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CLINICAL, TOXICOLOGICAL AND PATHOLOGICAL
ASPECTS OF ARSANILIC ACID POISONING IN
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CLINICAL, TOXICOLOGICAL AND PATHOLOGICAL ASPECTS OF ARSANILIC ACID POISONING IN SWINE

by

Arlo Elmer Ledet

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Dean of Graduate College

Iowa State University
Ames, Iowa

1970
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INTRODUCTION

The use of organic arsenicals in diets of swine for growth promotant, prophylaxis, and therapeutic effects is a common practice. The list of indications for the use of organic arsenicals and the benefits derived from them is quite substantial but the specific action of these drugs is poorly understood.

Arsanilic acid possesses a wide margin of safety when used as recommended. Toxic levels are 10 times the growth promotant or prophylactic dosage and 2.5 times the therapeutic dosage. Unfortunately, widespread use of arsanilic acid in the diets of swine has resulted in cases of toxicosis.

Numerous field cases of arsanilic acid toxicity have been investigated and confirmed by the Iowa Veterinary Diagnostic Laboratory, Ames, Iowa. In these cases toxicity occurred because of indiscriminate or accidental overdosage via the feed or because the drug was added to drinking water without consideration of arsanilic acid in the ration. Toxicosis has also occurred from the proper administration of a therapeutic dose to pigs with severe diarrhea. In these field cases a syndrome of incoordination and paraparesis was observed. Preliminary histopathologic studies indicated that clinical signs were caused in part by demyelination of peripheral nerves.
There is a paucity of published information on some aspects of arsanilic acid toxicosis such as arsenic content of nervous tissue and its relationship to the pathogenesis of peripheral neuropathy. Also data on possible retention of arsenic in edible tissues following prolonged ingestion of toxic levels of arsanilic acid are of public health interest. In view of the desirability for more complete information in these areas, experimental production of arsanilic acid toxicity in swine was undertaken in order to:

1. more fully characterize the clinical syndrome,
2. ascertain the gross and microscopic lesions,
3. determine the location of arsanilic acid in certain tissues,
4. assay arsenic content of tissues at various stages of the toxic syndrome,
5. measure the levels of arsenic retained in tissues after cessation of prolonged ingestion of toxic dose,
6. describe possible mechanisms of arsanilic acid toxicosis, and
7. propose a pathogenesis of peripheral neuropathy.
REVIEW OF LITERATURE

Various forms of arsenic, both organic and inorganic, have been used for many years for medicinal purposes as a general tonic as well as a treatment for specific diseases in man and animals. The medicinal virtues of these agents have been acclaimed for some 2000 years, however, the efficacy of these drugs by modern standards would probably cause their early usage to be classified as empiric.

Arsenicals were used for the treatment of syphilis, tuberculosis, malaria, diabetes, asthma, rheumatism, arthritis, neuralgia, anorexia, skin diseases, and blood disorders, Vallee et al. (1960), and Frost (1967). During the Middle Ages arsenic gained fame as a criminal poison, Goodman and Gilman (1956). In modern times inorganic arsénicals are used in many industrial and commercial activities. They are used in the manufacture of glass, metal alloys, and paper. Many pesticides, herbicides and defoliants contain inorganic arsenic.

In the United States organic arsenicals were made commercially available for animal feeding in 1945. By 1964 it was estimated that about 50 percent of the swine and 90 percent of the chickens raised were fed some form of organic arsenical (Reuber, 1966).

There are many reports in the literature regarding the effects of feeding organic arsenicals to poultry and swine
(Frost et al., 1955). To date no one has been able to assign a nutritional role to these compounds. The value status which arsenicals have achieved in animal production can only be justified on the basis of disease control. The factor of disease control perhaps explains the wide variation in growth promoting ability of the organic arsenicals as reported in the literature.

Morehouse and Mayfield (1946) studied the effect of 4-hydroxyphenylarsonic acid and 3-nitro-4-hydroxyphenylarsonic acid on experimental coccidiosis infection in chickens. These two compounds were effective in reducing hemorrhage and mortality from the coccidium Eimeria tenella when given as a preventive in drinking water.

The first reports of growth promoting effects of arsenicals appeared in the literature in 1949. Bird et al. (1949) and Morehouse (1949) found that the addition of organic arsenicals to diets of chickens not only improved health but also increased feed efficiency. Morehouse (1949) also claimed an average of 15.1 days earlier egg production over control birds. The 3-nitro-4-hydroxyphenylarsonic acid was the effective form used in these experiments.

Carpenter (1951) reported investigations on the effect of 3-nitro-4-hydroxyphenylarsonic acid on growth of swine. Dietary levels of .005, .01 and .02 percent stimulated
growth of weaned pigs. These pigs were reported to have smoother and glossier hair coats than controls. Symptoms of toxicity were observed at the .01 and .02 percent levels after 2 to 4 weeks. With toxicity, growth rate decreased due to interference with ingestion of feed. In the same report, Carpenter indicated that in trials using 3-nitro-4-hydroxyphenylarsonic acid and arsanilic acid at the 0.005 percent level, no growth stimulation occurred in pigs.

Becker et al. (1952) suggested that responses to arsenic supplementation depend upon the disease level in the herd the same as growth responses to antibiotics. Bridges et al. (1954) reported two experiments involving 84 weanling pigs in which bacitracin, penicillin and arsanilic acid were fed singly or in combination. None of these substances significantly increased the growth rates above the controls in either experiment. However, in the second experiment a significant improvement in feed efficiency occurred in all groups. The authors pointed out that all of the experiments were conducted under excellent sanitary conditions which may account for the failure of the supplements to produce a marked increase in growth rate.

In a study with baby pigs fed purified diets Schendel and Johnson (1955), showed that when the diet contained 0.009 percent of arsanilic acid pigs gained a total of 6.16 kg over a four-week period as compared with a gain of 4.16
kg for control pigs.

Hanson et al. (1955) did a study with 80 pigs fed arsanilic acid from weaning to market weight at 0.0033 to 0.01 percent level. Growth rate was improved with the arsanilic acid but the differences were not considered significant. There were no differences in feed efficiency due to feeding arsanilic acid.

A study of the influence of environment on the response of chickens to growth stimulants was conducted by Morrison et al. (1954). In two groups of 50 chickens in an old and new environment 3-nitro-4-hydroxyphenylarsonic acid and penicillin both produced significant growth responses in the old environment only. In the new environment only penicillin produced a significant growth response. No growth response was obtained with arsanilic acid alone in either environment. The level of each of the drugs was as follows, 3-nitro-4-hydroxyphenylarsonic acid 90 gm per ton feed, arsanilic acid 90 gm per ton feed, and penicillin 10 gm per ton feed.

McDonald (1955) also reported the failure of arsanilic acid to give an active growth response in chickens under conditions in which penicillin did.

Libby and Schaible (1955) noted a steady decline in the apparent efficiency gained from feed additives. They contend that this was not due to a buildup of resistant
organisms but rather to an overall improvement of the environment. Control animals benefited from feeding antibiotics and arsenicals by being exposed to a lower level of potential organisms. Evidence presented indicated an overall improvement in performance of control animals over a four year period. This report further substantiates the idea that growth promoters act primarily by reducing the level of disease agents in a group of animals rather than as a specific growth stimulant.

The organic arsenicals have been widely used in swine for prevention and treatment of swine dysentery. Boley et al. (1951) reported on the efficacy of arsanilic acid in controlling a natural outbreak of swine dysentery. Robinson (1951) used arsanic acid derivatives for the same purpose. Carpenter and Larson (1952) reported that the arsenicals, 3-nitro-4-hydroxyphenylarsonic acid and 4-nitro-phenylarsenic acids had a prophylactic and curative effect on swine dysentery when administered via the drinking water. These compounds however, did not prevent recurrence of the disease. Smith et al. (1961) used arsanilic acid at a level of 250 parts per million in feed or 175 parts per million in drinking water as a therapeutic and prophylactic agent for swine dysentery. These levels were effective in reducing the incidence of scours, lowering mortality and improving feed efficiency.
Feed Additive Compendium (1969) lists the approved level of arsanilic acid as 45 to 90 gm per ton of feed to stimulate growth and improve feed efficiency in growing swine. Levels of 225 to 360 gm per ton are permissible for 5 to 6 days for control of swine dysentery.

In an attempt to explain the toxicity of pentavalent organic arsenicals the idea of degradation and reduction to inorganic trivalent forms has been perpetuated. Voegtlin and Thompson (1923) studied the differences in toxicity and parasiticidal activity of trivalent, pentavalent and arsenobenzene types of arsenicals. They believed that differences in activity were due to the change of these compounds into trivalent oxides and that their effectiveness, as parasiticides, was determined by the rate of this change. Eagle and Doak (1951) reported on the parasiticidal activity of arsenoso versus arsonic acids. They indicated that arsenoso compounds have direct activity while arsonic acid became active by virtue of conversion to arsenoso in the animal body. The arsenoso compounds are trivalent and the arsonic acids pentavalent compounds.

Moody and Williams (1964) studied the fate of arsanilic acid and acetylarsonic acid in hens. They found that arsanilic acid was excreted unchanged by hens and that there was no evidence that arsanilic acid was changed to any other compound or converted to inorganic arsenic.
Evidence was based on paper chromatographic characteristics of arsanilic acid and excretion products.

Overby and Fredrickson (1963) and Overby and Straube (1965) studied the metabolic stability of arsanilic acid used in medicated feeds of chickens. The $^{14}$C:$^{74}$As isotope ratio was determined in tissues, organs and excreta of chicks given doubly labeled arsanilic-$1^{-14}$C-$74$As acid. The method used was sensitive enough to detect metabolic degradation of the compound to inorganic arsenic because as little as 1 percent of arsenate-$74$As added to the dose resulted in significant increases and decreases in isotope ratios in the tissues. Trace quantities of orally administered inorganic arsenate were selectively retained in the tissues. In four chicks consistent changes were not observed 24 hours after the last dose of arsanilic-$1^{-14}$C-$74$As acid. Arsanilic acid was found unchanged in excreta and neither arsenate metabolic products nor reduced forms of arsanilic acid were found in the tissues.

Overby and Fredrickson (1965) studied the localization and type of arsenic excreted and retained by chickens fed $74$As-labeled arsanilic acid, sodium arsenate and sodium arsenite. The nature of transport forms of arsanilic acid and inorganic arsenate were compared by paper chromatography, paper electrophoresis, and ion exchange chromatography. Arsanilic acid was excreted faster than inorganic forms and
had less affinity for body tissues. The biological half-life of arsanilic acid in blood was about 36 hours compared to more than 60 hours for arsenate. Two forms of "tissue-bound" arsanilic acid and three of arsenate were characterized. None of the arsenate products was found in the arsanilic acid fed birds. One "tissue-bound" form, compound A, was found in proventricular lining and feathers, and was convertible to arsanilic acid with NaOH. The second "tissue-bound" form, compound B, was chemically very similar to arsanilic acid. It was found as the only radioactive component in kidney and liver. Bile and intestinal contents contained compound B and arsanilic acid in equal proportions while excreta contained arsanilic acid and only trace amounts of compound B.

Since the effectiveness of a drug is dependent upon the length of time which it is retained in the body, the rate and route of arsenical elimination is important. In general, from the literature, it would appear that the organic arsencilals are poorly absorbed from the digestive tract but once absorbed they are quickly excreted primarily by the kidneys. When these same compounds are given parenterally they are rapidly eliminated in urine and to a lesser extent in feces.

Voegtlin and Thompson (1923) using rats, reported that from 80 to 90 percent of the arsenic in the organic pentavalent compounds was eliminated in the urine within the first
6 hours following intravenous injection. They believed that organic pentavalent compounds were excreted unchanged.

Hogan and Eagle (1944) found that as much as 40 percent, of pentavalent organic arsenicals injected intravenously into rabbits, was excreted in the urine within 1 hour.

After intravenous injection of four forms of organic arsenicals into rabbits, Chance et al. (1945) found that the ratio between the total amounts of arsenic excreted in the urine and feces varied widely but the urinary output predominated in all experiments. Arsonic acids were cleared from the body more rapidly than the corresponding arsenoxides. Of the latter, unsubstituted phenylarsenoxide was the more slowly excreted. Crawford and Levvy (1947) found that 90 percent of the phenylarsonic acid injected intravenously in rabbits was excreted in the urine.

In contrast to previous reports on the routes of excretion for pentavalent organic arsenicals, Overby and Frost (1960), reported that following feeding of arsanilic acid to swine, at levels of 30, 60, and 90 gm per ton of feed, more of the arsenic was excreted in feces than in urine. It should be noted that these forementioned studies involved the injection of arsenicals rather than oral administration.

Moody and Williams (1964) found that arsanilic acid
was more rapidly excreted by hens after intramuscular injection than after oral dosing. At an oral dose level of 50 mg/kg approximately 64 percent of the dose was excreted in 1 day while after injection of the same dosage approximately 83 percent was excreted in 1 day. They suggested that arsanilic acid was poorly absorbed by the hen. Acetylarسانیلیc acid given orally in doses of 50 mg/kg was largely excreted unchanged, but a small proportion, 5 to 6 percent, was deacetylated to arsanilic acid. However, if acetylarسانیلیc acid was injected intramuscularly into hens it underwent more extensive deacetylation, 36 percent. This occurred only in the kidney. Deacetylation was associated with the mitochondrial fraction of kidney homogenates. These findings suggested that acetylarسانیلیc acid was poorly absorbed in the hen.

Arsenic is a transition element, a metalloid. It forms alloys with metals and reacts readily with carbon, hydrogen and oxygen. Nonmetal properties permit arsenic to form divalent, trivalent and pentavalent acids. Arsenic is often involved in interactions with compounds of phosphorous, sulfur and selenium in biological systems (Frost, 1967).

Inorganic arsenicals are generally regarded as being more toxic than organic and trivalent forms more toxic than pentavalent. The most toxic forms are trivalent inorganic compounds and the least toxic pentavalent organic compounds.
Voegtlin and Thompson (1923) studied the toxicity and parasiticidal activity of several arsenicals in relation to the rate of their excretion. They found considerable variation in the ability to excrete arsenicals between individuals of the same species. Trivalent oxide forms of arsenicals were excreted the slowest and pentavalent compounds were excreted the most rapid. The rate of excretion decreased as the toxicity and parasiticidal activity increased.

Hogan and Eagle (1944) suggested that variability in systemic toxicity of arsenicals was primarily determined by the degree of binding by the tissues. They found that in a series of phenyl arsenoxides, varying twentyfold in toxicity, that each was bound by red blood cells in vitro in proportion to its systemic toxicity. A similar variation was found in the amount of arsenical bound by circulating red blood cells following intravenous injection. Kidney and liver levels of arsenic 24 or 48 hours after intravenous injection of arsenoxides followed a similar pattern.

Eagle and Doak (1951) studied the biological activity of arsenobenzenes on the basis of their chemical structure. They found that phenyl ring compounds were not only less toxic when they contained acid radicals but were also less effective. Amine-substituted groups however reduced toxicity but did not reduce effectiveness. They suggested
that toxicity and therapeutic activity of arsenoso compounds were determined by the amounts which combined with cellular components and that cytotoxic effects were due to reactions with sulfhydryl groups of essential enzymes.

Gordon and Quastel (1948) studied the use of trivalent arsenic compounds for the detection of enzyme systems containing reactive thiol groups. They found that pentavalent compounds affected hepatic esterase but not other enzymes investigated.

Vallee et al. (1960), in a review article, described some of the enzyme systems with which arsenic may interfere. Cytochrome oxidase, lactic dehydrogenase, sucrose phosphorlase and the sulfhydryl enzymes were listed as being susceptible to arsenic in some form.

Hanson et al. (1955) reported that pigs fed arsanilic acid retained levels of arsenic in their tissues in relation to the amount fed. Hepatic retention was approximately twice as high as renal retention. Arsenic retention in the muscle, fat, and skin was relatively low with 0.37, 0.45, and 0.68 μg/gm respectively the highest individual values obtained.

Brüggemann et al. (1965) reported that despite increasing intake of arsanilic acid and other drugs, residues in the tissues remained at a constant low level. A linear increase did not occur and only a slight increase in residue
was found when drug levels were increased up to 10 times recommended levels. Scheidy et al. (1953) was unable to produce high levels of arsenic in the tissues of swine by feeding 140 mg of sodium arsanilate per 30 pounds of body weight daily for one week. In a study of arsenic toxicity in cattle, Peoples (1964) found no evidence that arsenic was a cumulative poison. He was unable to detect arsenic in blood, milk or tissues of cattle fed arsenic trioxide. Urinary excretion was nearly equal to daily dietary intake.

Overby and Frost (1962b) fed chickens acetone-dried liver powder made from livers of swine fed arsanilic acid at a level of 500 parts per million in the ration. They found no evidence of arsenic accumulation in either species. A similar study by Overby and Frost (1962a) using rats showed no accumulation of arsenic due to ingestion of tissues from animals fed arsanilic acid. Calesnick et al. (1966) fed human subjects tissues of chickens which were receiving arsanilic-74As acid in their diets. There was a rapid fecal and urinary excretion of 74As by the participants. The same subjects were fed arsanilic-74As acid and tissue-74As with no statistical differences between the urinary and fecal recoveries of tissue-74As and pure arsanilic-74As acid. It was suggested that arsanilic acid remaining as tissue residue was not physiologically different from free arsanilic acid.
Notzold et al. (1956) produced experimental toxicosis in swine with arsanilic acid. Weanling pigs fed 8 times the recommended level for growth promotion developed an acute toxicosis characterized by depressed feed intake, sanguineous stools, irritability, cannibalism, and complete paralysis. Death occurred in an average of 29 days on this diet.

Oliver and Roe (1957) reported a field case of arsanilic acid toxicity in swine which had accidently been fed 15 times the recommended level for growth promotion. The clinical signs observed were varying degrees of incoordination and some blindness. Consciousness, temperature and appetite were not significantly affected. Recovery was uneventful following removal of arsanilic acid from the diet, however some of the pigs apparently remained blind.

Jubb and Kennedy (1963) described poisoning in swine by organoarsenicals. Cutaneous erythema, blindness, vestibular disturbances, ataxia and hyperaesthesia were listed as typical changes. No gross lesions were described. Peripheral neuropathy and degeneration of optic nerves and tracts were listed as the primary microscopic lesions.

Harding et al. (1968) reported findings in experimentally induced arsanilic acid poisoning in pigs. Clinical signs were incoordination, loss of sight, and eventual paresis. All animals remained alert and continued to eat
as long as they were able to get to the feed. Gross
lesions were limited to distension of the urinary bladder
in a few cases of prolonged poisoning. Microscopic lesions
were essentially the same as described by Jubb and Kennedy.

Vorhies et al. (1969) investigated decreased water
consumption as a factor affecting the toxicity of arsanilic
acid for swine. Pigs fed 19 percent protein diets con­taining 0.02 percent arsanilic acid and given water varying
quantitatively from 120 ml daily to 1,644 ml daily had no
signs or lesions of arsenic toxicosis. Typical signs and
lesions of toxicosis were produced in two pigs given amounts
of arsanilic acid equivalent to 0.10 percent of the diet
while water consumption was restricted. It was suggested
that the quantity of water consumed may be a factor in
arsanilic acid toxicity.

Peterson and Murray (1965) described three basic types
of peripheral demyelination experimentally produced in tissue
cultures of fetal rat dorsal root ganglia. Demyelination
was produced by crushing injury, diphtherial toxin or
thallium poisoning. Crushing injury produced typical
Wallerian degeneration with primary damage to the axon and
its myelin sheath.

Diphtherial toxin produced segmental demyelination with
primary damage related to individual Schwann cells. The
myelin lesions were scattered with neighboring segments of
myelin undamaged. With continued exposure to diphtherial
toxin or with higher levels demyelination was increased
and neuronal damage began to appear. The pattern of
demyelination changed to a Wallerian type when neuronal
damage was produced.

Thallium poisoning caused a vacuolization of axons
without disruption of the myelin sheath. Prolonged
exposure to thallium produced degeneration of neuronal
somas, breakdown of myelin and death of Schwann cells.

It was concluded that neuronal somas, axons, and
Schwann cells differed in their individual sensitivity
and response to particular toxic agents. Also that damage
to one will influence the behavior of the others in ordered
time sequence and result in different patterns of
demyelination.

Yonezawa and Iwanami (1966), produced thiamine
deficiency in cultured nervous tissue by application of
the antimetabolites, oxythiamine and pyrithiamine. Depending
upon the concentration of antimetabolites, deficiency changes
were classified basically into two groups, acute and chronic.
The acute form exhibited necrotic changes of neuron somas,
oligodendroglia, Schwann cells and satellite cells. Myelin
sheaths were also damaged in a characteristic fashion, which
differed from the Wallerian type of degeneration. In the
chronic form degeneration of myelin sheaths was the major
change and it was initiated in the area close to the Schwannian nucleus on peripheral fibers. It was inferred that nerve cells and myelin sheaths have a different threshold and tolerance for thiamine deficiency and that the major metabolic background of thiamine utilization in myelin-supporting cells differs from that of neuron somas.

Fullerton (1966) produced chronic peripheral neuropathy by lead poisoning in guinea-pigs. The most common histopathological change noted was a mixture of segmental demyelination and axonal degeneration. In some animals only segmental demyelination was found and in others axonal degeneration was the sole finding. When both demyelination and axonal degeneration occurred in the same fiber it was indistinguishable from Wallerian degeneration. It was suggested that large heavily myelinated fibers were more severely affected than small lightly myelinated ones.

Pleasure et al. (1969) reported that axoplasmic flow was interrupted in cats with neuropathy induced by acrylamide. It was not interrupted in normal cats or in cats with neuropathy induced by tri-orthocresyl phosphate. Axoplasmic flow was determined by injections of tritiated L-leucine and autoradiography. Proteins flowed from lumbo-sacral motor neurons along the ventral roots and from ganglion cells toward the spinal cord along the dorsal roots at about 1.5 mm per day. Transport of intracellular
proteins from site of synthesis to site of function is accentuated in motor neurons by the extreme length of their axonal processes. It was hypothesized that interruption of protein transport could cause axonal degeneration.
MATERIALS AND METHODS

Experimental Animals and Housing

Swine were obtained from the Iowa State University Swine Nutrition Farm, Ames, Iowa.

Animals for Trials I, II, and III were selected for uniformity of size without regard to sex. They varied in weight from 36 to 41 pounds. These animals were housed in isolation units at the Veterinary Medical Institute, Iowa State University.

Animals for Trial IV were selected to provide minimum size with maximum maturity. Two pigs, 6 weeks of age and weighing 11 and 12 pounds each were utilized in this trial. The pigs were housed in isolation quarters approved for radioisotope research, located in the Department of Veterinary Microbiology, Iowa State University. Each animal was held in a wire cage which was placed over a steel pan lined with a double layer of plastic in order to facilitate the collection of urine. To further contain the radioisotope a heavy sheet of plastic, 8' x 12', was placed on the concrete floor under the animal which received radioactive material.

Experimental Ration

Arsanilic acid was provided by Salsbury Laboratories, Charles City, Iowa, as a 10 percent arsanilic acid in a
corn meal carrier. Two pounds of 10 percent arsanilic acid were added to 198 pounds of a standard 16 percent protein grower ration and mixed in a rotating drum for 8 minutes. Random samples of feed containing the drug and drug-free feed were analyzed for arsenic content. The experimental ration had 900 gm of arsanilic acid per ton which was 10 times the recommended prophylactic level.

The standard ration used in this study was obtained from the Iowa State University Swine Nutrition Farm.

## Ingredients

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<td>Ground yellow corn</td>
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<tr>
<td>50% soybean oil meal</td>
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<tr>
<td>Vitamin premix</td>
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<tr>
<td>Calcium carbonate</td>
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<td>Dicalcium phosphate</td>
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<tr>
<td>Iodized salt</td>
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<tr>
<td>Trace mineral mix</td>
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**100.00 lbs.**

## Calculated analysis

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<td>Vitamin D2</td>
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<td>Riboflavin</td>
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Ca Pantothenate 6.8 mg
Niacin 18.2 mg
Choline chloride 407.0 mg
Vitamin B12 10.0 mcg

Tritium Labeled Arsanilic Acid

Arsanilic acid\textsuperscript{1} was custom labeled with tritium by New England Nuclear\textsuperscript{2} with a catalytic exchange labeling technique. The custom labeled material was purified by thin layer chromatographic procedures. Purified tritium labeled arsanilic acid containing 3 millicuries of radioactivity was mixed with nonlabeled arsanilic acid to make a 10 ml solution containing 200 mg of arsanilic acid\textsuperscript{3}.

Necropsy Procedures

Animals from Trials I, II, and III were stunned with 110 volt AC electric shock and exsanguinated by severing the subclavian arteries and veins.

In order to collect and fix tissues as quickly as possible two people performed all necropsies. The head was disarticulated at the atlas and one person removed the brain and optic nerves while a second person removed the spinal

\textsuperscript{1}Abbott Laboratory, North Chicago, Illinois.
\textsuperscript{2}New England Nuclear, Boston, Massachusetts.
\textsuperscript{3}Procedures carried out by Dr. J. J. O'Toole, Department Veterinary Pathology, Iowa State University, Ames, Iowa.
cord and major peripheral nerves.

To expose the spinal cord and dorsal root ganglia the following technique was devised. The carcass was positioned on the ventral abdominal surface with the limbs spread apart for support. A deep incision was made from the tail head to the anterior cervical vertebrae. The muscles were dissected away from dorsal and lateral spinous processes and displaced laterally to expose the dorsal aspects of the spinal column. A light weight meat cleaver was used to cut away the dorsal arch of the spinal canal, starting at the cervical vertebrae and working caudal. As the cutting proceeded the dorsal aspect of the spinal column was reflected away from the carcass to aid in controlling the depth of the cut. With care and practice the spinal cord and dorsal root ganglia could be exposed undamaged in minutes after death of the animal. Routine necropsy techniques were used for the remainder of the carcass.

Tissues collected for arsenic determination were sealed in plastic cups and frozen. Samples of liver, kidney, skeletal muscle, rib, blood, spinal cord, peripheral nerves, cerebellum, cerebrum, and brain stem were collected.

Tissues collected for histopathologic evaluation were fixed by immersion in 10 percent buffered formalin. Peripheral nerves were pinned to a paraffin sheet prior to immersion in formalin to reduce contraction and distortion
during fixation. Brain, spinal cord, dorsal root ganglia, sciatic nerves, obturator nerves, femoral nerves, radial nerves, optic nerves, liver, kidneys, adrenal glands, lung, pancreas, stomach, duodenum, lymph nodes, spleen, skin, heart, and skeletal muscles were collected from each animal in Trial I.

Pigs from Trial IV were anesthetized with sodium pentobarbital and killed by exsanguination. Necropsy techniques were used as described above with slight variations required for safe handling of radioactive materials. The necropsy was done on a double layer of plastic sheeting. All radioactive tissues and contaminated disposable supplies were wrapped in double sheets of plastic and placed in plastic bags for disposal of by the Radiological Safety Group. The work area and contaminated instruments were washed with Radiacwash \(^1\) and water.

Samples of liver, kidney, spinal cord, cerebrospinal fluid and bile were collected for liquid scintillation counting. Control animal tissues were not collected for liquid scintillation counting.

Samples of kidney, liver, spinal cord, dorsal root ganglia, optic nerves, optic tracts, and peripheral nerves were collected for autoradiographic procedures. One set

\(^1\)Atomic Products Corporation, Long Island, New York.
of tissues was fixed in 10 percent buffered formalin and one set was placed in glass vials and quick frozen by immersion in liquid nitrogen.

Histologic Procedures

Tissues were fixed in formalin, embedded in paraffin, sectioned at 6 μ and stained with Harris hemotoxylin and eosin Y.

Selected sections of peripheral nerves were stained by Bodian's method for nerve fibers and nerve endings (Bodian, 1936). This method selectively stained axons black thus making it easier to identify axonal fragmentation.

Arsenic Analysis

Arsenic residue in tissues of swine fed arsanilic acid was measured as total arsenic. The technique was described by Winkler (1962) for the determination of total arsenic in biological materials.

Tissue samples of 1 to 10 gm in weight were digested in nitric and sulfuric acids to volatilize all free chlorides and thus prevent the loss of arsenic as As Cl₃. The final digestate was placed in an arsine generator and the arsine gas captured in silver diethyldithiocarbamate in pyridine as AsH₃. Arsenic determinations were made by measuring absorbance at 540 μm with comparison to a standard curve.
Statistical Methods

Arsenic content of the various tissues from pigs within the 9 time periods was analyzed using analysis of variance techniques. The primary sources of variance to be investigated were: tissues, time periods, and the interaction of tissues by time periods. Sums of square and mean squares for these sources were used as indicators of existing differences among mean arsenic content of subgroups within each source. Appropriate F-tests were made in order to determine significant mean differences within each source of variation. Since arsenic content determinations were made on 10 tissues from each pig the statistical analysis was of the split-plot design with pigs treated as plots (units) and tissues from pigs as subplots (subunits).

Scintillation Counting Procedures

Radioactivity of the various tissues and fluids was measured by liquid scintillation counting using a Packard tri-carb liquid scintillation spectrometer\(^1\).

Samples of 0.05 ml whole blood, 0.1 ml urine, 0.5 ml cerebrospinal fluid, 0.1 ml serum, .05 ml clotted blood, and 0.1 ml bile respectively were solubilized in NCS\(^2\).

---


NOS solubilized samples were counted in 18 ml of PPO-POPOP\textsuperscript{1} scintillator solution (50 parts PPO and 0.625 parts POPOP in toluene).

Tissues were prepared for scintillation counting by maceration with scissors followed by digestion in Hyamine\textsuperscript{2}. Paired samples of 50 mg kidney, 50 mg liver, and 100 mg spinal cord respectively, were digested in 1 ml of Hyamine. The digested samples were counted in 18 ml of scintillator solution made up by mixing 8 gm PPO, 0.6 gm dimethyl POPOP, 150 gm naphthalene, 100 ml 2-ethoxyethanol and 20 ml ethylene glycol q.s. in 1,4-dioxane to 1 liter.

**Autoradiographic Procedures**

Samples of optic nerve, optic tract, sciatic nerve, lumbosacral cord, dorsal root ganglia, kidney and liver were placed in glass vials for quick freezing in liquid nitrogen. Frozen sections were cut at 8 \textmu m in a cryostat at -15\textdegree to -20\textdegree C and mounted directly from the microtome knife onto glass slides. The tissue sections were air dried, fixed in acetone for 10 minutes then air dried for 6 hours at room temperature. After drying for 6 hours the slides were dipped in NTB-3\textsuperscript{3} nuclear track emulsion, diluted 1:2

\textsuperscript{1}PPO, 2-5 diphenyloxazole; POPOP, Para-bis [-2-(5 Phenyllozaoyl)] benzene.

\textsuperscript{2}Packard Instrument Co. Inc., Downers Grove, Illinois.

\textsuperscript{3}Eastman Kodak Co., Rochester, New York.
in distilled water, dried in the air and packed in light-proof boxes with a drying agent.

Tissue samples, from areas adjacent to those mentioned above, were fixed in 10 percent buffered formalin, paraffin embedded, cut at 5 μ on a microtome, mounted on glass slides and dried in an oven at 80° C for 6 hours. These sections were deparaffinized in xylene, rehydrated, then dipped in NTB-3 nuclear track emulsion as described above.

After exposure for 27 days at 5° C the autoradiograms were developed for 2 minutes, in Dektol¹ diluted 1:2 in distilled water, at 15° C and fixed for 8 minutes. Developed autoradiograms were stained through the emulsion with Harris hematoxylin and eosin Y stain and permanently mounted.

Experimental Design

**Trial I**

**Continuous high level feeding of arsanilic acid**  
Thirty pigs, average weight 38 pounds, were placed in 10 groups of 3 pigs each. Nine groups were fed a ration containing 900 gm of arsanilic acid per ton and a control group was fed the same basic ration without the drug. The animals were housed in pens with concrete floors. Feed and fresh water were available free choice.

All animals were examined daily and the following obser-
vations recorded: appetite, stools, hearing, vision, sensitivity to touch, muscular coordination, and body temperature.

Hearing was evaluated by making a noise and observing the response. Vision was evaluated by moving a white towel slowly past the pen being careful not to attract attention by sound or air currents. Turning the head in the direction of the moving object was considered evidence of vision. Coordination was evaluated by observing the gait and by manual manipulation of the animals forcing them to regain balance or fall to one side. Sensitivity to touch was tested by probing the animals with the fingers or a wooden pointer in areas of the body away from the pigs field of vision.

One group of three pigs was killed and necropsied on day 4, 7, 10, 13, 16, 19, 21, 23, and 27 after the initiation of continuous arsanilic acid feeding. Histopathologic evaluations and arsenic determinations were performed on tissues from each pig.

**Trial II**

**Withdrawal of arsanilic acid after high level feeding**

This trial was designed to determine if pigs fed arsanilic acid at 10 times the recommended level would require longer than the specified 5 day withdrawal period to reduce tissue levels of arsenic to acceptable concentra-
Six pigs, average weight 40 pounds, were fed a ration containing 900 gm of arsanilic acid per ton. The ration was fed free choice with access to fresh water. On the 20th day this feed was replaced by an arsanilic acid free feed. Two pigs were killed and subjected to necropsy on days 3, 6, and 11 after withdrawal of the drug. Kidneys, liver, skeletal muscles, from the medial thigh, blood, rib, spinal cord, peripheral nerves and brain from each pig were assayed for arsenic content.

**Trial III**

**Clinical recovery study**

This trial was designed to determine if pigs which had developed severe posterior paresis, due to arsanilic acid poisoning, could recover within a reasonable period of time.

Six pigs averaging 40 pounds were fed a ration containing 900 gm of arsanilic acid per ton. The ration was fed free choice with access to fresh water. Examinations and observations were performed daily as described in Trial I.

When an animal developed posterior paresis to the extent that it was reluctant to, or unable to rise to a standing position the pig was placed on an arsanilic acid free ration. Pigs were killed and necropsied on day 10,
12, 17, 20, 32, and 38 following the change in diet. Prior to death each animal was examined for any change in clinical condition. Gross lesions were evaluated at necropsy.

**Trial IV**

**Studies with tritium labeled arsanilic acid**

Two pigs, six weeks of age and weighing 5 and 5.5 kg each, were used for this study. Feed was withheld from both animals for 24 hours preceding and for 24 hours during this study to reduce fecal output.

The smallest pig received the tritiated arsanilic acid in order to achieve maximal levels of radioactivity in the tissues with the limited supply of labeled drug. A total dose of 200 mg arsanilic acid containing 3 mC radioactivity was administered intravenously in two injections 12 hours apart. This dose was equal to 40 mg arsanilic acid and 600 mC radioactivity per kg body weight. The control animal received 40 mg arsanilic acid per kg body weight but no radioactivity.

A 0.10 ml sample of blood was taken hourly from the ear vein of the test animal for the first 12 hours. A 50 ml syringe was used to collect and measure the urine excreted each hour for the first 12 hours. Urine excreted in the second 12 hour period was collected and measured at
the end of the study. The contents of the urinary bladder at necropsy were added to the final collection. No blood or urine was collected from the control animal.
RESULTS

Trial I

Continuous high level feeding of arsanilic acid

Clinical signs The clinical signs observed in this trial reflected the variability in the response of a biological system to a stimulus. Due to this variation in response the clinical signs described are qualitative rather than quantitative. In describing the sequence of events in the toxic syndrome the observance of a particular clinical sign in one individual was considered to be the onset even though the same sign might not manifest itself in the entire group for several days.

Several pigs developed a roughened hair coat on day 3 of the experiment. Within 24 to 36 hours these animals had a mild diarrhea characterized by fluid stools. This condition persisted for 2-3 days and was followed by constipation with firm feces coated with excessive amounts of mucus. Following this transient diarrhea, of 2-3 days, normal stools were noted for the remainder of the experiment.

Cutaneous hyperemia and hyperesthesia were evident in several animals by day 5. Since all pigs were white, cutaneous hyperemia could readily be detected. Hyperesthesia was manifested by the pigs squealing and becoming excited when handled or probed. As early as day 6 several pigs that had cutaneous hyperemia and hyperesthesia were mildly
incoordinated. When these animals were forced to run around the pen, they moved with a swaying gait often slipping and staggering when attempting to change direction. Manual manipulation caused these animals to make exaggerated movements in regaining balance.

In this experiment, feeding high levels of arsanilic acid did not seem to affect the pigs appetite. It was noted, however, that with the onset of incoordination many animals had difficulty with prehension of feed and water. This was seen in the majority of the animals that became toxic. These same animals would eagerly ingest food and water placed in their mouths. Several animals were maintained by hand feeding for up to 10 days after loss of prehensile ability.

Signs of incoordination became progressively more severe and by day 15 several animals had developed posterior paresis. These pigs could rise to a standing position with difficulty and would do so only when prodded. They preferred to rise on the fore feet and remain in a sitting position while eating or drinking (Figure 1). Several pigs had quadriplegia by day 18. The characteristic repose was with the fore limbs drawn posteriorly and the hind limbs drawn anteriorly (Figures 3 and 4). Some pigs with quadriplegia remained on one side and made vain attempts at righting themselves (Figure 2). All animals in this trial
Figure 1. Pig with posterior paresis in typical sitting position. Animal had been fed arsanilic acid (900 gm/ton) for 18 days.

Figure 2. Pig with quadriplegia lying in lateral recumbency. Animal had made vain attempts at rising to an upright position. Arsanilic acid (900 gm/ton) had been fed for 20 days.
Figure 3. Pig with quadriplegia following 20 days of feeding arsanilic acid (900 gm/ton). The pig had developed posterior paresis on the 17th day of Trial I.

Figure 4. Pig with advanced quadriplegia manifested by nearly total lack of muscle control in the limbs. The pig had been fed arsanilic acid (900 gm/ton) for 25 days.
remained bright and alert and responded to auditory stimulation. One animal wandered aimlessly as though blind but attempts to prove blindness by quietly moving a white towel past the animal were inconclusive. Several pigs developed a peculiar head tilt. The direction of the tilt was inconsistent and no special significance could be assigned to it.

No unusually high or low rectal temperatures were recorded. There was a range of daily fluctuation between 101° to 104° F.

The onset of clinical signs in relation to the length of time that toxic levels of arsanilic acid were fed is illustrated in Table 1. Clinical signs from day 20 through day 27 were essentially the same as those recorded on the 20th day.

Arsenic residue Arsenic content determinations were made on tissues from each pig in Trial I. The mean arsenic content of 10 tissues is presented in Table 2.

Statistical analysis of the data in Table 1 is presented in Table 3. Significance was tested with the F-test at the .01 level. The F-values indicate a significant difference in: (1) mean arsenic content of tissues among the time periods, (2) mean arsenic content among the various tissues, and (3) time trends of mean arsenic content of tissues.
Table 1. Sequence of onset of clinical signs of toxicosis in pigs fed arsanilic acid at 10 times the recommended prophylactic level

<table>
<thead>
<tr>
<th>Days fed arsanilic acid at 900 gm/ton ration</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
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<th>17</th>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Diarrhea</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Firm feces</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Cutaneous hyperemia</td>
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<td>+</td>
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</tr>
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<td>+</td>
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</tr>
<tr>
<td>Incoordination</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Posterior paresis</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Quadriplegia</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>
Table 2. Arsenic levels\textsuperscript{a} in tissues of pigs fed arsanilic acid at 900 gm/ton ration

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>4</th>
<th>7</th>
<th>10</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>T\textsuperscript{c}</td>
<td>5.92</td>
<td>6.27</td>
<td>6.67</td>
<td>15.70</td>
</tr>
<tr>
<td>Liver</td>
<td>T</td>
<td>5.50</td>
<td>7.73</td>
<td>7.57</td>
<td>10.93</td>
</tr>
<tr>
<td>Muscle</td>
<td>N\textsuperscript{d}</td>
<td>0.41</td>
<td>1.17</td>
<td>0.56</td>
<td>0.67</td>
</tr>
<tr>
<td>Blood</td>
<td>T</td>
<td>1.65</td>
<td>1.70</td>
<td>1.81</td>
<td>2.98</td>
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<tr>
<td>Rib</td>
<td>N</td>
<td>0.35</td>
<td>0.51</td>
<td>0.37</td>
<td>0.89</td>
</tr>
<tr>
<td>Peripheral nerve</td>
<td>T</td>
<td>0.60</td>
<td>1.20</td>
<td>0.97</td>
<td>1.06</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>T</td>
<td>0.26</td>
<td>0.36</td>
<td>0.42</td>
<td>0.59</td>
</tr>
<tr>
<td>Brain stem</td>
<td>T</td>
<td>0.37</td>
<td>0.38</td>
<td>0.69</td>
<td>0.93</td>
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<tr>
<td>Cerebellum</td>
<td>T</td>
<td>0.24</td>
<td>1.03</td>
<td>0.68</td>
<td>0.74</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>N</td>
<td>0.18</td>
<td>0.34</td>
<td>0.49</td>
<td>0.63</td>
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<tr>
<td>Time period means</td>
<td>1.547</td>
<td>2.068</td>
<td>2.023</td>
<td>3.513</td>
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</tbody>
</table>

\textsuperscript{a}Data in each group represents a mean tissue level in parts per million from three pigs.

\textsuperscript{b}Overall tissue means averaged over time periods exclusive of control animals.

\textsuperscript{c}Trace—less than 0.02 ppm.

\textsuperscript{d}Negative to test.
<table>
<thead>
<tr>
<th></th>
<th>16</th>
<th>19</th>
<th>21</th>
<th>23</th>
<th>27</th>
<th>Means $^b$</th>
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<tr>
<td></td>
<td>8.38</td>
<td>8.33</td>
<td>6.30</td>
<td>6.13</td>
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<tr>
<td></td>
<td>9.83</td>
<td>9.67</td>
<td>4.57</td>
<td>5.63</td>
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<td>6.360</td>
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<td></td>
<td>0.61</td>
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<td>0.44</td>
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<td></td>
<td>2.10</td>
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<td>1.03</td>
<td>1.23</td>
<td>1.73</td>
<td>1.797</td>
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<td>0.77</td>
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<td></td>
<td>1.53</td>
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<td>1.01</td>
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<td>1.15</td>
<td>1.117</td>
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<tr>
<td></td>
<td>0.99</td>
<td>0.74</td>
<td>0.84</td>
<td>0.79</td>
<td>0.75</td>
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<tr>
<td></td>
<td>1.06</td>
<td>1.04</td>
<td>1.09</td>
<td>0.89</td>
<td>1.14</td>
<td>0.863</td>
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<tr>
<td></td>
<td>1.27</td>
<td>1.23</td>
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<td>1.00</td>
<td>0.972</td>
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<tr>
<td></td>
<td>0.74</td>
<td>0.82</td>
<td>1.14</td>
<td>1.10</td>
<td>0.93</td>
<td>0.597</td>
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<td></td>
<td>2.728</td>
<td>2.672</td>
<td>1.826</td>
<td>1.861</td>
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Table 3. Statistical analysis of arsenic content of tissues in Trial I

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of square</th>
<th>Mean square</th>
<th>F-value</th>
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</thead>
<tbody>
<tr>
<td>Time periods</td>
<td>8</td>
<td>87.24</td>
<td>10.91</td>
<td>7.68*</td>
</tr>
<tr>
<td>Pigs within time periods</td>
<td>18</td>
<td>25.50</td>
<td>1.42</td>
<td></td>
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<tr>
<td>Tissues</td>
<td>9</td>
<td>2114.01</td>
<td>234.89</td>
<td>234.19*</td>
</tr>
<tr>
<td>Time period x tissue interaction</td>
<td>72</td>
<td>293.38</td>
<td>4.07</td>
<td>4.06*</td>
</tr>
<tr>
<td>Tissues x pigs within time periods</td>
<td>162</td>
<td>162.41</td>
<td>1.003</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>269</td>
<td>2682.54</td>
<td>252.29</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at the 0.01 level.
Relationships between duration of drug intake and arsenic levels in tissues is illustrated in Figures 5, 6, and 7. The level of arsenic in blood is plotted in each of the above figures as a parameter common to all areas of the body.

Comparative amounts of arsenic in blood, muscle and rib may be seen in Figure 5. Blood arsenic consistently exceeded that present in muscle and rib. Muscle arsenic content exceeded 1 part per million in only one group even though arsanilic acid was fed at 10 times the recommended level for 27 days.

Comparative amounts of arsenic content of kidney, liver, and blood may be seen in Figure 6. Kidney and liver retained more arsenic than any other tissues. Blood arsenic remained relatively low as it closely paralleled kidney and liver levels. The sudden reduction in arsenic level between days 13 and 16 reflected the onset of difficulty in prehension of feed.

A comparison of arsenic in blood, peripheral nerves, and central nervous system is illustrated in Figure 7. Spinal cord, brain stem, cerebellum, and cerebrum arsenic levels were averaged and are shown as central nervous system. Arsenic in nervous tissue increased more slowly and did not fluctuate as rapidly as arsenic levels in the blood.
Figure 5. Mean parts per million arsenic in blood, muscle, and rib during continuous high level feeding of arsenic acid. Each point on the graph represents the mean of three pigs.
Figure 6. Mean parts per million arsenic in blood, kidney, and liver during continuous high level feeding of arsanilic acid. Each point on the graph represents the mean of three pigs.
Tissue levels arsenic in ppm

Days fed Arsanilic acid 900 gm/ton ration

- BLOOD
- KIDNEY
- LIVER
Figure 7. Mean parts per million arsenic in blood, peripheral nerves, and central nervous system during high level feeding of arsanilic acid. Each point on the graph represents the mean of three pigs. Mean arsenic content of spinal cord, brain stem, cerebellum, and cerebrum was plotted as a composite of central nervous system
Gross pathology Gross lesions attributable to arsanilic acid toxicosis were not evident. Severe distension of the urinary bladder occurred in several animals that had quadriplegia and were recumbent for 4 days or longer. Decubital ulcers were common in all animals with posterior paresis or quadriplegia.

Histopathology Detectable histopathologic lesions were confined to myelinated nervous tissue. The peripheral nerves, optic nerves, and optic tracts were consistently affected.

The neurons of dorsal root ganglia and the ventral gray column were examined for degenerative changes referable to peripheral nerve damage. In spite of the severe axonal and myelin sheath damage noted in peripheral nerves of some pigs, no pathologic changes were recognized in the cell bodies based on comparisons with controls.

Slight changes were noted in the myelin sheaths of optic nerves and peripheral nerves as early as day 10. Pathologic alterations were condensation and contraction of myelin sheaths. The areas of contracted myelin were stained more deeply eosinophilic than surrounding myelin. On day 13 the lesions were more pronounced and on day 16 definite demyelination was detected. It was characterized by fragmentation and condensation of myelin into globules followed by disintegration of axis cylinders. The final
result was ovoids of myelin containing axonal fragments. With the appearance and progressive development of demyelination there was a marked increase of inflammatory cells, mononuclear and polymorphonuclear, first within blood vessels and later among the nerve fibers. Most of the polymorphonuclear cells were of the eosinophile type. The mononuclear cells had dense, darkly staining nuclei and were of the lymphoid type. In some areas the mononuclear cells were intimately associated with swollen Schwann cells but this was not consistently evident.

Figure 8 illustrates the histological appearance of a peripheral nerve from control animal. Note the neurokeratin network of the myelin sheaths surrounding the faintly gray axons. Nuclei of Schwann cells are rather large and oval shaped. Figure 9 illustrates the early and Figure 10 the advanced lesions in peripheral nerves associated with arsanilic acid toxicosis. Note the presence of many darkly staining nuclei in Figures 9 and 10 compared to Figure 8. A pyknotic Schwann cell nucleus can be seen in Figure 10 between the arrows labeled with a number 3.

Figures 11 and 12 illustrate peripheral nerves from control animal stained by Harris hematoxylin and eosin and by Bodians method respectively. In Figure 13 Bodians method was used to demonstrate axonal disintegration.
Figure 8. Sciatic nerve from control pig. An axon appears as a faint gray line between the arrows 1. Note darkly stained neurokeratin network arrow 2. Harris hematoxylin and eosin Y stain. X480

Figure 9. Sciatic nerve from pig fed arsanilic acid (900 gm/ton) for 16 days. Note contraction of myelin around intact axon, arrow 1, myelin fragment, arrow 2, and myelin ovoid, arrow 3. Harris hematoxylin and eosin Y stain. X480

Figure 10. Sciatic nerve from pig fed arsanilic acid (900 gm/ton) for 27 days. Animal had developed quadriplegia. An estimated 60 percent of the nerve fibers were damaged. Note axon with myelin contracting around it, arrow 1, fragment of myelin, arrow 2, and fragments myelin containing fragment of axon, arrow 3. Fragmented axon is stained darker than the myelin. Harris hematoxylin and eosin Y stain. X480
Figure 11. Sciatic nerve from control pig. Note intact axon between arrows. Harris hematoxylin and eosin Y stain. X375

Figure 12. Sciatic nerve from control pig. Note intact axon between arrows. Bodian's stain. X375

Figure 13. Sciatic nerve from pig fed arsanilic acid (900 gm/ton) for 16 days. Note fragmented axon of degenerating fiber (arrows). Bodian's stain. X375
Demyelination in the optic nerves and optic tracts was less obvious but essentially paralleled lesions in the peripheral nerves. Affected optic tracts and nerves appeared more cellular than those in controls. The appearance of increased cellularity was in part due to the swelling of neuroglial cells causing them to become more prominent.

The histological appearance of optic tracts from a control animal can be seen in Figures 14, 15, and 16, and from an animal which had ingested toxic levels of arsanilic acid for 27 days in Figures 17, 18, and 19. Pathologic alterations seen in the optic tracts of the latter animal were, neuroglial cells with shrunken and pyknotic nuclei, fragmentation of myelin sheaths, and diffuse vacuolization of the tissue.

The histological appearance of optic nerves from a control animal can be seen in Figures 20, 21, and 22, and from an animal fed toxic levels of arsanilic acid for 27 days in Figures 23, 24, and 25. Histopathologic changes noted in the animal receiving arsanilic acid were pyknotic nuclei, fragmentation of myelin sheaths and the presence of many vacuoles in the tissue.

Pathological alterations were evident in the heavily myelinated fibers of peripheral nerves by days 10-13. These alterations had progressed to definite demyelination by day 16. The earliest degenerative change seen in Schwann
Figure 14. Optic tract from control pig. Harris hematoxylin and eosin Y stain. X80

Figure 15. Optic tract from control pig. Harris hematoxylin and eosin Y stain. X160

Figure 16. Optic tract from control pig. Harris hematoxylin and eosin Y stain. X415
Figure 17. Optic tract after 27 days of high level arsanilic acid (900 gm/ton) feeding. Note appearance of increased cellularity and vacuolation. Harris hematoxylin and eosin Y stain. X80

Figure 18. Optic tract after 27 days of high level arsanilic acid (900 gm/ton) feeding. Note intact axon with contracted myelin, arrow 1, and myelin fragments, arrow 2. Harris hematoxylin and eosin Y stain. X160

Figure 19. Optic tract after 27 days of high level arsanilic acid (900 gm/ton) feeding. Note intact axon with contracted myelin, arrow 1, fragment of myelin, arrow 2, and myelin ovoid containing fragment of axon, arrow 3. Harris hematoxylin and eosin Y stain. X415
Figure 20. Optic nerve from control pig. Harris hematoxylin and eosin Y stain. X80

Figure 21. Optic nerve from control pig. Harris hematoxylin and eosin Y stain. X160

Figure 22. Optic nerve from control pig. Harris hematoxylin and eosin Y stain. X415
Figure 23. Optic nerve from pig fed arsanilic acid (900 gm/ton) 27 days. Note appearance of increased cellularity and presence of vacuoles. Harris hematoxylin and eosin Y stain. X100

Figure 24. Optic nerve from pig fed arsanilic acid (900 gm/ton) 27 days. Note myelin fragments, arrow 1. Harris hematoxylin and eosin Y stain. X200

Figure 25. Optic nerve from pig fed arsanilic acid (900 gm/ton) 27 days. Note myelin contracted around axon, arrow 1, and vacuolation, arrow 2. Harris hematoxylin and eosin Y stain. X480
cells associated with initial demyelination was nuclear swelling and deeply eosinophilic cytoplasm. With more advanced demyelination pyknosis and eventually karyorrhexis and karyolysis of Schwann cells were noted. The Schwann cells of nonmyelinated fibers did not appear to be affected in a similar manner. Demyelination was less apparent in the fibers within the dorsal root ganglia.

An evaluation of peripheral nerve degeneration and associated clinical signs is listed in Table 4. Note the progressive development of clinical signs and the pathologic changes in peripheral nerves associated with the duration of feeding toxic levels of arsanilic acid.

**Trial II**

**Effect of withdrawal of arsanilic acid after high level feeding**

*Tissue retention of arsenic* Arsenic determinations were made on tissues of pigs that had ingested arsanilic acid at 10 times the recommended level (900 gm/ton) for 19 days and then were fed an arsanilic acid free ration for 3, 6, or 11 days.

Data from arsenic determinations on tissues of 9 pigs are presented in Table 5. The data shown for day 0 were taken from day 19 in Table 2 and are a mean tissue arsenic in 3 pigs. Data presented for each of the days 3, 6, and 11 represents mean arsenic content of tissues from 2 pigs.
Table 4. Percentage of peripheral nerve fiber degeneration and clinical sign manifested by animals.

<table>
<thead>
<tr>
<th>Day</th>
<th>Clinical sign</th>
<th>Sciatic %</th>
<th>Femoral %</th>
<th>Obturator %</th>
<th>Radial %</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Incoordination</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>Incoordination</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>Incoordination</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>16</td>
<td>Incoordination</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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<td>16</td>
<td>Incoordination</td>
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<td>5</td>
<td>1</td>
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<td>Incoordination</td>
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<td>2</td>
<td>1</td>
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<tr>
<td>19</td>
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<td>5</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>19</td>
<td>Incoordination</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>Incoordination</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>21</td>
<td>Quadriplegia</td>
<td>30</td>
<td>10</td>
<td>5</td>
<td>5</td>
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<tr>
<td>21</td>
<td>Posterior paresis</td>
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<td>10</td>
<td>10</td>
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<td>21</td>
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<tr>
<td>23</td>
<td>Posterior paresis</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>23</td>
<td>Posterior paresis</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>10</td>
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<tr>
<td>23</td>
<td>Quadriplegia</td>
<td>25</td>
<td>20</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>27</td>
<td>Quadriplegia</td>
<td>60</td>
<td>15</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>27</td>
<td>Quadriplegia</td>
<td>60</td>
<td>10</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>27</td>
<td>Quadriplegia</td>
<td>40</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

*Percentage of fibers having undergone degeneration was estimated by random counting of normal and damaged fibers.*
Table 5. PPM\textsuperscript{a} arsenic retained in tissues after feeding arsanilic acid (900 gm/ton) for 19 days

<table>
<thead>
<tr>
<th></th>
<th>Days after arsanilic acid withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.33</td>
</tr>
<tr>
<td>Liver</td>
<td>9.67</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.92</td>
</tr>
<tr>
<td>Blood</td>
<td>1.94</td>
</tr>
<tr>
<td>Rib</td>
<td>0.46</td>
</tr>
<tr>
<td>Peripheral nerve</td>
<td>1.57</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>0.74</td>
</tr>
<tr>
<td>Brain stem</td>
<td>1.04</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.23</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>0.82</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Mean parts per million in tissues of three pigs at day 0 and two pigs each at day 3, 6, and 11.
Arsenic levels decreased rapidly in edible tissues following removal of arsanilic acid from the diet. Skeletal muscles retained less than one-third of a part per million of arsenic 3 days after drug withdrawal.

Figures 26, 27, and 28 illustrate relative levels of arsenic retention by various tissues. Skeletal muscle, rib, and blood arsenic means are compared in Figure 26. There was a rapid decrease in arsenic content in all three tissues following removal of arsanilic acid from the diet.

Mean arsenic content of liver, kidney, and blood are compared in Figure 27. Arsenic levels decreased rapidly in these tissues after 3 days of withdrawal. Kidney and liver arsenic levels remained relatively stable after the first 3 days and were 3 to 4 times greater than blood arsenic 11 days after arsanilic acid withdrawal.

Arsenic in blood, peripheral nerves, and central nervous system are compared in Figure 28. Mean arsenic content of spinal cord, brain stem, cerebellum, and cerebrum are plotted as a composite of the central nervous system. Blood arsenic decreased rapidly while nervous tissue arsenic was more stable. Approximately 50 percent of the arsenic in nervous tissue was still retained 11 days after withdrawal.

Figure 29 illustrates the mean percent arsenic retained in tissues 3, 6, and 11 days after removal of arsanilic acid
Figure 26. Mean arsenic content of blood, skeletal muscles, and ribs after withdrawal of arsanilic acid from diet following 19 days of feeding 900 gm/ton ration
DAYS AFTER ARSANILIC ACID WITHDRAWAL

TISSUE LEVELS ARSENIC IN PPM

BLOOD

MUSCLE

RIB
Figure 27. Mean arsenic content of blood, kidney, and liver after withdrawal of arsanilic acid from diet following 19 days of feeding 900 gm/ton ration.
DAYS AFTER ARSANILIC ACID WITHDRAWAL

TISSUE LEVELS ARSENIC IN PPM

- BLOOD
- KIDNEY
- LIVER

DAYS AFTER ARSANILIC ACID WITHDRAWAL
Figure 28. Mean arsenic content of blood, peripheral nerves, and central nervous system after withdrawal of arsanilic acid from diet following 19 days of feeding 900 gm/ton ration
BLOOD  PERIPHERAL NERVE  CENTRAL NERVOUS SYSTEM

Tissue levels arsenic in ppm

DAYS AFTER ARSANILIC ACID WITHDRAWAL
Figure 29. Mean percent arsenic retained in tissues of pigs 3, 6, and 11 days after removal of arsanilic acid from diet. Mean percentage based on mean arsenic level in tissues following feeding of arsanilic acid (900 gm/ton) for 19 days. Nervous tissue represents a composite of determinations made on peripheral nerves, spinal cord, cerebellum, brain stem, and cerebrum.
NERVOUS TISSUE
MUSCLE
KIDNEY
LIVER
BLOOD

DAYS AFTER ARSANILIC ACID WITHDRAWAL
from the diet. The peripheral and central nervous system arsenic levels are shown as mean nervous tissue arsenic.

**Trial III**

**Clinical recovery from arsanilic acid toxicosis**

Clinical recovery Six pigs were fed a ration containing 10 times the recommended level of arsanilic acid (900 gm/ton) until they had a marked clinical paresis so that they were reluctant to or unable to rise to a standing position. The shortest time period required to produce paresis was 15 days and the longest period 21 days.

After development of paresis the pigs were maintained on an arsanilic acid free ration for periods of 10 to 38 days. None of the 6 pigs made a significant clinical improvement after removal of arsanilic acid from the diet. Two pigs developed a more severe paresis after removal of the drug, however, 1 of these 2 pigs had a suppurative cystitis and bilateral hydronephrosis at necropsy. The results of Trial III are presented in Table 6.

**Trial IV**

**Studies with tritium labeled arsanilic acid**

Blood clearance and urinary excretion Blood and urine samples were collected each hour for 12 hours following the intravenous administration of 1.5 mC radioactivity in 100 mg arsanilic acid. The levels of radioactivity in
Table 6. Clinical recovery trial from arsanilic acid toxicosis

<table>
<thead>
<tr>
<th>Pig</th>
<th>Days fed drug</th>
<th>Signs at drug withdrawal</th>
<th>Days off drug</th>
<th>Signs prior to necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>18</td>
<td>Posterior paresis</td>
<td>10</td>
<td>Quadriplegia</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>Posterior paresis</td>
<td>12</td>
<td>Unchanged</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>Posterior paresis</td>
<td>17</td>
<td>Unchanged</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>Quadriplegia</td>
<td>20</td>
<td>Unchanged</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>Quadriplegia</td>
<td>32</td>
<td>Unchanged</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>Posterior paresis</td>
<td>38</td>
<td>Quadriplegia</td>
</tr>
</tbody>
</table>

Suppurative cystitis and bilateral hydronephrosis found at necropsy.

The various collections, as determined by liquid scintillation counting, are compiled in Table 7.

There was a rapid decrease in levels of radioactivity in the blood. Four hours post-injection only 0.2 percent as much radioactivity remained in the blood as was present at one hour post-injection.

Urinary excretion during the first four hour period accounted for approximately 52 percent of the initial dose. The percent of initial dose excreted via the urine over the 12 hour period is illustrated in Figure 30.
Table 7. Blood clearance and urinary excretion of tritiated arsanilic acid administered intravenously

<table>
<thead>
<tr>
<th>Post injection (hour)</th>
<th>Blood dpm/ml</th>
<th>Volume (ml)</th>
<th>Urine dpm/ml</th>
<th>Initial dose&lt;sup&gt;a&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16,900,000</td>
<td>17</td>
<td>3,060,000</td>
<td>1.56</td>
</tr>
<tr>
<td>2</td>
<td>5,320,000</td>
<td>10</td>
<td>115,800,000</td>
<td>34.70</td>
</tr>
<tr>
<td>3</td>
<td>4,220,000</td>
<td>37</td>
<td>9,400,000</td>
<td>10.45</td>
</tr>
<tr>
<td>4</td>
<td>350,000</td>
<td>46</td>
<td>3,800,000</td>
<td>5.26</td>
</tr>
<tr>
<td>5</td>
<td>380,000</td>
<td>20</td>
<td>4,800,000</td>
<td>2.88</td>
</tr>
<tr>
<td>6</td>
<td>180,000</td>
<td>14</td>
<td>14,800,000</td>
<td>6.05</td>
</tr>
<tr>
<td>7</td>
<td>150,000</td>
<td>50</td>
<td>1,520,000</td>
<td>2.27</td>
</tr>
<tr>
<td>8</td>
<td>147,000</td>
<td>26</td>
<td>2,930,000</td>
<td>2.27</td>
</tr>
<tr>
<td>9</td>
<td>107,000</td>
<td>64</td>
<td>400,000</td>
<td>.79</td>
</tr>
<tr>
<td>10</td>
<td>100,200</td>
<td>26</td>
<td>321,000</td>
<td>.25</td>
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<tr>
<td>11</td>
<td>83,200</td>
<td>31</td>
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<td>.68</td>
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<td>12</td>
<td>117,600</td>
<td>20</td>
<td>1,720,000</td>
<td>1.03</td>
</tr>
<tr>
<td>Totals</td>
<td>361</td>
<td></td>
<td>68.19</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Initial dose contained 1.5 mC radioactivity (3.33 x 10<sup>9</sup> disintegrations per minute).
Figure 30. Urinary excretion of radioactive material for 12 hours following the intravenous administration of 1.5 mC radioactivity in 100 mg arsanilic acid
PER CENT INITIAL DOSE EXCRETED IN URINE

HOURS POST INJECTION

1 2 3 4 5 6 7 8 9 10 11 12
Distribution of radioactivity at necropsy

Samples of blood, cerebrospinal fluid, spinal cord, bile, urine, liver, and kidney were collected at necropsy for liquid scintillation counting. The pig had received a second intravenous injection of 1.5 mCi radioactivity in 100 mg arsanilic acid 12 hours after the first injection which was 12 hours prior to necropsy. Levels of radioactivity detected in the samples collected at necropsy plus the activity present in the composite 24 hour urine sample are listed in Table 8.

The highest levels of radioactivity detected were in urine and bile. Urinary excretion of radioactive materials during the 24 hour period accounted for nearly 75 percent of the total dose administered. Renal and hepatic tissue contained similar levels of radioactivity per gm weight. At necropsy the kidneys weighed a total of 12 gm and the liver weighed 170 gm. Therefore on a total weight basis the liver contained approximately 12 times as much radioactivity as the kidneys.

Whole blood, serum, and blood clot levels of radioactivity were determined and were found to be approximately equal. Cerebrospinal fluid and spinal cord contained the lowest levels of radioactivity of the fluids and tissues collected in this trial.
Table 8. Distribution of radioactivity at necropsy

<table>
<thead>
<tr>
<th>Location</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>141,000 dpm/ml</td>
</tr>
<tr>
<td>Clotted blood(^a)</td>
<td>153,000 dpm/ml</td>
</tr>
<tr>
<td>Serum</td>
<td>144,000 dpm/ml</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>42,600 dpm/ml</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>38,200 dpm/gm</td>
</tr>
<tr>
<td>Bile</td>
<td>2,795,000 dpm/ml</td>
</tr>
<tr>
<td>Urine(^b)</td>
<td>5,180,000 dpm/ml</td>
</tr>
<tr>
<td>Liver</td>
<td>362,000 dpm/gm</td>
</tr>
<tr>
<td>Kidney</td>
<td>348,500 dpm/gm</td>
</tr>
<tr>
<td>Composite urine(^c)</td>
<td>11,070,000 dpm/ml</td>
</tr>
</tbody>
</table>

\(^a\)After removal of serum.

\(^b\)Collected from bladder.

\(^c\)451 ml collected over 24 hours.

**Autoradiographic findings**

Autoradiographs of the various tissues were prepared with permanent mounts for counting of silver grains. Average net counts of silver grains were calculated for an area 50 \(\mu^2\) in four autoradiographs of each tissue. The following net counts were obtained: liver 7, kidney
5.5, optic nerve 1.5, optic tracts 2, medulla 2, spinal cord 2.5, dorsal root ganglia 1, and sciatic nerve 1.5. The silver grains were spread diffusely over the tissue sections with no apparent foci of concentration.
This study was designed to experimentally produce arsanilic acid toxicosis in pigs in order to evaluate the clinical syndrome, gross and microscopic lesions, and to measure arsenic content of various tissues.

The clinical signs were essentially in agreement with the descriptions reported by Oliver and Roe (1957), Jubb and Kennedy (1963), and Harding et al. (1968). However, there were some differences. One was that symptoms of blindness occurred in only one of 39 pigs fed toxic levels of arsanilic acid. The test used for evidence of vision suggested that the pig was not totally blind, but no clinical evaluation was made to determine the degree of impaired vision. Some degree of impairment would be expected because of the histopathologic changes present in the optic nerves and optic tracts. The findings in this study suggest that loss of sight is not as common in arsanilic acid toxicosis as the aforementioned workers have reported. It should be noted that the reports in the literature do not specify the criteria used for determination of blindness. Evaluation of blindness is difficult because an intoxicated and incoordinated animal mimics blindness by its manner of gait.

A roughened hair coat consistently preceded the onset of diarrhea by 24-36 hours. Pigs often develop rough hair
coats prior to or during the onset of diarrhea. This is probably due to a change in fluid balance and possibly indicates mild dehydration. Hyperesthesia and incoordination usually occurred 24-72 hours following the development of diarrhea. The observance of this sequence of clinical signs should be considered suggestive of arsanilic acid toxicosis in swine receiving this drug in feed or water.

Oliver and Roe (1957) and Harding et al. (1968) reported that the removal of arsanilic acid from the diets of pigs showing toxic incoordination, resulted in rapid and nearly complete recovery. In Trial III the removal of arsanilic acid from the diets of pigs which had developed posterior paresis resulted in either no clinical improvement or the progressive development of a more severe paresis. These results suggest that symptoms of posterior paresis marks a point in the toxic syndrome after which demyelination will probably continue to develop despite removal of the drug from the diet. Irreversible changes in the Schwann cells at this stage could account for the lack of clinical improvement. Another possibility is that retention of arsanilic acid may cause a potentiation of the toxicity to nervous tissue. Since regeneration of myelin may require 2-6 months and the pigs in Trial III were maintained for only 38 days after removal of the drug, it cannot be assumed that they would not have eventually recovered. These pigs
would require more than a reasonable period of time for recovery from an economic standpoint.

In this experiment, pigs which developed incoordination also suffered a loss of prehensile ability. Loss of the latter function became more severe in conjunction with the development of posterior paresis and quadriplegia. Affected pigs would eagerly ingest feed and water placed in their mouths which indicated that there was no loss of appetite because of the high levels of arsanilic acid in the ration but rather that there was a decreased ability to ingest feed. Apparently the cranial nerves controlling the muscles used in prehension are also affected in arsanilic acid toxicosis.

Tissue arsenic content was determined at 9 different time periods in Trial I in order to determine if there were differences among the time periods. The results indicated that there was a significant difference in mean tissue arsenic among the time periods. These differences reflected variations in the relative rates of arsanilic acid intake and excretion. During the first 13 days arsanilic acid intake exceeded excretion which resulted in maximum mean tissue arsenic levels by the 13th day. After the 13th day mean tissue arsenic levels decreased by approximately one-fourth. This decline coincided with the clinical onset of incoordination and diminished prehensile ability which caused
a decreased feed intake.

Arsanilic acid excretion studies in Trial IV indicated that 2 hours after an intravenous administration 36 percent of the dose was excreted via the urine. During a 12 hour period urinary excretion accounted for 68 percent of the initial dose. At necropsy there was evidence of arsanilic acid excretion via the bile but the levels of radioactivity per ml was only 20 percent of that in a ml of the 24 hour urine sample. These results indicate that arsanilic acid injected intravenously in pigs is rapidly eliminated in the urine and to a lesser extent in bile. This is essentially in agreement with similar studies done with rats, Voegtlin and Thompson (1923), Hogan and Eagle (1944), and with rabbits, Chance et al. (1945), Crawford and Levvy (1947). The evidence of rapid excretion of arsanilic acid supports the suggestion that variations in the rate of arsanilic acid intake could account for differences in mean arsenic content of tissues among the time periods. Due to the rapid elimination of arsanilic acid from the body any changes in the rate of intake would quickly be reflected in the mean levels retained.

Arsenic determinations in Trial I also indicated that it accumulated at different rates and to different levels in the various tissues. The retention of different levels of arsenic by the various tissues is in agreement with previous
reports (Scheidy et al., 1953; Hanson et al., 1955; Harding et al., 1968). These reports failed to include arsenic levels in nervous tissue and did not indicate the relative rates of arsenic accumulation. In Trial I there were significant differences in the rates of arsenic accumulation by the various tissues. Arsenic content of nervous tissue did not reach maximum levels until days 19 and 21. All other tissues assayed reached maximum levels of arsenic on or before day 13. The delayed attainment of maximum mean arsenic level in nervous tissue coincides approximately with the development of lesions in this tissue. Time trends in the accumulation of arsenic by nervous tissue suggests an increased binding of arsanilic acid as a result of pathological changes.

Arsenic retention by tissues, following prolonged feeding of arsanilic acid at high levels, was investigated in Trial II. Mean tissue arsenic was determined 3, 6, and 11 days following removal of arsanilic acid from the diet. Mean percent of arsenic retention was calculated on the basis of arsenic in tissues on the 19th day of feeding arsanilic acid at the rate of 900 gm/ton ration. Three days after removal of arsanilic acid from the diet skeletal muscle, kidney, liver, and blood retained 31, 34, 32, and 13 mean percent arsenic respectively. The mean percent arsenic retained by these tissues remained essentially constant
during the following 8 days. Arsenic retention by nervous tissue differed markedly. Nervous tissue retained 100, 93, and 57 percent arsenic on days 3, 6, and 11 post withdrawal respectively. This relatively slow rate of arsenic release suggests some form of arsanilic acid binding unique to nervous tissue. This is supported by the results of this study indicating that lesions from arsanilic acid toxicosis are primarily limited to the nervous tissue.

Vorhies et al. (1969) indicated that water deprivation may be a factor in arsanilic acid toxicity. Some form of water deprivation could be a factor in the field cases of arsanilic acid toxicosis in which pigs with severe diarrhea are ingesting therapeutic levels of the drug. Animals with severe diarrhea usually become dehydrated and therefore have a decreased urinary output. In view of the findings in Trial IV that excretion of arsanilic acid is primarily via the urine it is certainly conceivable that anything that would reduce urine output could increase toxicity. Another factor to consider is the possibility of increased rate of absorption from the intestinal tract as a result of inflammation. Moody and Williams (1964) reported that arsanilic acid was poorly absorbed from the digestive tract of hens. There is no reason to suppose that swine absorb arsanilic acid much more readily than hens. Also it is doubtful if an inflamed intestine would absorb arsanilic
acid more readily than a normal intestine. Therefore decreased excretion via the urine probably plays a more important role in the development of arsanilic acid toxicosis than does increased absorption.

The results from the autoradiographic study in Trial IV were inconclusive for the determination of specific localization of arsanilic acid in the tissues. The distribution of silver grains indicated that arsanilic acid was present throughout the tissues. Relative counts among the various tissues tended to reflect the variation in the levels of radioactivity detected in those tissues by liquid scintillation counting.

The failure to determine specific localizations of arsanilic acid was probably due to the lack of any tissue specificity for this drug. A possible solution to the problem might be long term administration of tritiated drug, 15 to 20 days, followed by a 3 day withdrawal period. This would allow the excretion of approximately 70 percent of the arsanilic acid from all tissues except nervous tissue according to findings in Trial II of this study.

Results in Trial IV with tritiated arsanilic acid indicated that urine and bile are two important routes for the elimination of arsanilic acid. Because of the relative levels of radioactivity in each and the volumes excreted, urine would have to be considered the primary route of
arsanilic acid excretion in pigs.

Approximately equal levels of radioactivity in whole blood, serum, and clotted blood indicated that arsanilic acid was evenly distributed between cellular and fluid components. Hepatic and renal tissues contained similar levels of radioactivity on a per gm basis. The liver weighed 170 gm and the kidneys weighed 12 gm thus on a total weight basis the liver contained nearly 12 times as much radioactivity as the kidneys. Cerebrospinal fluid and spinal cord were the least radioactive of the fluids and tissues collected in this trial.

The absence of detectable gross lesions attributable to arsanilic acid toxicosis is in agreement with Jubb and Kennedy (1963) and Harding et al. (1968). Several animals which had quadriplegia and were recumbent for 4 days or longer did have severe distension of the urinary bladder. One of these pigs had a suppurative cystitis and bilateral hydronephrosis which was not considered unusual under the circumstances of paralys's and prolonged recumbency.

Detectable histopathologic lesions were confined to the myelinated nervous tissue. The peripheral nerves, optic nerves, and optic tracts consistently had myelin sheath and axonal degenerations. In spite of the severe lesions noted in the peripheral nerves of some pigs no referable pathologic changes were recognized in the neuron
somas located in the dorsal root ganglia or ventral gray column. Peripheral neuropathy in the absence of recognizable pathological changes in the neuron soma suggests that the etiological factors exert their actions on the cells or mechanisms which maintain myelin sheaths and axons.

Pleasure et al. (1969) reported that axoplasmic flow was interrupted in peripheral neuropathy induced by acrylamide in cats. Axoplasmic flow was determined by the use of tritium labeled L-leucine and autoradiography. In normal cats proteins flowed from lumbo-sacral motor neurons along the ventral roots and from the dorsal root ganglion cells toward the spinal cord at about 1.5 mm per day. Interruption of this flow would be accentuated in the motor neurons due to the extreme length of their axonal processes.

Peterson and Murray (1965) described three basic types of peripheral demyelination experimentally produced in tissue cultures of fetal rat dorsal root ganglia. Demyelination was produced by crushing injury, diphtherial toxin, or thallium poisoning. Crushing injury produced Wallerian degeneration with primary damage to the axon and myelin sheath. Diphtherial toxin produced a segmental demyelination with primary damage related to individual Schwann cells. Neuron soma damage occurred with increased levels of diphtherial toxin. With neuron soma damage the pattern of demyelination changed from segmental to Wallerian type.
Thallium poisoning caused axonal vacuolization without disruption of the myelin sheath. It was concluded that neuronal somas, axons and Schwann cells differed in their individual sensitivity and response to various toxic agents.

Yonezawa and Iwanami (1966) induced thiamine deficiency in cultured nervous tissue by the application of antimitabolites, oxythiamine and pyrithiamine. With low concentrations of antimitabolite they could induce a chronic form of thiamine deficiency. Degeneration of myelin sheaths was the major change. This change was initiated in the area close to the Schwannian nucleus on peripheral fibers. With high concentrations of antimitabolites an acute form of thiamine deficiency was induced. The changes noted were necrosis of neuron somas, oligodendroglia, Schwann cells and satellite cells. It was inferred that there was a different threshold and tolerance for thiamine deficiency in myelin-supporting cells and neuron somas.

In the present study early peripheral nerve degeneration was characterized by condensation and contraction of myelin without apparent axonal damage. Later in the toxic syndrome there was fragmentation and condensation of myelin into globules followed by disintegration of axons. The final result was ovoids of myelin containing axonal fragments. In peripheral nerves the more heavily myelinated fibers were affected first and had the most severe lesions.
Schwann cells associated with damaged fibers were in various stages of degeneration. Some were swollen and had eosinophilic cytoplasm while others had pyknotic nuclei or were karyorrhectic. Schwann cells of unmyelinated fibers did not appear to be affected.

Fullerton (1966) produced chronic peripheral neuropathy by lead poisoning in guinea pigs. Changes noted were segmental demyelination or axonal degeneration. When both occurred in the same fiber it was indistinguishable from Wallerian degeneration. Large heavily myelinated fibers appeared to be more severely affected than small lightly myelinated fibers.

In view of the findings of Peterson and Murray (1965), Yonezawa and Iwanami (1966), and Fullerton (1966), it would seem that myelin-supporting cells, axons, and neuron somas differ in their sensitivity and response to an individual toxic agent. The findings in the present study would suggest that there is a difference in sensitivity and response, on the part of the neuron soma, axon and myelin-supporting cells, to arsanilic acid. Further these findings indicate that myelin-supporting cells are the most sensitive to toxic levels of arsanilic acid.

The presence of peripheral demyelination with necrosis of Schwann cells and a lack of recognizable pathological change in the nerve cell bodies indicates that toxic levels
of arsanilic acid induces a primary demyelination disease in swine.
SUMMARY

The clinical, toxicological, and pathological aspects of arsanilic acid were studied in swine.

Toxicosis was induced in 27 pigs, average weight 38 pounds, by feeding arsanilic acid at the level of 0.1 percent of the total ration. One group of three pigs was killed and necropsied on day 4, 7, 10, 13, 16, 19, 21, 23 and 27 after the initiation of continuous arsanilic acid feeding. Clinical observations were noted daily. Histopathologic and arsenic determinations were performed on tissues from each pig.

The clinical syndrome of toxicosis was characterized by transient diarrhea and incoordination early and posterior paresis and quadriplegia later. Individual variations in susceptibility to the toxic effects of arsanilic acid were noted.

Detectable histopathological changes were confined to the optic tracts, optic nerves, and peripheral nerves. Major lesions noted were necrosis of myelin-supporting cells and degeneration of myelin sheaths and axons. Lesions were first noted after 10 days of feeding the experimental ration. There was an obvious increase in the severity of the lesions with the progression of the toxic syndrome.

Arsenic content of liver, kidney, blood, skeletal muscle, ribs, brain, spinal cord, and peripheral nerves
was determined for each pig. Maximum mean arsenic levels were present in liver, kidney, skeletal muscle, rib, and blood on or before day 13 of the experiment. Maximum mean arsenic levels in the nervous system were not attained until days 19 and 21.

Six pigs, average weight 40 pounds, were fed arsanilic acid at the level of 0.1 percent of the total ration for 19 days at which time they were fed an arsanilic acid free diet. Two pigs were killed and necropsied 3, 6, and 11 days following withdrawal of the drug. Tissues from each pig were assayed for arsenic content. Three, 6, and 11 days after the withdrawal of arsanilic acid skeletal muscle, kidney, liver, and blood retained approximately 31, 34, 32, and 13 mean percent arsenic respectively. Nervous tissue retained 100, 93, and 57 mean percent arsenic 3, 6, and 11 days post-withdrawal respectively.

Arsanilic acid toxicosis was induced in six pigs by feeding arsanilic acid in the ration at the 0.1 percent level. The pigs were maintained on this ration until they had developed severe posterior paresis or quadriplegia at which time they were fed an arsanilic acid free diet. Pigs were killed and necropsied on day 10, 12, 17, 20, 32, and 38 post-withdrawal of drug. Four pigs had no clinical improvement and two pigs developed a more severe paralysis after withdrawal of the drug.
One six week old pig weighing 5 kg was used for studies with tritiated arsanilic acid. A total dose of 3 mC radioactivity in 200 mg of arsanilic acid was administered intravenously in two equal injections 12 hours apart. Urinary excretion over a 24 hour period accounted for 75 percent of the total dose administered. A much lesser amount was excreted via the bile. Autoradiographic procedures did not give conclusive evidence as to the specific localization of arsanilic acid in tissues.

These studies support the conclusion that arsanilic acid toxicosis induces a primary demyelinating disease in swine.
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