A and B. The ratio of imidocarb clearance to creatinine clearance indicates net reabsorption by the renal tubules of the drug filtered by the glomeruli.

Excretion in bile:

A relatively high concentration of imidocarb was found in the bile of 5 sheep killed at various intervals after intramuscular injection of the drug (Table 3). In 24 hours the bile/plasma ratio for imidocarb was about 2.3:1. Up to 3.4 µg/ml could be detected in the bile 32 days after administration of the drug. Using spectrophotometry and thin-layer chromatography, there was

Tables 6 A&B. Renal clearance of imidocarb in sheep.

Table 6 A

Sheep	Urine Vol. ml/min	Urine Imidocarb µg/ml	Urine Creatinine mg%	Plasma Imidocarb µg/ml	Plasma Creatinine mg%
1	0.57	145.4	231.8	10.23	1.37
2	0.43	151.8	306.6	6.48	1.38
3	0.39	379.8	268.7	10.57	1.28
Means	0.46	225.6	269.0	9.09	1.34

Table 6 B

Sheep	Clearance (ml/min.) imidocarb	Clearance (ml/min.) creatinine	Clearance Ratio
1	8.19	96.26	.085
2	10.07	82.27	.077
3	13.98	95.54	.170
Means	9.72	91.36	.111

no hint as to the presence of intermediary metabolites in the bile.

Excretion in the milk:

The results of analysis of the milk of a lactating ewe are presented in Table 7. Imidocarb was detected as early as 15 minutes after treatment. Milk concentrations were less than plasma concentrations during the first 4 hours, but thereafter exceeded plasma values. Peak milk concentration occurred 12 hours after treatment. The ratio of peak milk concentration to plasma concentration was 2.4:1. Imidocarb disappeared rather slowly from milk after 12 hours, decreasing at approximately the same rate as plasma concentrations. The disappearance rate constants, K_e, from milk was 0.0063 $hour^{-1}$ and 0.0045 $hour^{-1}$ for plasma. Determinations beyond 1 week could not be made because lactation ceased. The mammary glands and milk of 5 ewes used in the distribution study consistently contained high levels of imidocarb throughout the 32-day period of the study (Table 3, p61).

Aliquot samples of milk fed to nursing lambs had imidocarb concentrations of 1.14 to 3.27 µg/ml. No drug could be detected in the blood plasma of the lambs. The total amount of drug fed with milk was not

Table 7. Imidocarb concentrations in the plasma and milk of a lactating ewe after a single intramuscular administration of the drug (4.5 mg/kg of body weight).

Post treatment time (hours)	Concentration (µg/ml)	
	Plasma	Milk
0.25	3.24	trace
0.50	3.67	1.03
1	3.60	1.49
2	3.38	1.61
3	3.67	3.45
4	5.00	4.24
6	2.66	4.99
12	3.44	8.20
24	1.98	4.97
48	2.13	3.95
72	2.45	2.92
96	2.06	3.13
168	1.27	1.71

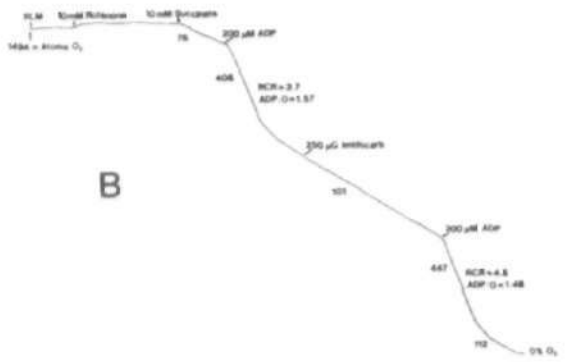
determined since the lambs were allowed to suckle the ewes overnight.

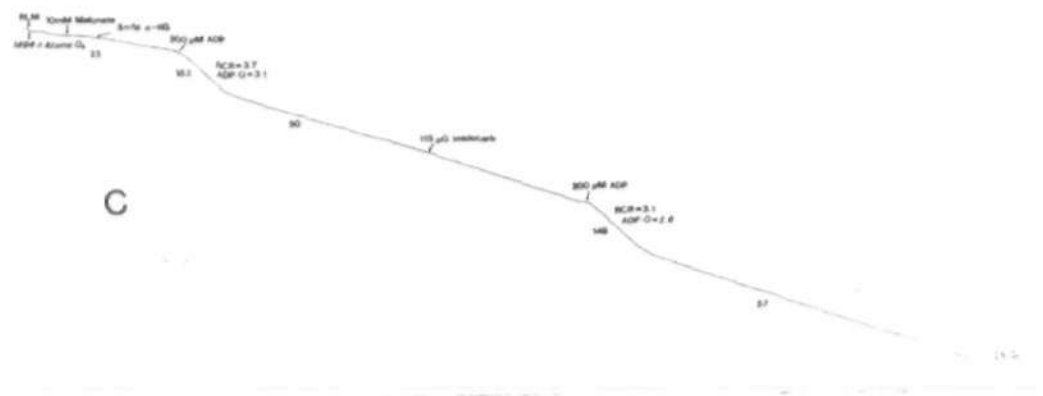
Effect on mitochondrial respiration:

To test the hypothesis that imidocarb may uncouple oxidative phosphorylation and inhibit cellular respiration, rat liver mitochondrial respiration was measured in the presence of imidocarb, using alpha-ketoglutarate and succinate as respiratory substrates. Inhibition of oxygen consumption was not observed (Figs. 12A, B, and C) when imidocarb was added to the reaction mixture during State 4 mitochondrial respiration. Respiratory control ratios and ADP/oxygen ratios were not decreased by imidocarb at concentrations of 38-383 µg/ml. The mitochondrial preparations used in these studies had respiratory control ratios ranging between 2.2 and 4.8; the ADP/oxygen ratios were approximately 1.5 for succinate and 2.8 for alpha-ketoglutarate.

Figs. 12A, B&C--Effects of different concentrations of imidocarb on rat liver mitochondrial respiration. Reaction mixtures (3.0 ml, 27°C) contained 0.5 ml of rat liver mitochondrial preparation. Imidocarb was added in 5-50 µl of water. Malonate, alpha-ketoglutarate (α-KG), succinate and ADP were added in 30 µl of water; rotenone was added in 5 µl of water. Figs. 12A and B show the effects of 38.3 and 83.3 µg/ml of imidocarb respectively, on succinate oxidation. Fig. 12C shows the effect of 38.3 µg/ml of imidocarb on NADH-linked respiration. Respiratory rates are given below each State as nanoatoms of oxygen consumed/minute.


A


B


C

CHAPTER V

DISCUSSION

Assay procedures:

Aromatic amidines are readily and quantitatively extracted from basic aqueous solutions by specific organic solvents (14, 29, 30, 85). In this study, the best solvent system for extraction of unchanged imidocarb was found to be a mixture of isoamyl alcohol and hexane (2:3 v/v). Various protein precipitants were tried in developing the present assay procedure. Compounds such as trichloroacetic acid, phosphotungstic acid, saturated barium hydroxide and zinc sulphate produced unsatisfactory emulsions. Four normal NaOH in the presence of isoamyl alcohol-hexane gave a basic solution and was found to be satisfactory protein precipitant. The sample and the extracting solvent mixed in a ratio of 1:10 (v/v) was agitated vigorously; this agitation avoids emulsion formation and has been reported to be remarkably efficient for direct solvent extraction of drugs (57). Careful regulation of the pH of the final solution in determining the ultraviolet absorption spectrum increased the efficiency of the determination. Of various acids tested at different concentrations, 0.82 N HCl was the best for back-

extraction; it also provided the optimal peak absorbance.

When 1 ml of 0.82 N HCl or 1 ml of plasma or 1 gram of unspiked tissue was put through the assay procedure, a small amount of absorbance was invariably present. In a series of 11 consecutive measurements using HCl, plasma, kidney, liver and fat, background absorbance values of 0.016-0.022 were found. Most of the background reading was attributable to the solvent system used, probably a result of ultraviolet absorbing impurities in the solvent system. Thus, provided that these blanks were used as reference, the small error that the background absorbance could have introduced was eliminated. A concentration of 0.2 µg/ml of imidocarb in 0.82 N HCl, or 1.0 µg/ml in plasma, or 5.0 µg/Gm in tissue gave an absorbance of about twice the background reading. These levels were considered the lowest levels of accurate detection of imidocarb in aqueous solutions, plasma and tissues, respectively (Figs. 2 and 3, pp 46 and 47).

Imidocarb is reported to be tightly bound to components of liver, kidney, and muscle (62). This tight binding has been advanced as an explanation for the low recovery rates (82.1% for liver, 86.5% for brain, 87.5% for kidney and 90.5% for muscle) from these

tissues. The high recovery rate (96.2%) from the urine would tend to support this contention.

Because of the possibility that imidocarb may be bound to blood cells, the assay procedure was successfully applied to whole blood and washed red blood cells. The red blood cells were washed in veronal buffer in order to remove imidocarb that was adhering to the outside of the cells. Addition of 1 ml of water to 1 ml of heparinized whole blood or to 1 ml of washed red blood cells prevented emulsion formation and improved the recovery rate.

Absorption:

The low standard deviation (0.2 - 1.8 µg/ml) of the plasma levels of imidocarb following intramuscular administration of the dipropionate salt indicate a high uniformity between the different sheep in rates of absorption, diffusion to and from the various tissues. Absorption was moderately rapid and probably complete suggesting that imidocarb remained highly non-ionized at the pH values at the site of injection and in the plasma. Peak concentrations between 7.0 and 9.9 µg/ml were achieved between 1 and 4 hours. In a similar experiment when cattle were given 6 mg/kg intramuscularly, peak plasma levels ranged from 4.5 and 8.5 µg/ml

and occurred between 20 and 30 minutes (61). Whereas, in horses 2 intramuscular injections of imidocarb dihydrochloride (6 mg/kg) administered at 24-hour intervals produced peaks on the 3rd and 6th days of only 0.25 µg/ml each (28). The higher values for sheep and cattle may be attributed primarily to more rapid and complete absorption of the dipropionate salt. Local reactions at sites of injection following intramuscular or subcutaneous administration have been reported for the dihydrochloride salt (28, 71, 81). However, in the present study, the dipropionate salt did not cause local irritation. Adams (1) has observed local irritation that persisted for 17 days after an intramuscular injection of 16 mg/kg of the dipropionate salt in horses. While this dose is very high, it is possible that horses differ from cattle and sheep in their ability to absorb the drug and in their susceptibility to its irritant properties.

Plasma levels:

The disappearance rate of imidocarb from sheep plasma following intramuscular injection was prolonged. This is in contrast to the very rapid decrease of diminazene aceturate (5), pentamidine (52), and stilbamidine (32) from the plasma of dogs and mice. This

difference most likely is related to differences in rates of biotransformation and excretion of the various drugs. Species differences have not been observed with imidocarb which is characteristic of drugs that are not substantially metabolized in the body (59). After 4 hours, the plasma level of imidocarb dropped sharply, which is probably a reflection of the rapid diffusion into tissues and excretion of the drug. This kinetic behavior is characteristic of other basic drugs that have been studied (87). Further evidence that imidocarb is sequestered in vascular and extra-vascular spaces is provided in the present study. The high apparent volume of distribution indicates a high degree of localization outside the plasma. In a limited study with cows and sheep treated intramuscularly, imidocarb was found to concentrate within the red blood cells to the same extent as it does in the plasma.

Several investigators (32, 39, 69, 84) have reported the rapid plasma clearance of various diamidines following intravenous or intraperitoneal injection. In this study when 2 mg/kg of body weight of imidocarb was injected intravenously into 3 sheep, the mean plasma concentration was about 10.8 µg/ml at 15 minutes and decreased very rapidly to 1.9 µg/ml in 1

hour, and was less than 1.0 µg/ml in 4 hours (Fig. 8, p56). The plasma rate constant of elimination during the first hour was very rapid (1.74 hours^{-1}) indicating rapid excretion and/or transfer of imidocarb into extravascular tissues to establish distribution equilibrium conditions. However, some of the extravascular tissues apparently do not release the drug at a rate sufficient to maintain the distribution equilibrium. This may explain the changes in the rate constant of elimination observed during the first few hours following administration. Such changes are reported to occur when elimination becomes dependent upon the slow release of drugs from the so called "deep" tissues (20).

There is very little data available on blood concentration of imidocarb, although it is important from the standpoint of dosage regimen. It seems reasonable from the results obtained by Brown and Berger (10), Callow and McGregor (11), Todorovic and colleagues (81) on the treatment of cattle babesiosis and those of Kuttler (51) on the treatment of anaplasmosis with imidocarb that a distinction must be made between suppressive, curative and prophylactic concentrations of imidocarb in the animal body. Although curative doses for the various species of

Babesia and Anaplasma, have been reported by the above investigators, the plasma levels of these doses were not given. It is difficult to decide whether the high peak plasma concentrations maintained for a short span of time or the lower concentrations maintained for long periods are responsible for effecting the cure (54).

It was reported that a dose of 5 mg/kg of imidocarb injected intramuscularly or subcutaneously produced 100% sterilization of cattle infected with the Kenyan strain of Babesia bigemina, and protected the cattle for 6 months (7). The peak plasma concentrations were probably about 7-9 µg/ml. Todorovic et al (81) found 2 mg/kg of body weight of imidocarb given intramuscularly to be highly efficacious in the treatment of acute infections of B. bigemina and B. argentina of Colombian origin, and to protect the cattle for over 3 months. The peak plasma level was probably 3-4 µg/ml. In the present study, the peak plasma concentration of 7.0-9.9 µg/ml had declined to 0.56-1.02 µg/ml after the first month. These results suggest that the suppressive concentration of imidocarb in plasma may be well below 1 µg/ml, probably 0.5 µg/ml. The suppressive dose of 2 mg/kg of body weight reported by several investigators for Babesia (7, 11, 81) probably

maintains concentration of 0.5-1.0 µg/ml for several weeks. The question of the timing of multiple dose, therefore, becomes important as suggested by Frerichs et al (28).

Reference to Fig. 6, p52, will show that uniform decay in plasma levels of imidocarb did not begin until the 3rd day following intramuscular injection of 4.5 mg/kg of body weight. The value observed on the 28th day is somewhat below what is considered to be the accurate range of the instrument for the assay method used. The regression equation was, therefore, calculated from the data obtained between the 3rd and 21st days inclusive (Fig. 7, p53) and is shown below:

$$\text{Log}_{10}\hat{Y} = .7270 - .0277\ (X)$$

$$r = .9803$$

The disappearance of imidocarb from the plasma after the 3rd day, therefore, followed first-order kinetics and will fit the equation:

$$C = C_o e^{-k_e t}$$

Where: C_o = initial concentration at t_o

C = concentration at time t

e = base of natural logarithms

K_e = rate constant of elimination from plasma.

Since the half-life (t 1/2) of the drug is 10.9 days, K_e can be calculated to be 0.0637 day^{-1} according to the equation:

$$K_e = 0.693/_{t\ 1/2}$$

The apparent volume of distribution of drug (Vd) can be calculated by using the y-intercept (C_o) of the regression equation and the dose administered (Q) by the equation:

$$Vd = Q/_{C_o}$$

$$= \frac{210.2\ mg}{5.33\ mg} \times L = 39.42\ L$$

The dose required to produce a given drug concentration in the plasma can, therefore, be related to the apparent volume of distribution (18) as follows:

Total Dose = Vd x body wt. x desired conc.

Where: Vd is expressed in L/kg,
body weight in kg,
desired concentration in mg/L and
the total dose will be in mg.

The interval between doses required to maintain a drug concentration in plasma above a certain minimal value (C_{min}) is related to the initial plasma concentration and the first-order disappearance constant (64) as defined by the equation:

$$\{\ln [(1 + C_o)/ C_{min}]\}/K_e$$

If it is assumed that 0.5 µg/ml is the minimal suppressive plasma level for most babesial infections, the intervals between various dosages may be calculated as shown in Table 8. The calculated values illustrate that doubling or tripling the dose may not significantly increase intervals between doses. This would tend to indicate that dosage levels of 2-3 mg/kg of body weight given intramuscularly as recommended previously in the treatment of cattle babesiosis (10, 11, 81) are probably adequate. The present data would also support this dose for curative purpose since high peak levels are reached in blood plasma and blood corpuscles with possible concentration in Babesia.

It is probable from Kuttler's results (51) that Anaplasma cannot sequester imidocarb and, therefore, a high concentration must be maintained in the blood. Since the rate of excretion is high within the first

Table 8. Estimated dose intervals necessary for maintenance of plasma levels of imidocarb above 0.5 µg/ml in sheep.

Intramuscular dose mg/kg	Initial plasma conc. [Co] mg/L	Intervals in days
2.0	2.4	30.1
3.0	3.6	34.8
5.0	5.9	41.2
6.0	7.1	43.7
10.0	11.8	50.9

24 hours and slow thereafter, the dose administered must be limited to avoid toxic concentrations. Small repeated doses at 2-3 day intervals may be safer and just as effective as single large doses.

Plasma protein binding:

The nature of the interaction of imidocarb with sheep plasma proteins was not found to be similar to that observed with such basic drugs as amphetamine (3), chloramphenicol (18), and phenobarbital (86). The degree of binding at therapeutic plasma levels may depend on drug concentration so long as the concentration of imidocarb is low relative to the number of available binding sites on protein. Protein binding sites appear to become saturated at plasma concentrations of 7.4-18.9 µg/ml with a theoretical maximum binding of about 68.9% as shown in Fig. 9A, p60. A maximum binding of 62.2% was obtained experimentally (Table 2, p58). The moderately high plasma protein binding may be a factor of importance in the extraordinary persistence of imidocarb in the body.

Distribution:

Imidocarb was unusually persistent and widely distributed in the body. Nimmo-Smith and Ince (61)

have reported detectable levels in various tissues in cattle for up to 6 months. The extensive localization observed in tissues is not unusual since it is a property common to a large variety of basic compounds (e.g., antrycide, mepacrine, stilbamidine, chloroquine, etc.). However, its highly selective nature is noteworthy. The initial concentration in the kidney and in the liver have also been observed in cattle (61), horses and donkeys (28). The high kidney concentrations at 6 and 24 hours correlate very closely with the high urine level obtained during the same periods and may reflect more the drug trapped within the lumen of the renal tubules. The appreciable decline in 32 days indicates lack of strong binding by the kidney and confirms the finding by Frerichs et al (28) that imidocarb may be concentrated and resorbed unchanged from the renal tubules. The high liver levels follow closely the bile concentrations of imidocarb and this would indicate the importance of biliary excretion of imidocarb. The results of the assay methods did not show any metabolites of imidocarb in the bile or liver. Compared to the kidney, the liver tended to bind the drug more tightly, hence there was still a high proportion of the drug remaining in the liver at 32 days. The high

concentrations in the kidney and liver may be a major factor in the nephro- and hepato-toxicity of imidocarb reported by Adams (1) and Corrier (16). Further experiments on the relationship between kidney and liver capacities and imidocarb toxicity are desirable.

The brain concentrated imidocarb and the level reached within 24 hours did not decrease significantly over the 32-day period of the experiment. The slow rate at which imidocarb entered the brain as evidenced by the low level at 6 hours might be due to difficulty of the drug in crossing the blood brain barrier. The wide distribution of imidocarb within the central nervous system and its persistence seem to follow the pattern described for stilbamidine (15, 58, 65, 73, 77). The unusually high concentration of ^{14}C-labelled imidocarb in the pituitary may be related to the high vascularity of this organ.

Localization of imidocarb in the kidney, liver and other tissues should be independent of the nature of the salt administered. Several workers (28, 61, 63) have reported on the persistence of considerable residues of imidocarb in tissues when the dihydrochloride salt was injected.

In one cow[m] treated sucutaneously with the dihydroxy-naphthoate salt at a dose rate of 2.5 mg/kg, 15 days post treatment, the liver, kidney, and plasma contained approximately 40 µg/Gm, 30 µg/Gm and 1.7 µg/ml, respectively. The subcutaneous site of injection had a high drug residue (1,618 µg/Gm). Nimmo-Smith and colleague (62) have observed that imidocarb was widely distributed and persistent in tissues after intravenous administration of the dipropionate salt. The duration of prophylaxis afforded by imidocarb, if dependent on localization in tissues, should therefore be independent of the nature of the salt administered. The presence of the dihydroxy-naphthoate salt at site of injection may not be essential for the persistence of imidocarb in the body or for the maintenance of prophylaxis. Todorovic et al (81) demonstrated prophylaxis for at least 21 days against B. bigemina in cattle treated intravenously with the dihydrochloride salt at a dose rate of 3 mg/kg.

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m. tissues supplied by Burroughs-Wellcome, Research Triangle Park, N.C.

Excretion:

Urinary excretion of imidocarb in sheep was rapid during the first 24 hours but reaching a maximum in about 6 hours; thereafter it tended to fall exponentially in close correlation with the plasma levels (Fig. 11, p68). Chromatograms of urine, bile, milk and isoamyl alcohol-hexane extracts of liver, kidney and other tissues did not indicate the presence of metabolites or conjugation products of imidocarb. Perhaps, biotransformation products, if present, are not readily defected by the assay methods used.

The rate of renal clearance of imidocarb has been shown to be considerably below glomerular filtration rate and up to 90% of the amount filtered is reabsorbed. The reabsorptive process that is available to imidocarb is comparable in efficacy with that for certain amino acids such as arginine, suggesting that this may be an active transport system. Peters (67) has reported that strong organic bases are actively transported by the renal tubules since various bases have been shown to depress the simultaneous tubular excretion of others and that in the renal slice at least, the inhibition appears to be a competitive one.

The most important criterion for an active process

is reabsorption against a concentration gradient. Calculation of the ratio of urinary to plasma concentration of imidocarb for every clearance period revealed that the ratio was always considerably greater than unity. These results would indicate that non-ionic passive diffusion is the cause of reabsorption of imidocarb. The rate of renal excretion of a number of organic bases in man and dog has been shown to vary inversely with the pH of the urine (56). When the pH of the tubular fluid is different from that of plasma in the peritubular capillaries, the ratio of non-ionized to ionized form will be different in the two compartments. When the fluid in the tubular lumen is rendered more alkaline than that of the plasma, there will be more of the non-ionized form in the lumen than in the peritubular blood and the resulting concentration gradient will favor passive reabsorption.

It is implicit in such a passive mechanism that it should not exhibit reabsorption maxima (i.e., Tm's), since no rate limiting transport mediator is involved. However, the deleterious physiological effects of imidocarb, like other known strong organic bases, precludes determination of the upper limit for the quantity of the compound that may be reabsorbed. This

dual mechanism of active transport and volume and pH-dependent passive reabsorption has been demonstrated for organic acids in the dog (89), and Torreti and colleagues (82) have reported that it seems to be a plausible hypothesis for organic bases.

Biliary excretion appears to be an important avenue for the elimination of imidocarb after intramuscular injection in sheep. This finding is in agreement with the report by Schanker and Solomon (74) that a number of strong organic bases can be excreted into the bile in high concentrations, and the finding by Reid and coworker (69) that relatively high amounts of injected radioactive stilbamidine were excreted through the bile of mice during the first 24 hours. The level of imidocarb excreted via the urine and bile slowly declined each day without significant fluctuation. Enterohepatic recycling therefore, is probably not important because the diamidines as a class are poorly absorbed from the gastro-intestinal tract.

The detection of imidocarb in milk is important from the standpoint of a public health hazard. Launoy et al (52) were unable to detect pentamidine in the milk of nursing rats, whereas imidocarb was promptly detected

in the milk of lactating ewes. Imidocarb base is virtually water insoluble but highly soluble in the more polar organic solvents (dimethyl sulfoxide, methyl alcohol, isoamyl alcohol, etc.). The rapid penetration into milk can therefore be explained by the low degree of ionization and high lipid solubility. Nonionic passive diffusion is considered the principal way by which drugs penetrate from blood into milk (68). The apparent volume of distribution of imidocarb is approximately equal to the total body water. Such a value is indicative of widespread distribution of the drug in the body. In this context, the mammary gland and its contained milk could slow the elimination of imidocarb from the body by acting as a retention site. The mammary gland concentration of imidocarb remained relatively high throughout the 32-day period of this experiment.

It is possible that differences in pH between blood and milk may account for the high concentrations of imidocarb found in milk. Being a basic drug, the percentage of the non-ionized fraction may be greater in plasma than in milk. A proportion of the diffusible, non-ionized drug is converted into non-diffusible, ionized form, due to the lower pH (6.5-6.8) of milk,

and is trapped in milk and is unable to enter the general circulation. Miller et al (55) have reported that basic drugs produce milk ultrafiltrate to plasma ultrafiltrate ratios of 1.0 or higher. This report is supported by the present experiment where peak milk to plasma ratio of imidocarb was approximately 2.4:1.

Effect on mitochondrial respiration:

Adams (1) and Corrier (16) have demonstrated by electron microscopy the affinity of imidocarb for mitochondria. Since imidocarb is partitioned equally between plasma and red blood cells, it may penetrate into Babesia in the event of an infection. No direct evidence of localization of the drug within Babesia is available at present, but it is known that Babesia do possess a mitochondrion-like structure that performs mitochondrial functions (90). This hypothesis may not, however, explain the mechanism by which imidocarb exerts its action against Babesia, since it was not found in the present study to uncouple rat liver oxidative phosphorylation or interfere with ADP oxygen uptake.

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The author is married to former Zenebu Salawu and has a daughter, Oiza Ozioroko. The typist for this dissertation is Mrs. Zenebu Aliu to whom I express my deep and fond appreciation.