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Clinical, morphological, histochemical
and clinical pathological studies
of anamú (Petiveria alliacea) poisoning in cattle

by

Alfonso Ruiz

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY
Major Subject: Veterinary Pathology

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In Charge of ~~Major~~ Work

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For the Major Department

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INTRODUCTION

Colombia, a primary cattle-producing country of South America, is situated in the tropical zone with most of it north of the equator, and it has both Atlantic (1,600 kilometers) and Pacific (1,300 kilometers) coastlines. The legendary Andes divide in Colombia to form 3 ranges: the east range 1,200 kilometers in length, the west range with 1,095 kilometers, and central range with 1,000 kilometers.

The mountain ranges which have a south-north direction afford variable climatic zones and between the ranges are fertile valleys. The altitude of the mountains causes different ecologic regions, each of which has its own fauna and flora. The coastal plains of the north and the llanos orientales form a major portion of the tropical lowlands of Colombia. Temperatures range from 6-10 C. (Boyacá, Nariño departments) to 33-37 C. (Córdoba, Magdalena, Chocó) and the humidity ranges from 30% to 90% (Chocó).

The country has variable climatological conditions producing several ecological regions, each of them useful for specific types of agricultural and animal industries. Some zones are usable only for forestry whereas others are more suitable for agriculture.

It has been recognized that cattle production has occupied an important place in Colombian economy because of

excellent grazing areas. Statistical studies published by the National Department of Statistics (DANE, 1969), illustrate the importance of cattle production in Colombia as noted in Table 1.

Table 1 Agricultural production data of Colombia

Total area in plant and animal production	(23,535,687 Ha.)	56,756,682.577 Acres
Area in annual crops	(1,630,761 Ha.)	4,029,510.431 Acres
Area used in perennial crops	(1,861,708 Ha.)	4,600,280.458 Acres
Pastures with natural grasses	(15,664,344 Ha.)	38,706,594.024 Acres
Pastures with improved grasses	(2,025,308 Ha.)	5,005,536.068 Acres
Total number of cattle in Colombia		17,700,179 Head

From the above data, it is apparent that much land is used by the cattle industry. This also suggests that the future Colombian economy may depend on increasing and improving animal production. Colombia has a low stocking rate (animal/hectarea) which is affected by many factors such as infectious, parasitic, nutritional and toxicologic diseases. Many diseases caused by viruses, bacteria and rickettsiae have been identified and are known to cause many deaths in cattle. Important economic losses of the livestock industry are also caused by internal and external parasites. Nutritional diseases are primarily caused by

mineral (calcium and phosphorus) and protein deficiencies often leading to inanition and poor carcass quality.

In the last 2 years, plant toxicities have become of prime importance in the raising of beef cattle. This assumption is based in part upon the following information:

1. Pastures (15,664,393 Ha.) are covered by native grasses and a great variety of weeds. The lands are grazed throughout the year, usually without rotation. This management is practiced by most farmers as few can afford the investment of improved pastures (Table 1).
2. The drought of the summer months (December to April) transforms the natural and improved grasses to unpalatable feed for cattle. Therefore, the animals eat any fresh plant without selectivity and accidental poisoning is usually the final result.
3. If cattle with symptoms of intoxication are removed from these pastures, they recover after a short period of time when placed in weed-free pastures.

Furthermore, infectious agents were not found to be the cause of deaths of animals that died from grazing on the above weedy pastures.

These observations strongly suggested that poisonous plants were one of the main causes of cattle losses in

those regions. These pastures had a wide variety of recognized poisonous plants.

Circumstantial evidence has incriminated two plants, Mascagnia concinna (cansaviejo) and Petiveria alliacea (anamú) as being the cause of a toxicity in cattle. Preliminary trials conducted by Ruiz and Roberts (1971) indicated that Mascagnia concinna was incapable of producing disease in spite of its cyanide content, but Petiveria alliacea in combination with Mascagnia concinna or singly did develop the disease syndrome in cattle.

Furthermore, it has been known for a long time in Colombia by the ganaderos (cattlemen) that this condition in cattle only infrequently terminated in death. Often these animals became so incoordinated and their locomotion so impaired that they could not continue to walk. They usually made a spontaneous recovery after varying periods of time if they had access to water and feed free of the anamú plant.

However, no information could be found in the literature or from any source concerning the tissue damage in cattle caused by this poisonous plant. Clinically these animals appeared to have suffered severe muscle damage. Reflection on this disease syndrome prompted the following questions:

Do these animals following recovery from this condition make the same weight gains of that of nonaffected animals?

How does it affect the carcass quality?

Does the reparative process of the muscle tissue damage result in extensive fibrosis of the meat?

Does this condition cause some permanent impairment of mobility which would have an adverse effect on the animal's ability to graze on the rugged terrain and the movement of the cattle over long distances which frequently occurs in Colombia?

It became apparent that comparative studies of the natural and experimental disease would have to be done. A better understanding of the pathogenesis of this intoxication was essential in order to answer the above questions.

The present study was undertaken to evaluate clinical, biochemical and morphological alterations found in both the natural and experimental disease. Selected clinical pathological tests were investigated with the hope of finding a laboratory aid for the diagnosis and differentiation of anamú toxicity from other diseases. Attempts were made to identify the active principle in the plant and relate its chemical structure and metabolic products to the physical alterations in cell components.

LITERATURE REVIEW

General Features

In Colombia 2 syndromes have been associated with poisonous plants. One, known as "cattle fall", occurs mainly during the summer after the animals are driven long distances. If the animals are forced to move rapidly, they stagger and fall down. This syndrome has been associated with cyanide poisoning because of the symptomatology, mainly anoxic signs, and the prevalence of Mascagnia concinna which has the property to accumulate cyanogenic glycosides (Gómez, 1970).

Another syndrome, "cachectic - muscular dystrophy", is less known by the farmers and veterinarians, probably because it is not so spectacular as "cattle fall". However, it is probably more important since the number of affected animals is high and its clinical course is prolonged and often inapparent. It is characterized by reduced weight gains, lameness in some animals, incoordination, and muscular weakness. They may eventually become cachectic.

Characteristics of *Petiveria alliacea* (anamú)

Taxonomic description Perennial erect herb up to 1 meter tall, with glabrous stem, somewhat woody at the base. Leaves oblanceolate, acuminate, 2.5 to 15 centimeters long and 1 to 5 centimeters wide, alternate. Stipules linear, glabrous (Fig. 1, 2). Flowers small, in racemes 10 to 35

Fig. 1. Petiveria alliacea (anamú)

Fig. 2. Petiveria alliacea (anamú)



centimeters long. Each flower has 3 basal bracts: the central one larger, acuminate, and the lateral ones obovate. Stamens, 8, irregularly fused at the base; anthers bilocular, separated in the base and apex. Ovary obovate, green, glabrous with 4 hooked bristles at the tip. Fruit, green, obovate, bilobulate at the apex, each lobule bearing 2 apical spines (Perez, 1956; Romero, 1965; Standley, 1937).

Classification

Class: Angiospermae

Subclass: Dicotyledonae

Family: Petiveriaceae

Genus: Petiveria

Species: P. alliacea. Linneus

(Perez, 1956; Core, 1955; Hutchinson, 1959; Blohm, 1962).

Plant Distribution

Petiveria alliacea has been described in many countries of Central America and South America as Mexico (Bentham, 1968; Standley, 1930); Guatemala (Standley and Stayermark, 1958); Honduras (Standley, 1936, 1931; Yuncker, 1940); El Salvador (MIPRES, 1948); Costa Rica (Standley, 1937; Pittier, 1957); Colombia (Pérez, 1956; Romero, 1965; Pérez de Barradas, 1957); Venezuela (Blohm, 1962; Badillo and Schnne, 1965; Pittier, 1926); Perú (Macbride, 1936); Brazil (Hoehne and Kuhlmann, 1951; Angely, 1965); Argentina (Bertotto, 1964); Ecuador (Bentham, 1968); Guyana française (Lemée, 1955). It has also been reported in the United States (Britton and Millspaugh,

1920) and Cuba (Roig y Mesa, 1962); Jamaica and Bahamas (Hitchcock, 1893).

In Colombia, Petiveria alliacea has been found in warm climates up to 600 meters above sea level and especially in departments of Cordoba, Sucre, Bolivar, Magdalena, Cesar, Cundinamarca, Tolima, and Caqueta. These departments have large prairies which are used for beef cattle production, and unfortunately they also have the largest incidence of anamu (Map 1).

Toxic and Medicinal Properties

Petiveria alliacea has been used throughout many generations as a medicinal plant. It is reported that the Mayas crushed the seeds, placed them upon a leaf of the plant and applied them as a poultice to relieve "spells" of witchcraft in the sick (Standley, 1930).

In Central America the Tacuma Indians used the plant for its curare-like effects (MIPRES, 1948). Krukoff and Smith (1937) in their notes on the "Botanical components of curare" mentioned that this plant was a possible source of it.

West Indians of Central America often sniffed the odor of a piece of the root to relieve headache (Standley, 1931). It was used by the Mayas in domestic medicine, the crushed leaves being used as poultices to relieve rheumatism and to bring boils to a head. A decoction of the plant was used in fomentations to promote motion in paralyzed limbs.

It also was reported that extract of the plant was useful as an antispasmodic in hysteria and other nervous affections (Standley, 1930). Bertotto (1964) noted that in Argentina, the fluid extract of 20 gm. of roots in 1 l. of water boiled for 10 minutes, was used as a stimulant for appetite, taking 1 glass of this beverage before each meal. Also, he said that it was used in kidney diseases for its diuretic properties. A similar use was described in Peru by Macbride (1936).

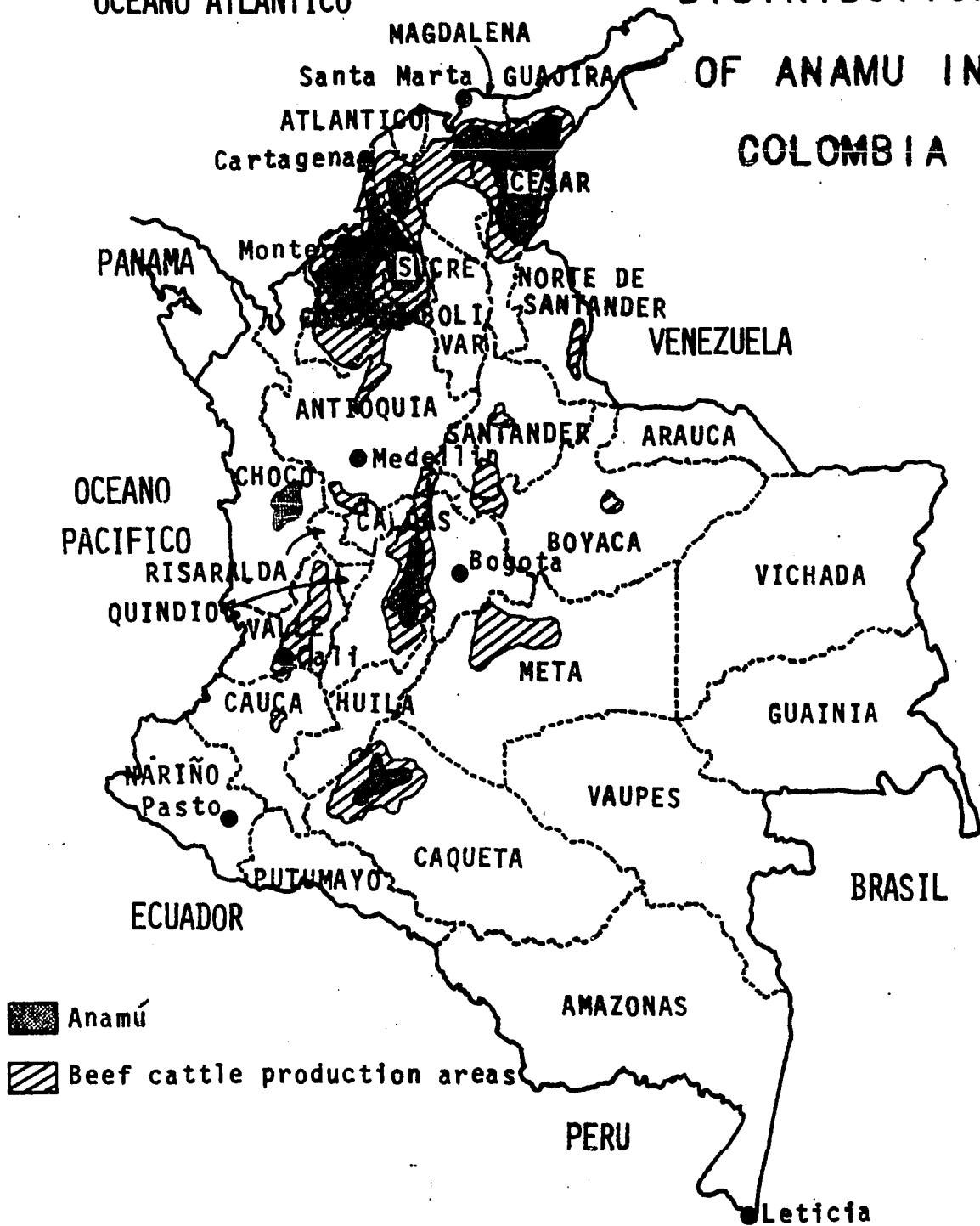
It has been used to produce abortion in humans and animals of Cuba (Roig y Mesa, 1962), El Salvador (MIPRES, 1948) and Colombia (Pérez, 1956).

In Colombia it has been used as vermifuge by drinking infusions of the leaves, and to relieve toothache by chewing roots of anamú (Pérez, 1956; Pérez de Barradas, 1957). Inhalation of steam from boiling stems and leaves was used to relieve bronchopneumonia (Romero, 1965).

The plant has a strong garlic odor which is imparted to the milk of cows that eat this plant (Pérez, 1956; Romero, 1965; Blohm, 1962; Pittier, 1957).

OCEANO ATLANTICO

DISTRIBUTION OF ANAMU IN COLOMBIA



MATERIALS AND METHODS

Animals

Eighteen 5- to 7-month-old Zebu-Romo sinuano cross-bred calves were purchased from anamu-free farms. They were isolated and adjusted to the new feed and environment for 10 days prior to the experimental trials. During this time weight and clinical evaluations were recorded and blood and fecal examinations were made. All animals were treated twice with the recommended dosages, as noted on the label, of Thiobendazole¹, Diethylcarbamazine citrate², and Sulfamethazine³.

Housing

All the calves were maintained in isolation. The units were cement floored with adequate self-feeders and waterers available. Cleaning of the units was done twice a day.

Food

A complete, cotton seed hull ration and mineralized salts were given free choice. The ration did not contain any antibiotics or feed additives.

¹Bovizole - Merck Laboratories.

²Luvorem - Cyanamide.

³Sulmet.

Source of Plant

Collection of fresh anamú material was made twice a week from farms, Hacienda "La Aldea" in Corozal and Hacienda "El Bonito" in Carmen de Bolívar, having suspected cattle syndrome of plant poisoning. In the laboratory the plant was kept frozen. At the time of daily feeding, plant components (stems and leaves) were thawed, chopped, weighed and administered orally by manual feeding (Fig. 3). The amount for each animal was 3 gm. per each kilogram of body weight, once a day.

Clinical Observations

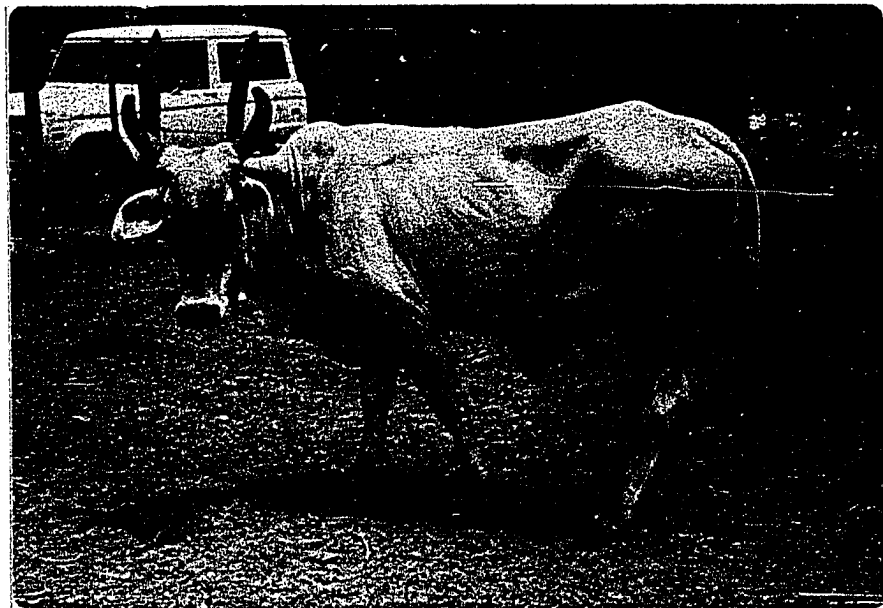
All animals were observed daily for clinical signs. Rectal temperatures were recorded at 9 A.M. and 4 P.M. every day. Heart and respiratory rates (1 minute duration) and rumen movements (2 minutes) were recorded every Monday and Thursday some 40 minutes after feeding of the anamú and every Thursday after exercising the animals.

Hematology

Blood samples were taken from the jugular vein each Tuesday and Friday before feeding. Approximately 15 ml. of blood were taken from each animal and collected into 2 different tubes, one containing an anticoagulant, ethylene diamino tetracetic acid (EDTA; 0.5 ml. of 1% solution per

Fig. 3 Administration of anamú.
Manual feeding of preweighted amount of anamú

Fig. 4 Natural case.
Flexion of hock and abnormal forward placement
of hind legs



5 ml. of blood), and the other without an anticoagulant. The blood was allowed to clot, then centrifuged at 1000 G for 15 minutes. The serum was drawn off, placed in separate screw-cap tubes, and frozen at -20 C.

From blood with the anticoagulant, the following determinations were made on the EDTA blood sample:

Packed cell volume

Microhematocrit method was used. Capillary tubes of 75 mm. x 10 mm. and International hematocrit centrifuge with its standardized reader were available for the test. Centrifugation was made at 11,000 r.p.m. for 5 minutes.

Hemoglobin

The Sheard and Sandford (1929) procedure for oxyhemoglobin determination was used. Twenty ml. of 0.1% sodium carbonate were placed into a 19 mm. cuvette. Then, 0.05 ml. of whole blood was added and mixed. Reading of the spectrophotometer (Coleman junior¹) was made at 545 mμ, with a reagent reference blank (0.1% sodium carbonate) set at 100% transmittance. The values were obtained from the accompanying calibration chart of the Coleman catalog.

White blood cell count

Diluting fluid used for the white blood cell count was 3% glacial acetic acid solution. Certified diluting pipettes

¹Coleman Instruments, Inc., Maywood, Illinois.

and hemocytometer were used to count the cells.

Differential leukocytic count (blood smear)

Immediately after taking the blood, a thin blood film was prepared and dried quickly. The film was fixed with absolute methyl alcohol for 5 minutes, air dried and stained with Giemsa's stain for 30 minutes.

From serum, the following tests were made:

Serum glutamic oxalacetic transaminase (SGOT) determination

This enzyme catalyzed the reaction:

Aspartate + α -Ketoglutarate $\xrightleftharpoons{\text{GOT}}$ Oxaloacetate + Glutamate

Determination of SGOT was made by using the set of Harleco¹ reagents and its procedure. In this procedure the oxaloacetic acid formed, partially decomposed to pyruvic acid which is then reacted with dinitrophenylhydrazine (DNPH), forming pyruvatedinitrophenylhydrazine complex. This complex, with the addition of 0.4 N sodium hydroxide, gives an intense brownish color which is read in the spectrophotometer.

The procedure Two-tenths ml. of serum were reacted with buffered substrate in 37 C. water bath for 1 hour. DNPH was added and permitted to react for 20 minutes. Then, 0.4 N sodium hydroxide was added for development of the brown

¹Harleco - Hartmen-Leddon Company, Philadelphia, Pennsylvania.

color which was read at 505 mμ in a spectrophotometer. Units of SGOT were read from % transmittance plotted in the calibration curve.

Glucose

A set of Harleco reagents and procedure were used to determine the amount of glucose in the serum. The procedure consists of adding orthotoluidine and glacial acetic acid to serum. A green color developed which was read in a spectrophotometer at 630 mμ. The serum glucose level was read directly from calibration graphs.

Blood urea nitrogen

A set of Merck¹ reagents and procedure was used. In principle, it is the Berthelot's reaction, in which urea is quantitatively transformed into ammonium carbonate by urease. The phenol, in presence of ammonium carbonate, is oxidized by sodium hypochlorite to form a blue color. The concentration of this color is determined by the spectrophotometer, and the calculations made as follows:

$$\text{B.U.N.} + \frac{\text{Serum sample absorbance}}{\text{Standard absorbance}} \times 18.7 \text{ mg./100 ml. of serum}$$

¹Merck - E. Merck, Darmstadt, Germany.

Urinalysis

Urine was collected every Wednesday and Friday before feeding, for the last 36 days of the experiment.

The following determinations (Benjamin, 1961) were made:

- a. Specific gravity: by urinometer
- b. pH: by nitrazine paper
- c. Glucose: by Benedict's test
- d. Protein: by Robert's test
- e. Blood: by Benzidine test
- f. Acetone: by Ross test
- g. Urobilinogen: by the method of Wallace and Diamond
- h. Indican: by Obermayer's method
- i. Chloride: by Fantus method
- j. Microscopic examination: centrifugation of the urine was made at 1000 G for 5 minutes to separate the sediment from the fluid portion of the urine, and the sediment was examined with the microscope.

Necropsy Procedures

The calves were killed by electrocution and complete necropsies were made.

All gross lesions were described and the following tissues were taken and fixed in 10% buffered formalin for microscopic examination:

Cardiovascular system

Myocardium: Right and left atria

Right and left ventricles

Thoracic aorta

Digestive system

Tongue (Dorsum linguae)

Esophagus

Pharynx

Duodenum

Parotid salivary gland

Liver

Mandibular salivary gland

Pancreas

Lymphatic system

Spleen

Muscular system

Diaphragm (pars costalis)

Intercostal muscle (6th and 7th intercostal space)

Cricoaerenoideus dorsalis muscle

Vastus medialis (Quadriceps femoris)

Gastrocnemius (tendo-muscular junction)

Nervous system

Cerebral cortex

Corpora quadrigemina

Cerebellar cortex

Thoracic and lumbar
segments of
spinal cord

Pyriform lobe

Thalamus

Ischiatic nerve

Medulla oblongata

Dorsal root ganglia

Respiratory system

Right dorsal turbinate

Lung (right and left caudal (diaphragmatic) lobes)

Urinary system

Kidney (left)

All tissues were dehydrated in increasing grades of ethanol, clarified in benzaldehyde, embedded in paraffin and sectioned at 5 μ for regular staining with Harris' hematoxylin and eosin stain (H and E; AFIP, 1968).

Histochemical Procedures

The following special stains were performed as described by the Armed Forces Institute of Pathology (1968): Cajal's; periodic acid Schiff (PAS); Oil red O (ORO); Pancreatic islet cell (Gomori's); and Jones' Kidney stains. Additional techniques included phosphotungstic acid hematoxylin stain (PTAH; Zugibe, 1970) and Marchi's myelin stain (Davenport, 1961).

Specific tissues stained by these procedures are listed in Table 2.

Electron Microscopy Procedures

Tissues for electron microscopic examinations were collected during necropsy and immediately fixed in 3.0% glutaraldehyde in a 0.1 M phosphate-buffered solution for 2

Table 2 Summary of histochemical studies of tissues

	Nerve endings Cajal	Reticulum stain (Jones)	Islet cells (Gomori)	Myelin (Marchi)	Unsat- urated lipids (ORO)	Glycogen (PAS)	Muscle striations (PTAH)
Heart	-	-	-	-	+	+	+
Skeletal muscle	+	-	-	-	+	+	+
Liver	-	-	-	-	-	+	-
Kidney	-	+	-	-	+	+	-
Spinal cord	-	-	-	+	-	-	-
Ischiatic nerve	-	-	-	+	-	-	-
Pancreas	-	-	+	-	-	-	-

hours and washed with 3 changes of 3% buffered sucrose solution. The pieces of tissue were transferred to a 0.25% osmium tetroxide buffered solution and fixed for 2 hours. Dehydration was accomplished in ascending concentrations of ethanol, each change of 70%, 85%, 95% and absolute alcohol for 15 minutes. Then, 3 changes were made in mixture of equal parts of propylene oxide-absolute alcohol, for 15 minutes each. The tissues were infiltrated with 2 changes of Maraglas¹ for 75 minutes each. The pieces of tissue processed in this way, were placed in number 0 gelatin capsules containing fresh Maraglas solution and left in the oven at 60 C. for 48 hours.

The capsules were trimmed and sections were cut with an LKB ultramicrotome² at 600 Å. The sections were placed on 200-mesh grids and stained with 2% Uranyl acetate and lead nitrate for 15 minutes each. Examination of the sections was made on a Jeol³ electron microscope, model No. JEM 7A.

Plant Chemistry

Screening analysis for the presence of alkaloids, glycosides, saponins and resins in the roots, stems and

¹Vaughn, Inc., Memphis, Tennessee.

²L.K.B. Produktes, A.B., Stockholm, Sweden.

³Japan Electron Optics Laboratory Company, Tokyo, Japan.

leaves of the anamú plant was done at ICA¹ research laboratory, Bogotá, and in the Chemistry Department, Iowa State University. Amino acid analysis from plant components was also made in the Chemistry Department of Iowa State University.

Design of Experiment

Selection of principals and the control animals was randomly made.

From the 18 calves, 3 groups were chosen, and 6 animals were randomly assigned to each group. Within each group, 4 principals were selected that received anamú (3 gm./kg. of body weight) and 2 calves served as controls.

Groups 1, 2, and 3 were killed 8, 30, and 60 days respectively after initiation of the experiment. The identification of the principals and controls can be noted in Table 3.

Table 3 Experimental design and animal identification

	Controls No.	Principals No.	Duration of experiment
Group 1	4 - 15	1 - 5 - 7 - 12	8 days
Group 2	2 - 17	3 - 6 - 11 - 14	30 days
Group 3	8 - 16	9 - 10 - 13 - 18	60 days

¹Instituto Colombiano Agropecuario, Bogotá, Colombia.

Field Cases

Four 5- to 6-month old Zebu calves (A, B, C, and D), donated by the owner of Hacienda "El bonito", were observed for 48 hours for clinical signs. Two blood samples were taken from each animal, the first one upon arrival and the second one 24 hours later. Blood and serum examinations were made in similar ways as previously described for experimental trials.

After 48 hours the animals were killed by electrocution, necropsied, and the tissues saved and processed exactly as in experimental cases.

RESULTS

Clinical Signs

Field cases

Calves of 4 to 5 months of age were most frequently affected. However, animals younger than 2 months and older than 1 year of age have been found to be susceptible.

The syndrome, itself, had silent and progressive development and the initial symptoms gave the appearance of a sudden onset when they were discovered by the ganadero (cattleman).

Several calves in the herd simultaneously had clinical signs of incoordination and sometimes a few adults. The most striking signs were related to the locomotor system. There was excessive muscular weakness which was better appreciated by putting some pressure on the lumbar region. The animals flexed the tarsal joints, went down, and assumed a sitting position. Heavy animals had difficulty in standing for long periods of time. When standing they adopted abnormal positions of the body and legs. There was abnormal forward placement of the hind legs (Fig. 4). They frequently spread their hind legs to increase support of their body. Also, they had slight flexion of their hocks and lateral rotation of the hocks (Fig. 5).

When walking, they had much incoordination, especially of the hind quarters; if the calves ran they soon fell down. If they were walking slowly, an abnormal and pronounced flexion of the hocks and knuckling of the pasterns were observed (Fig.6).

If the animals ran, they fatigued quickly and dyspnea was observed. Dysmetric movements were also observed. The animals moved their extremities without estimation of distances of their steps, and they often stumbled over low obstacles.

Several affected animals with the natural disease had a very peculiar noise at every respiratory expiration, similar to that heard in "roaring" of horses. This sign was more commonly found in young calves.

All the muscles were flabby, especially those big muscular masses of the shoulder, hip, and thigh. Muscular tonicity was greatly decreased. The reflexes were still present and skin sensitivity was normal.

Body temperature was found to be about normal.

A = 39.0 C.

B = 38.2 C.

C = 39.1 C.

D = 38.8 C.

Mean = 38.7 C.

Milk from lactating cows had a garlic odor similar to that of the plant. Ganaderos (cattlemen) noted that cows,

Fig. 5 Natural case. Outward
rotation and flexion of
hock joint

Fig. 6 Natural case.
Flexion of hock
joint and
knuckling of
pastern

Fig. 7 Principal No. 9, 35
days. Unilateral
rotation of hock
joint

Fig. 8 Principal No.
14, 18 days.
Flexion of hocks
and rotation of
tarsal joint



pigs, and sheep had a selective palatability for anamú over other plants during summer days.

Experimental cases

Most of the animals accepted the plant without difficulty. About 40 minutes after administration of anamú, several principals (No. 1, 3, 6, 9, and 12) were bloated and frequent eructations of a garlic odor were noted. These signs persisted for about 90 minutes. Eight days later other principals (No. 10, 13, and 18) were showing similar symptoms. These signs were spontaneous and irregularly manifested throughout the time of the duration of the research. The controls (No. 2, 4, 8, 15, 16, and 17) and principals (No. 5, 7, 11, and 14) did not show the above signs.

On the 16th day, principals (No. 9 and 18) had excessive bilateral lacrimal serous secretion (Fig. 9). This sign was inconsistently observed later in these 2 animals and in principals (No. 3, 6, 10, 11, 13, and 14). This was usually related to the eructation of the calves after eating the plant. The condition persisted for about 3 or 4 hours. It was also observed that on those days when the calves did not have eructation, they did not develop lacrimation.

Muscular weakness was the most striking feature of this particular syndrome. It was first observed in principal No. 18 on the 7th day. It was characterized by an instability

Fig. 9 Principal No. 10. Lacrimation. Forty minutes
after plant administration



of both tarsal joints, and the animal could not support its own body. Similar signs appeared in other principals (No. 6, 10, 12, and 13) on the 8th day.

On the 10th day, 2 principals (No. 3 and 9) had difficulties in walking. The first signs were slightly different from those observed in the other principals. These 2 animals had stiffness of the pelvic limbs, especially marked in the muscles of the thigh. The tarsal joints were rigid. On walking, very short steps were taken with the pelvic limbs. Four days later they developed similar signs of muscular weakness as previously described in other principals.

On the 12th day another principal (No. 14) had symptoms of muscular weakness and fatigue. Principal No. 11 did not exhibit symptoms throughout the 30-day experimental trial.

Five to 6 days after the first clinical sign of the muscular weakness was noted, other signs became apparent. The animals adopted abnormal positions of standing, such as semiflexion of the tarsal joints, abnormal bilateral or unilateral rotation of the tarsal joints (Fig. 7 and 8), continuous shifting in position of pelvic limbs when standing and leaning against the walls of the isolation units or other animals. When walking, the animals often staggered and rotated laterally their tarsal joints, interfering with normal locomotion. Frequently they tripped over low obstacles.

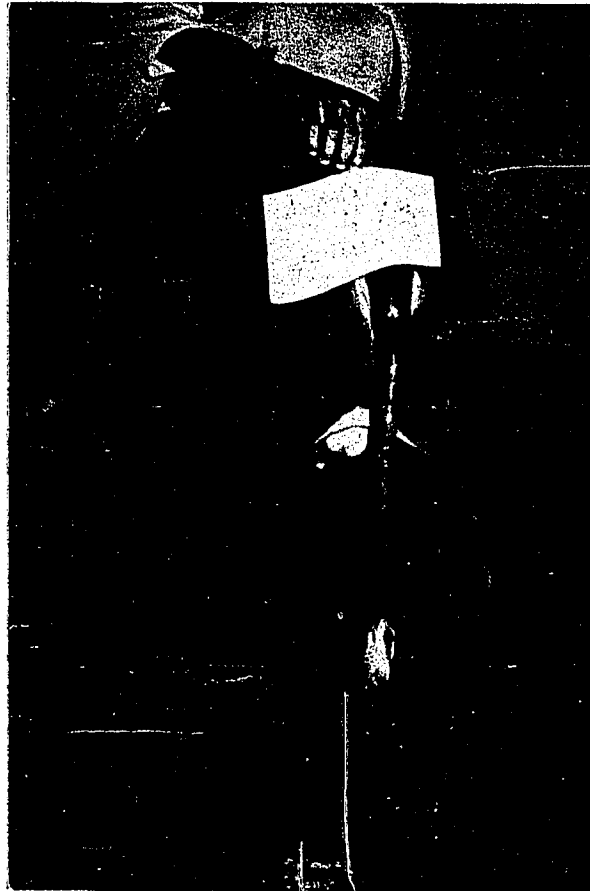
Slight pressure on the back caused the animals to flex their tarsal joints.

On the 20th day some principals (No. 3, 10, and 18) appeared to have recovered but 2 days later the symptoms reappeared with more intensity. The principals fatigued quickly and moved slowly in relation to the controls. These principals preferred to remain recumbent. If they stood up, they did it with some difficulty and usually spread their hind legs to assist in their own support (Fig.10). In addition to their staggering, the principals had marked locomotor ataxia of their pelvic limbs. These signs continued with about the same intensity until the calves were killed.

Around the 28th day the principals (No. 6, 9, 10, 13, and 14) had reduced muscle tonicity and muscular masses of the thigh, such as the biceps femoris, quadriceps, semitendinosus, and semimembranosus, were flabby, lacking their normal tension. This flaccidity was especially pronounced in principals of group 3 during the last 10 days of the trial.

Muscular atrophy was noted after some 28 days in principals No. 14 and 18. This was most apparent in the muscles of the thigh. In principal No. 9, it was noted by the 35th day, and in principal No. 13, it could not be detected until the 43rd day.

Fig. 10 Principal No. 13, 46 days. Spreading of hind
legs to assist in support



No inflammatory signs involving muscles, joints or tendons could be found by detailed clinical examination of the extremities and hip.

No nervous disorder was found on neurological examination. Tendon reflexes were still present and skin sensitivity was normal.

Polyuria and pollakiuria were other interesting findings. These were noted on the 15th day in principal No. 18. The following day other principals (No. 3 and 10) had pollakiuria. Urine from all these principals had the characteristic garlic odor of anamú. These signs persisted throughout the experiment. An increased water intake was observed in all these polyuric calves for the duration of the trial.

Diarrhea was observed on day 25 in principals (No. 6 and 9) and later in the other principals of groups 2 and 3, and became intermittent for the remainder of the trials.

Behavioral changes were noted in the principals. Sometimes they were depressed while on other occasions they were excited and irritable. These changes occurred irregularly and were difficult to predict.

The body temperatures of principals and controls remained within normal ranges throughout the experiment.

The principals had an elevated heart rate after feedings of the plant (Table 4 and Fig. 11).

Rumen movements were similar to those of control animals.

Table 4 Heart rate

	Animal Number	Time						H. R. Before Trial	
		8 day	15 day	25 day	35 day	45 day	55 day		Mean
Controls	2	80.0 ^a	76.0	72.0	— ^b	—	—	76.0	75.5 ^c
	4	76.0	—	—	—	—	—	76.0	78.0
	8	78.0	83.0	78.0	76.5	78.0	73.0	77.7	76.0
	15	76.0	—	—	—	—	—	76.0	70.0
	16	72.0	76.0	80.0	66.5	68.0	74.0	72.7	70.5
	17	84.0	84.0	88.0	—	—	—	85.3	86.0
Principals	1	81.0	—	—	—	—	—	81.0	85.0
	3	86.0	108.0	82.0	—	—	—	92.0	75.0
	5	75.0	—	—	—	—	—	75.0	75.0
	6	82.0	92.0	78.0	—	—	—	84.0	79.0
	7	82.0	—	—	—	—	—	82.0	70.0
	9	75.0	66.0	76.0	79.5	75.0	75.0	74.4	74.0
	10	84.0	82.0	90.0	88.0	94.0	81.5	86.5	70.0
	11	114.0	100.0	90.0	—	—	—	101.3	69.0
	12	78.0	—	—	—	—	—	78.0	70.0
	13	76.0	72.0	78.0	71.0	73.0	79.0	74.8	70.0
	14	96.0	108.0	96.0	—	—	—	100.0	72.0
	18	72.0	67.0	91.0	66.0	68.0	76.0	73.3	68.0

Mean	con- trols	77.7	79.7	79.5	71.5	73.0	73.5	75.8	76.0
	prin- ci- pals	83.4	86.9	85.1	76.1	77.5	77.9	81.1	73.1

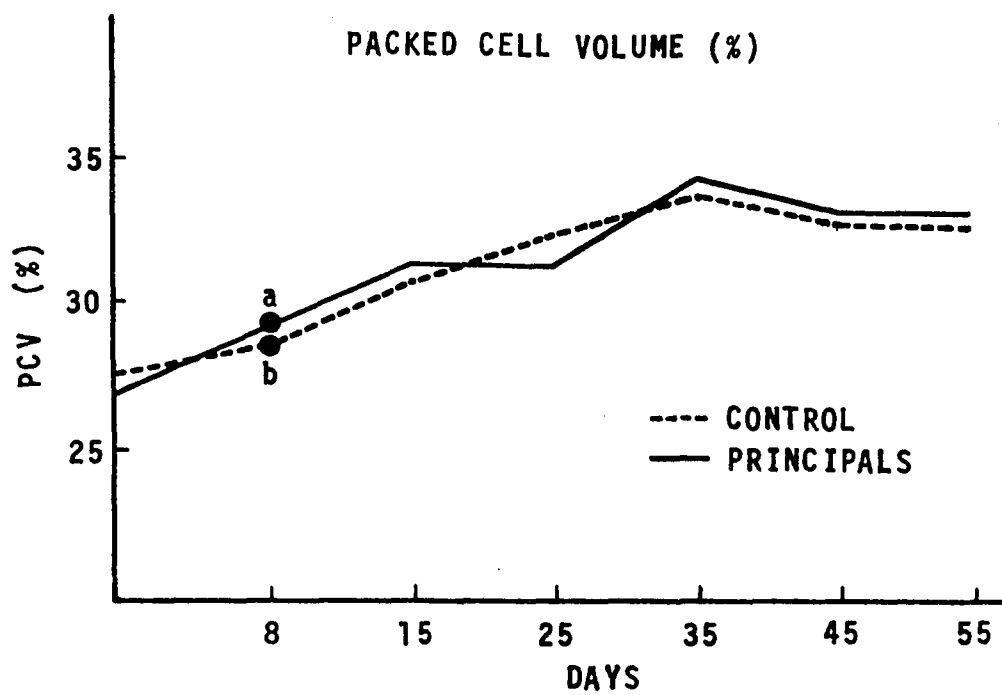
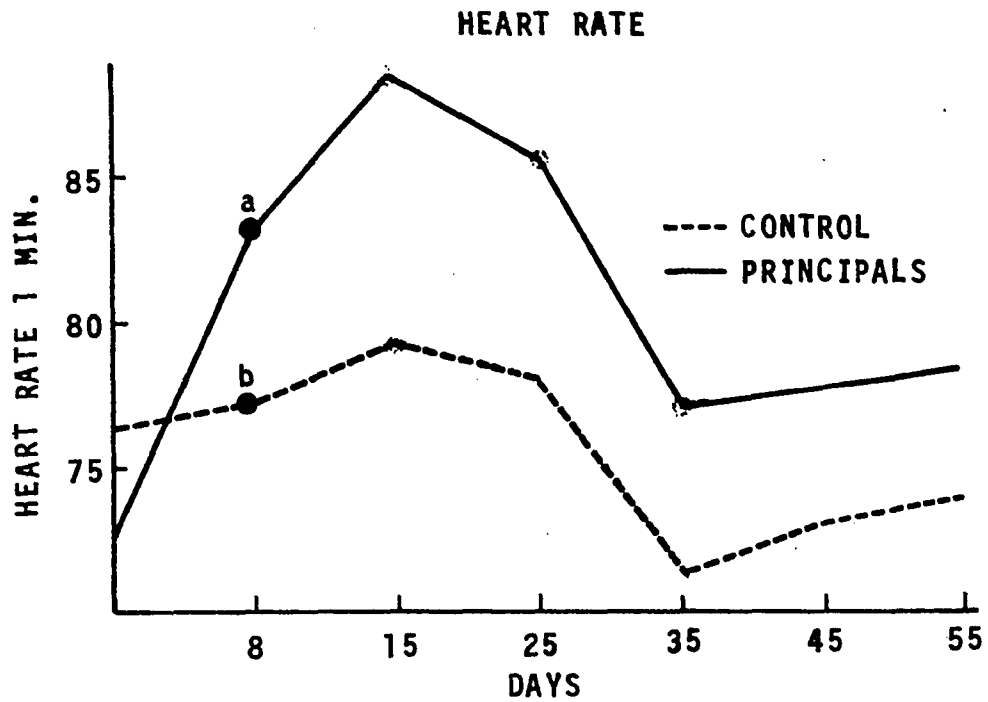
^aEach figure represents mean of a (Monday and Thursday) recording.

^bNo recording taken. Animal killed at that time.

^cMean of 2 observations.

Fig. 11 Heart rate (in 1 minute). Recorded 40 minutes
after plant administration

Fig. 12 Packed cell volume (%)



After forced exercise there was no appreciable difference of heart and respiratory rate recordings of principals and controls for the first 30-35 days. After the 35th day, some 30 to 60 seconds of forced exercise caused the principals to fall down. They were unable to rise for some 10 minutes. Then, voluntarily they got up with difficulty and had marked locomotor ataxia. They salivated profusely, had muscle tremors, had distended jugular veins, and micturated frequently.

Around the 29th day cardiac arrhythmia was noted upon auscultation in principals (No. 6, 10, 11, 13, 14, and 18). This arrhythmia consisted of a reinforcement of the second heart sound, producing irregularly altered intervals of tachycardia and bradycardia. The arrhythmia also was more noticeable after exercising the animals, and it still persisted after subcutaneous inoculation of 5 mg. of atropine to each principal¹.

Hematology

Field cases

The results obtained from blood and serum analysis of the 4 calves (A, B, C, and D) can be seen in Table 5.

¹A. Orrego, ICA, Monteria, Colombia. Private communication, 1971.

Table 5 Natural occurring cases of anamú intoxication
Hematological analysis

	A	B	C	D
Packed cell volume (%)	44 ^a	33	33	30
Hemoglobin (gm./100 ml.)	14.10	10.45	10.45	9.50
White blood cells (1 cmm.)	15,450	11,000	10,100	13,500
Neutrophils (%)	8	14	20	20
Lymphocytes (%)	84	86	74	75
Monocytes (%)	8	-	4	5
Eosinophils (%)	-	-	2	-
Basophils (%)	-	-	-	-
SGOT (<u>International Units</u>) 100 ml.	122	122	166	126
BUN (mg./100 ml.)	8.8	8.8	19.4	14.7
Glucose (mg./100 ml.)	57	39	36	42

^aAll values for all animals listed are a mean of paired samples.

Experimental cases

Packed cell volume (PCV) There was little difference in PCV values of controls and principals. In both cases, all the animals exhibited a progressive increase of PCV (Table 6 and Fig. 12).

Hemoglobin There was similar progressive increase of hemoglobin in both principals and controls (Table 7 and Fig. 13).

White blood cell count (WBC) The leukocytic count was progressively increasing in both principals and controls during the first 35 days, but after this time there was a rapid decrease in the count of the principals while the

Table 6 Packed cell volume (%)

	Animal Number	Time						Mean	PCV Before Trial
		8 day	15 day	25 day	35 day	45 day	55 day		
Controls	2	33.00 ^a	33.50	34.00	— ^b	—	—	33.50	35.42 ^c
	4	29.50	—	—	—	—	—	29.50	30.25
	8	27.50	31.00	32.00	34.66	32.00	32.25	31.56	28.12
	15	26.50	—	—	—	—	—	26.50	22.12
	16	26.50	29.50	32.33	32.00	34.00	33.00	31.22	25.62
	17	23.50	26.50	30.75	—	—	—	26.91	22.12
Principals	1	25.50	—	—	—	—	—	25.50	20.25
	3	31.00	30.00	30.00	—	—	—	30.33	28.55
	5	27.50	—	—	—	—	—	27.50	23.25
	6	28.50	30.00	29.75	—	—	—	29.41	30.87
	7	29.50	—	—	—	—	—	29.50	29.62
	9	27.00	29.50	29.66	32.00	33.66	33.25	30.84	27.00
	10	30.00	31.00	30.66	32.33	33.00	31.50	31.41	24.50
	11	31.00	34.00	36.25	—	—	—	33.75	28.75
	12	26.50	—	—	—	—	—	26.50	25.25
	13	26.50	28.50	30.33	34.66	36.00	32.75	31.45	26.25
	14	29.00	30.00	30.75	—	—	—	30.37	29.75
	18	30.50	31.50	30.66	35.00	34.66	34.25	32.76	29.00

Mean	con- trols	27.75	30.12	32.27	33.33	33.00	32.62	31.51	27.27
	prin- ci- pals	28.54	30.56	31.00	33.50	34.33	32.94	31.81	27.75

^aEach figure represents mean of paired samples.

^bNo sample. Animal killed at that time.

^cMean of 2 observations.

Table 7 Hemoglobin gm./100 ml.

	Animal Number	Time						Mean	Hb. Before Trial
		8 day	15 day	25 day	35 day	45 day	55 day		
Controls	2	9.87 ^a	10.80	10.37	— ^b	—	—	10.34	10.61 ^c
	4	9.10	—	—	—	—	—	9.10	9.28
	8	9.65	9.82	9.38	10.65	10.11	9.96	9.92	8.71
	15	7.55	—	—	—	—	—	7.55	6.82
	16	8.05	8.50	9.30	9.70	10.00	9.57	9.18	8.02
	17	6.65	7.55	9.01	—	—	—	7.73	6.63
Principals	1	8.10	—	—	—	—	—	8.10	7.43
	3	8.77	8.90	8.83	—	—	—	8.83	8.57
	5	9.07	—	—	—	—	—	9.07	7.48
	6	8.65	8.90	8.71	—	—	—	8.75	9.51
	7	8.80	—	—	—	—	—	8.80	9.15
	9	8.10	8.50	8.65	9.30	10.26	10.12	9.15	8.50
	10	8.77	9.05	9.01	9.40	9.66	9.50	9.23	8.00
	11	9.35	9.50	10.47	—	—	—	9.77	8.48
	12	7.67	—	—	—	—	—	7.67	7.70
	13	8.10	8.77	8.83	10.00	10.45	9.75	9.31	8.52
	14	8.62	8.35	8.86	—	—	—	8.61	8.98
	18	8.77	9.20	9.05	10.10	10.23	9.90	9.54	9.00

Mean	con-	8.48	9.16	9.51	10.17	10.05	9.76	9.52	8.34
	trols								
	prin-	8.48	8.89	9.05	9.67	10.15	9.81	9.34	8.27
	ci-								
	pals								

^aEach figure represents mean of paired samples.

^bNo sample. Animal killed at that time.

^cMean of 2 observations.

Table 8 White blood cells (1 cmm.)

	Animal Number	Time					Mean	W.B.C. Before Trial
		8 day	15 day	25 day	35 day	45 day	55 day	
Controls	2	12,875 ^a	11,150	12,050	— ^b	—	—	12,025 12,650 ^c
	4	11,250	—	—	—	—	—	11,250 12,212
	8	11,375	11,700	13,266	13,016	13,350	12,250	12,492 10,487
	15	8,250	—	—	—	—	—	8,250 8,200
	16	9,425	11,000	12,150	12,950	12,000	11,900	11,570 10,112
	17	7,825	9,800	9,987	—	—	—	9,204 8,525
Principals	1	9,350	—	—	—	—	—	9,350 7,650
	3	12,250	13,325	13,937	—	—	—	13,170 11,325
	5	9,275	—	—	—	—	—	9,275 10,687
	6	8,775	6,125	7,237	—	—	—	7,379 8,012
	7	14,300	—	—	—	—	—	14,300 13,425
	9	13,000	11,775	11,175	12,776	11,750	10,975	11,908 13,000
	10	9,625	11,575	10,300	11,300	8,550	9,487	10,139 9,375
	11	10,950	10,450	12,337	—	—	—	11,245 11,075
	12	10,700	—	—	—	—	—	10,700 10,000
	13	8,350	8,975	10,900	11,933	11,183	12,000	10,556 9,925
	14	8,475	10,675	11,062	—	—	—	10,070 11,612
	18	13,575	10,050	9,750	11,566	9,683	9,762	10,731 11,150

Mean	con-	10.333	10.912	11.863	12.983	12.675	12.075	11.806	10.364
	trols								
	prin-	10.718	10.368	10.837	11.893	10.291	10.556	10.777	10.603
	ci-								
	pals								

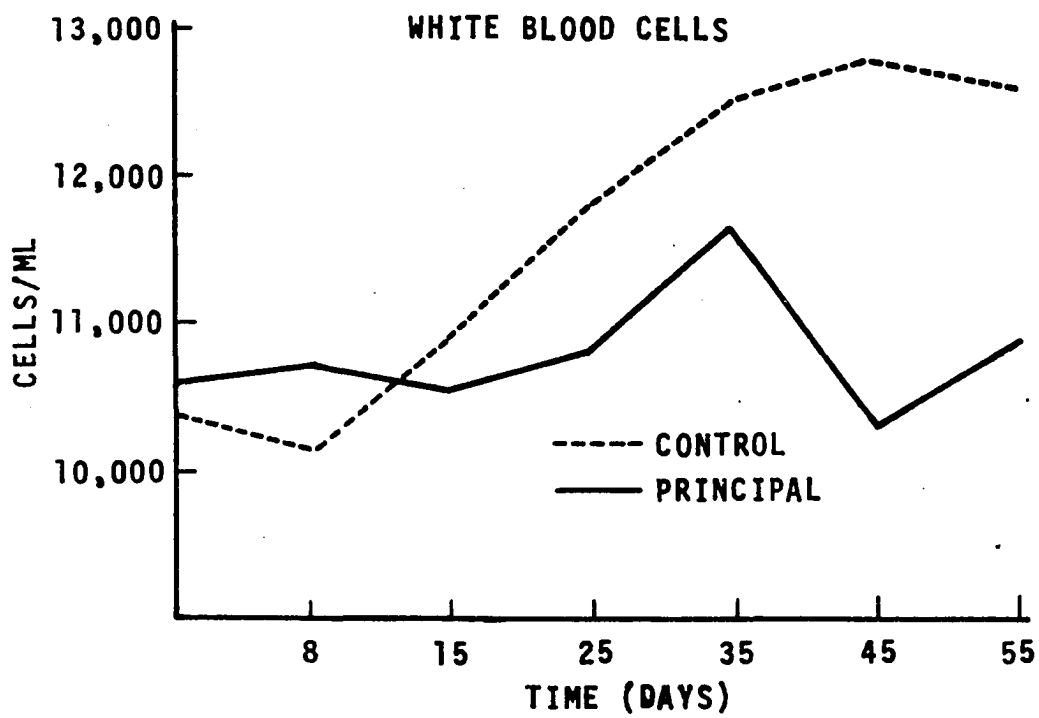
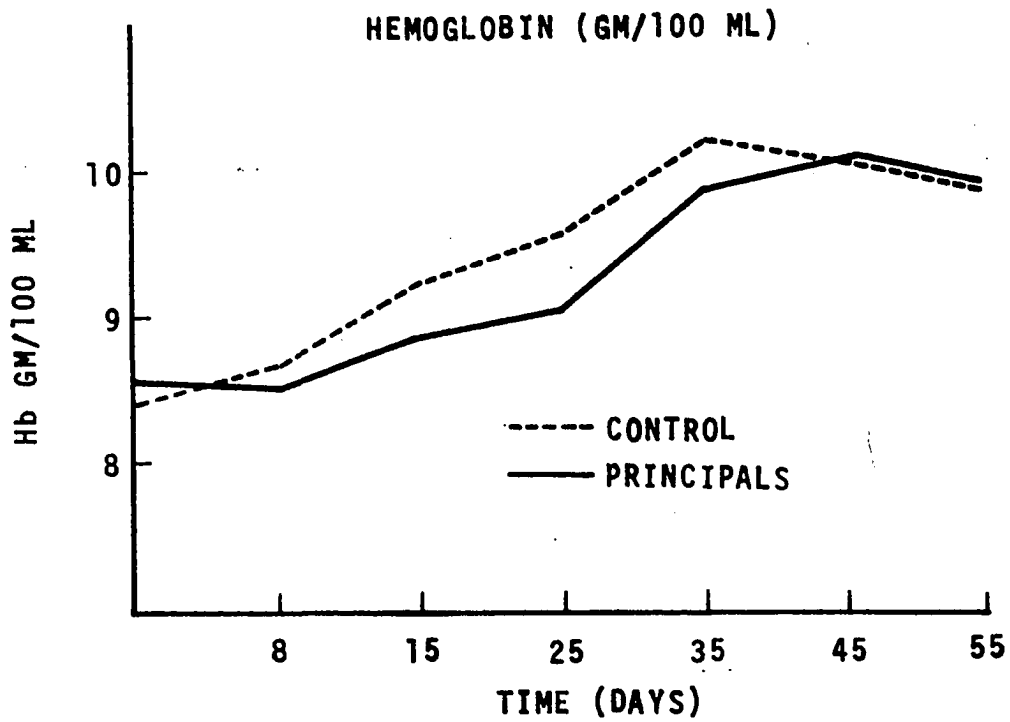
^aEach figure represents mean of paired samples.

^bNo samples. Animal killed at that time.

^cMean of 2 observations.

Fig. 13 Hemoglobin (gm./100 ml.)

Fig. 14 White blood cells (cells/cmm.)



values of the controls remained high (Table 8 and Fig. 14).

The controls (No. 8 and 16) had the largest mean increase. These animals belonged to group 3 that received the plant for 60 days. The principals from the same group (No. 9, 10, 13, and 18) had a decrease in their total leukocytic count to a mean of 1 600 cells during the last 20 days of the trial (Fig. 14). In general the controls had a larger increase in the number of leukocytes than the principals.

Differential leukocyte count Little differences were found in the differential leukocyte counts of principals and controls. There was an initial increase of neutrophils which was greater in principals than in controls. This increase was followed by a rapid decrease after the 15th day (Table 9 and Fig. 16). The lymphocytes were increasing progressively in both principal and controls with minor differences. The lymphocytosis tended to be lower in the principals than that in the controls (Fig. 15).

Serum glutamic oxaloacetic transaminase (SGOT) There was a very obvious difference between the SGOT values of the principals and controls. The principals had a progressive increase of SGOT more than twice its initial value, while the controls had an almost uniform SGOT value throughout the trial (Table 10 and Fig. 17).

Table 9

Anamú feeding trial
Differential leukocyte count (mean value) (%)

Time	Neutrophils		Lymphocytes		Monocytes		Eosinophils		Basophils	
	con- trols	prin- ci- pals	con- trols	prin- ci- pals	con- trols	prin- ci- pals	con- trols	prin- ci- pals	con- trols	prin- ci- pals
Day 0	18	18	77	74	3	4	2	4	-	-
Day 8	14	20	82	75	2	3	2	2	-	-
Day 15	20	26	75	69	3	3	2	2	-	-
Day 25	15	16	81	78	2	3	2	3	-	-
Day 35	13	17	84	78	1	2	2	3	-	-
Day 45	16	16	82	78	1	2	1	4	-	-
Day 55	13	19	84	75	1	2	2	4	-	-

Fig. 15 Lymphocytes (%)

Fig. 16 Neutrophils (%)

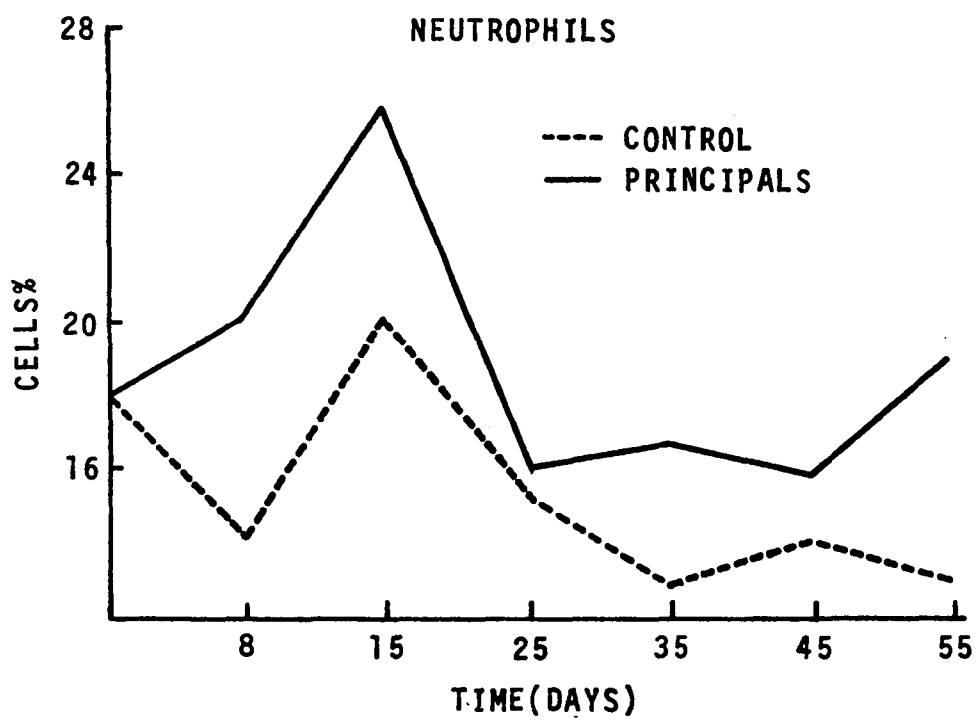
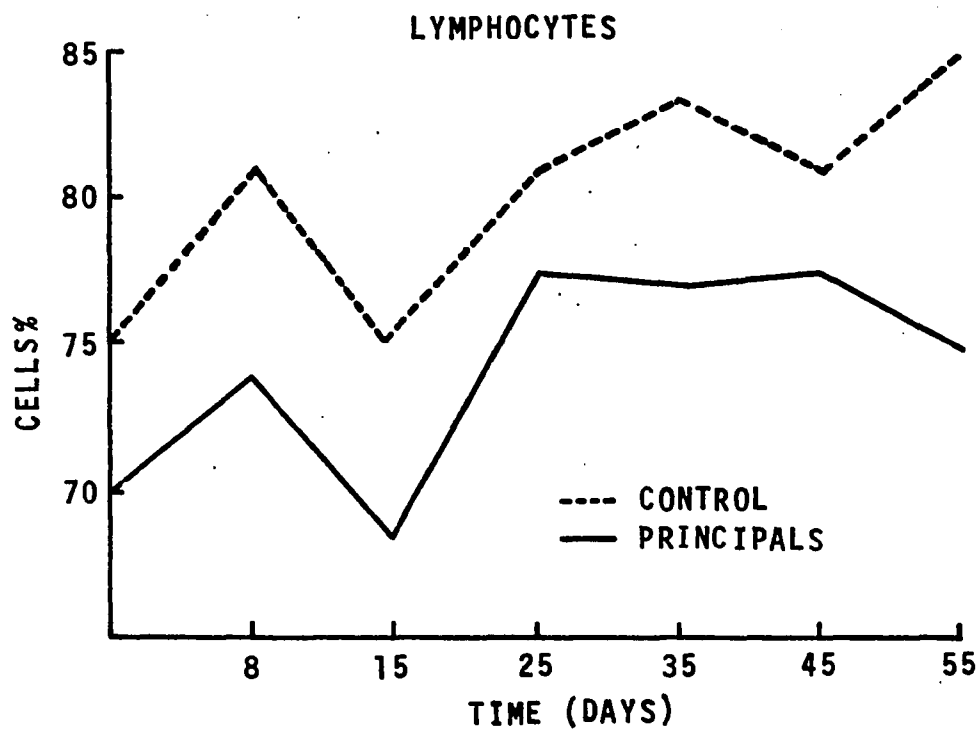


Table 10 Serum glutamic oxaloacetic transaminase (SGOT - units)

		Time						SGOT	
Animal Number		8 day	15 day	25 day	35 day	45 day	55 day	Before Trial	
Controls	2	84.00 ^a	70.00	74.66	— ^b	—	—	76.22	72.00 ^c
	4	94.00	—	—	—	—	—	94.00	72.25
	8	73.00	69.00	69.00	66.00	72.00	86.50	72.58	77.25
	15	98.00	—	—	—	—	—	98.00	70.75
	16	81.50	69.00	72.00	78.00	86.66	72.00	76.52	74.00
	17	66.50	66.00	72.00	—	—	—	68.16	80.50
Principals	1	75.50	—	—	—	—	—	75.50	79.66
	3	91.00	93.00	116.00	—	—	—	100.00	78.75
	5	83.50	—	—	—	—	—	83.50	44.00
	6	105.00	170.50	147.33	—	—	—	140.94	86.50
	7	130.00	—	—	—	—	—	130.00	76.25
	9	115.50	182.50	215.00	190.00	163.33	170.00	175.55	94.00
	10	97.00	122.00	174.33	110.00	105.33	86.00	115.77	85.00
	11	105.50	99.00	112.66	—	—	—	102.38	71.00
	12	71.00	—	—	—	—	—	71.00	73.25
	13	104.50	76.00	82.00	81.66	81.00	98.00	87.19	69.00
	14	110.00	138.00	167.66	—	—	—	129.55	88.50
	18	104.00	154.00	81.50	95.00	121.00	215.00	128.41	81.25

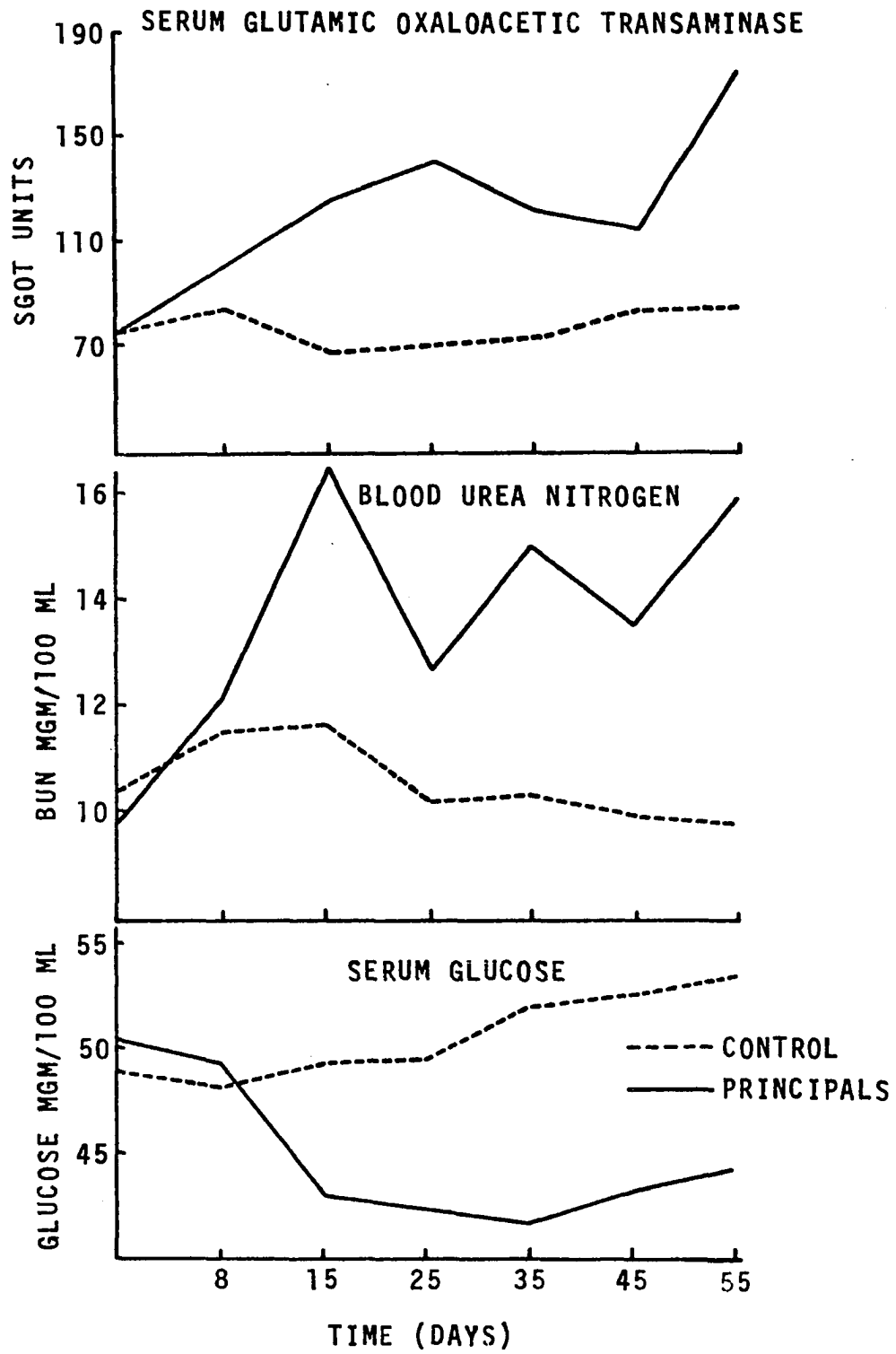
Mean	con- trols	82.82	68.50	71.91	72.00	79.33	79.25	75.63	74.45
	prin- ci- pals	99.37	129.37	137.06	119.06	117.66	167.25	153.29	71.26

^aEach figure represents mean of a paired sample.

^bNo samples. Animals killed at that time.

^cMean of 2 observations.

Fig. 17 Serum glutamic oxaloacetic transaminase
(International units; SGOT)
Blood urea nitrogen (mg./100 ml.) BUN
Serum glucose (mg./100 ml.)



Glucose A progressive fall of serum glucose level of the principals was noted throughout the trial. The controls had a constant level with a slight increase during the last 25 days (Table 11 and Fig. 17).

Blood urea nitrogen (BUN) Also, a significant difference was found between the BUN values of the principals and those of the controls. It became apparent that there was a progressive increase of BUN of the principals and a constant decrease in that of the controls (Table 12 and Fig. 17).

Urinalysis

In general urine from the principals had the characteristic garlic odor of anamú. The urine from the principals became very clear during the last 35 days of the trial (Fig. 18).

Specific gravity

In general, the specific gravity of the urine of the principals was lower than that of the controls.

Urine of some principals (No. 13 and 18) had specific gravity values lower than 1.010 during the last 20 days of the trial (Table 13 and Fig. 19).

pH

The principals of group 3 had an acid pH for the last 30 days of the trial. The controls of the same group had

Table 11 Glucose mg./100

		Time							
	Animal Number	8 day	15 day	25 day	35 day	45 day	55 day	Mean	Before Trial
Controls	2	52.00 ^a	46.50	49.25	— ^b	—	—	46.95	55.50 ^c
	4	48.00	—	—	—	—	—	48.00	45.00
	8	55.00	52.50	52.33	54.66	54.66	54.00	53.85	47.50
	15	45.00	—	—	—	—	—	45.00	42.00
	16	40.00	46.50	47.00	48.33	49.00	50.00	46.80	53.65
	17	49.50	50.50	49.75	—	—	—	49.91	47.75
Principals	1	48.50	—	—	—	—	—	48.50	46.25
	3	48.00	46.50	48.75	—	—	—	47.75	48.50
	5	57.50	—	—	—	—	—	57.50	53.25
	6	47.50	33.50	38.00	—	—	—	39.83	53.17
	7	47.50	—	—	—	—	—	47.50	49.50
	9	48.50	46.00	40.00	36.00	36.00	41.00	41.25	50.25
	10	43.00	48.00	42.50	45.00	48.00	46.00	45.41	55.00
	11	59.00	45.00	43.33	—	—	—	49.11	55.50
	12	45.00	—	—	—	—	—	45.00	51.50
	13	47.00	39.50	41.33	44.33	44.66	45.00	43.63	48.00
	14	46.50	34.00	40.00	—	—	—	40.16	47.75
	18	44.00	51.00	46.33	42.33	48.33	50.00	46.99	50.25

Mean	con-	48.25	49.00	49.68	51.49	51.83	52.00	50.37	48.56
	trols								
	prin-	48.73	42.93	42.53	41.91	44.24	45.50	44.30	50.74
	ci-								
	pals								

^aEach figure represents mean of a paired sample.

^bNo sample. Animal killed at that time.

^cMean of 2 observations.

Table 12 Blood urea nitrogen (BUN) mg./100

	Animal Number	Time						Mean	BUN Before Trial
		8 day	15 day	25 day	35 day	45 day	55 day		
Controls	2	9.56 ^a	11.05	9.92	— ^b	—	—	10.17	9.86 ^c
	4	11.91	—	—	—	—	—	11.91	10.47
	8	10.00	10.73	9.09	10.82	10.21	9.96	10.13	10.04
	15	9.86	—	—	—	—	—	9.86	10.50
	16	12.10	12.90	8.09	9.70	9.71	9.43	10.32	10.46
	17	16.22	12.67	13.58	—	—	—	14.15	11.79
Principals	1	11.40	—	—	—	—	—	11.40	9.49
	3	11.26	11.65	10.70	—	—	—	11.20	5.84
	5	13.47	—	—	—	—	—	13.47	8.61
	6	9.74	11.83	11.50	—	—	—	11.02	9.55
	7	10.97	—	—	—	—	—	10.97	12.39
	9	10.96	11.31	10.60	13.47	10.96	17.70	12.50	12.34
	10	21.15	21.00	16.45	20.13	13.26	15.52	17.92	13.92
	11	12.10	15.48	12.62	—	—	—	13.40	11.03
	12	9.42	—	—	—	—	—	9.42	8.70
	13	9.99	14.16	10.02	13.35	12.51	10.00	11.67	8.27
	14	10.69	19.96	13.71	—	—	—	14.78	8.81
	18	14.60	29.08	14.65	14.15	15.31	21.10	18.14	10.16

Mean	con-	11.60	11.83	10.17	10.26	9.96	9.69	10.58	10.52
	trols								
	prin-	12.14	16.80	12.53	15.27	13.01	16.08	14.30	9.91
	ci-								
	pals								

^aEach figure represents mean of a paired sample.

^bNo samples. Animal killed at that time.

^cMean of 2 observations.

Fig. 18 Principal No. 13, 45 days (right). Colorless urine collected during clinical state of polyuria and hyposthenuria. Normal urine from control on left

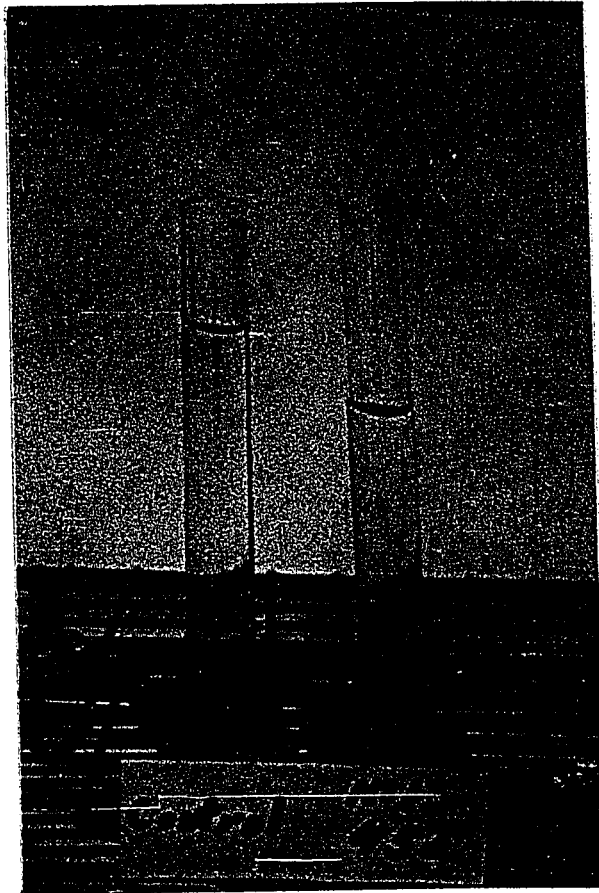


Table 13 Urine specific gravity

	Animal Number	Time					Mean
		35 day	40 day	45 day	50 day	55 day	
Con- trols	8	1.025 ^a	1.026	1.016	1.016	1.018	1.020
	16	1.016	1.016	1.017	1.018	1.016	1.016
Princi- pals	9	1.010	1.020	1.020	1.016	1.016	1.016
	10	1.017	1.014	1.011	1.017	1.018	1.016
	13	1.014	1.020	1.015	1.005	1.004	1.010
	18	1.016	1.009	1.014	1.013	1.014	1.012
Mean	con- trols	1.020	1.021	1.016	1.017	1.017	1.018
	prin- ci- pals	1.014	1.016	1.015	1.012	1.013	1.013

^aEach figure represents mean of a paired value.

a higher pH. The pH of some of the principals (No. 9, 10, and 18) was as low as pH 5.0.

None of the controls had a pH lower than pH 6.0 (Table 14 and Fig. 19).

Protein

During the last 30 days of the trial the urine from the principals of group 3 was intermittently positive for protein. The urine from the controls was protein negative.

Glucose

It was also present in the urine of the principals of group 3 in similar manner as protein, but without correlation with the protein finding. Glucose was not found in the urine of the controls.

Urobilinogen

There was a slight increase of urobilinogen in the principals (No. 9, 10, and 18). The urobilinogen in the other animals of group 3 was within the normal ranges.

Blood, acetone, and indican

Blood, acetone and indican were found negative in the urine of both principals and controls.

Chloride

The concentration of chloride in the urine of the principals remained lower than that of the controls until about the 45th to 50th day, when it became higher in the urine of the principals (Table 15 and Fig. 19).

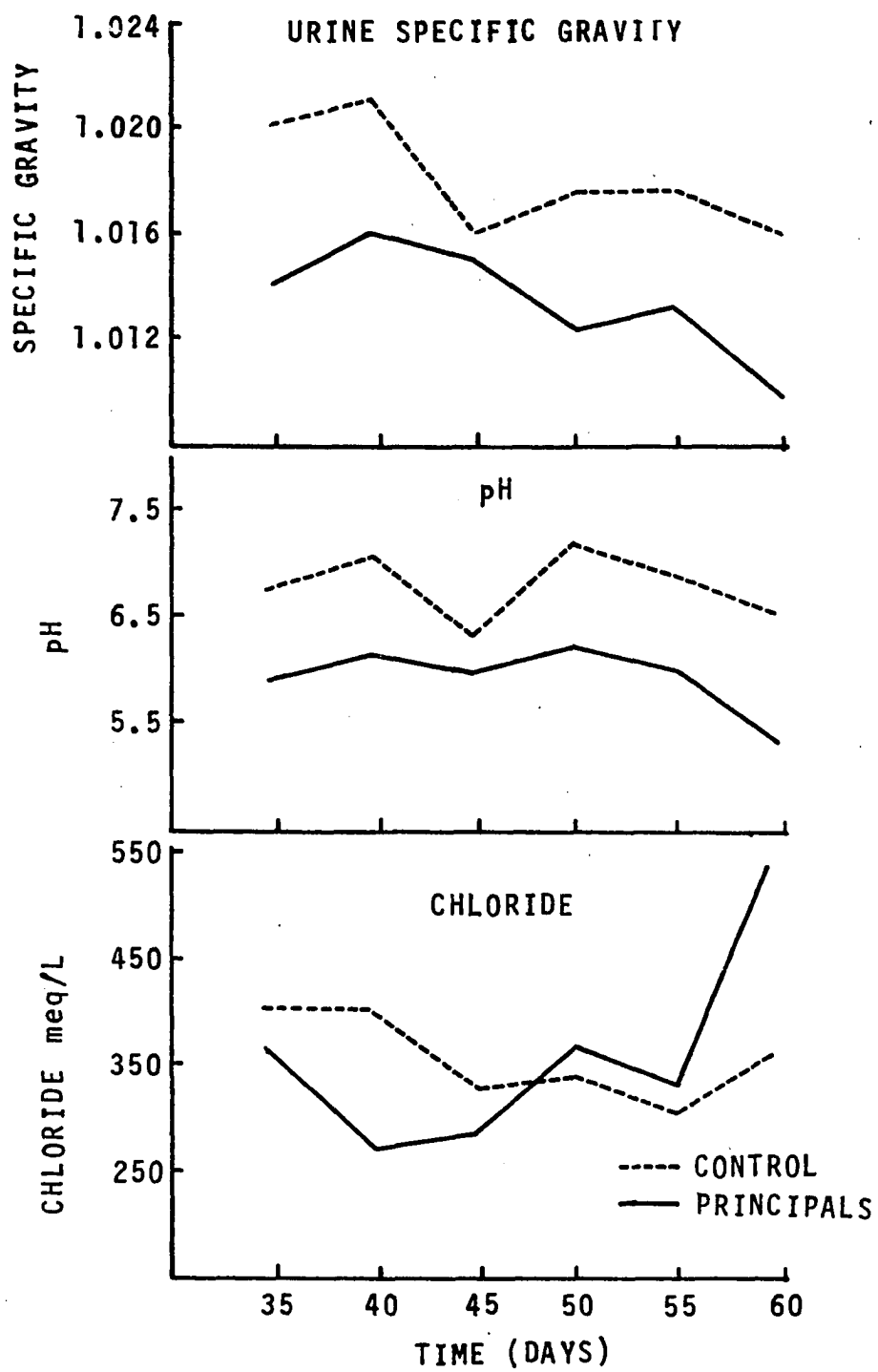
Table 14 Urine pH

	Animal Number	Time						
		35 day	40 day	45 day	50 day	55 day	60 day	Mean
Con- trols	8	7.0	8.0	6.0	7.8	7.0	6.6	7.0
	16	6.5	6.5	6.8	6.8	6.8	6.8	6.7
Princi- pals	9	5.0	7.0	6.0	7.1	6.5	5.0	6.1
	10	7.0	6.5	5.8	6.0	5.5	5.0	5.9
	13	6.7	6.0	6.5	6.1	6.3	6.3	6.3
	18	5.0	5.0	6.0	5.7	5.8	5.0	5.4
Mean	con- trols	6.7	7.2	6.4	7.3	6.9	6.7	6.8
	prin- ci- pals	5.9	6.1	6.0	6.2	6.0	5.3	5.9

Table 15 Urine chloride mEq/L

		Time						Mean
Animal	Number	35 day	40 day	45 day	50 day	55 day	60 day	
Con- trols	8	497	497	398	342	256	513	417
	16	342	342	171	342	342	256	299
Princi- pals	9	171	342	342	427	342	513	356
	10	171	171	199	456	427	768	398
	13	769	342	256	171	171	285	332
	18	342	206	356	427	399	627	392
Mean	con- trols	419	419	334	342	299	384	366
	prin- ci- pals	364	265	288	370	320	548	359

Fig. 19 Urine specific gravity. pH and chlorides (mEq/L)



It was noticed that some principals (No. 9, 10, and 18) had a progressive increase of chloride concentration in their urine. The other principal (No. 13) had a progressive decrease within the same period of time. The concentration of chloride in the urine of the controls remained about constant (Table 15).

A correlation between the high chloride concentration and the low pH in the same urine of the same principals was noted (Fig. 20, principal No. 10 as an example).

Urinary sediment

In general, the principals had a larger number of leukocytes and epithelial cells than the controls.

Most of the epithelial cells were small, round, and about 15 μ in diameter (Tables 16 and 17).

Casts were found in large amounts in the urinary sediment of the principals of group 3. These were mostly hyaline and granular. Granular casts were found more frequently and in greater number than hyaline ones during the last 20 days of the trial.

Crystals were also found in large numbers in urinary sediment of the principals (No. 9, 10, 13, and 18). These were of several classes: calcium carbonate, amorphous phosphates, hippuric acid, triple phosphates, and occasionally calcium oxalate crystals.

Amorphous phosphate and hippuric acid crystals were most frequently found.

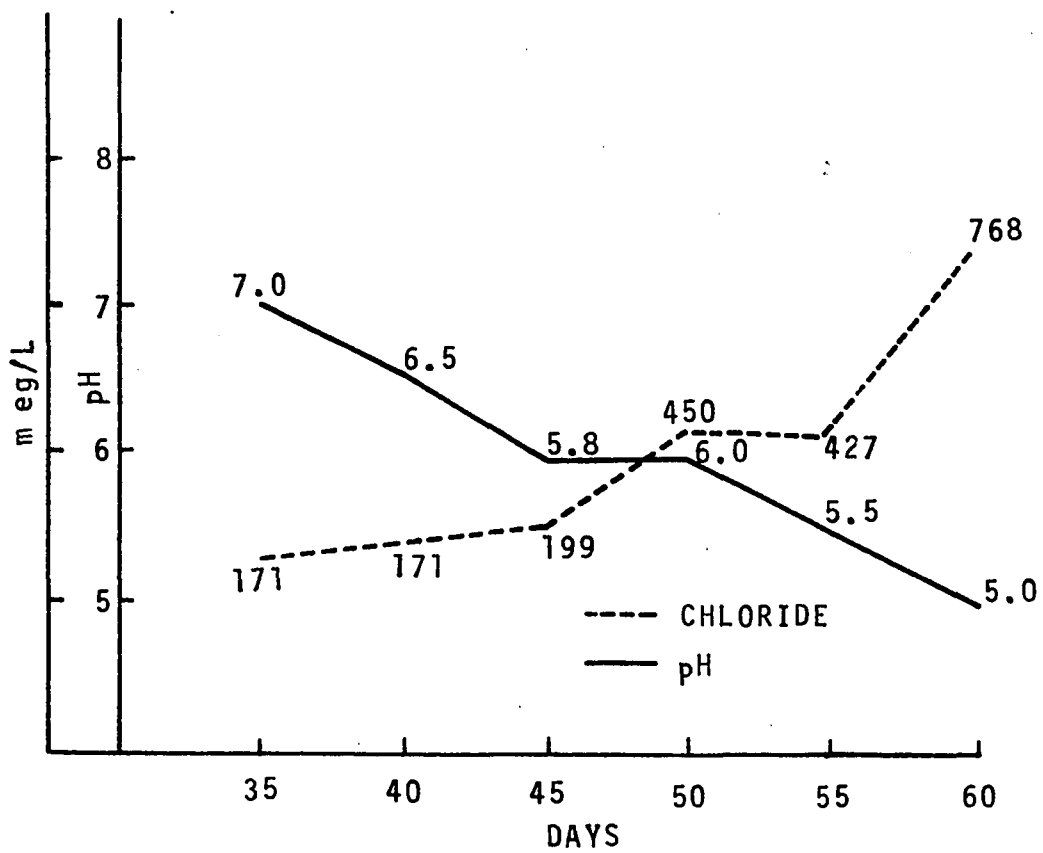
Table 16 Leukocytes in urine sediment cells/field

		Time						Mean
Animal Number		35 day	40 day	45 day	50 day	55 day	60 day	
Con- trols	8	4	—	—	—	—	2	1.0
	16	—	—	—	—	—	—	—
Princi- pals	9	4	—	—	2	—	4	1.6
	10	—	—	4	2	10	12	4.6
	13	8	8	—	4	4	—	4.0
	18	19	—	10	10	3	4	7.6
Mean	con- trols	2	—	—	—	—	1	0.5
	prin- ci- pals	7	2	4	5	4	5	4.5

Table 17 Epithelial cells in urine sediment cells/field

		Time						Mean
	Animal Number	35 day	40 day	45 day	50 day	55 day	60 day	
Con- trols	8	4	—	—	2	—	1	1.0
	16	—	4	8	2	—	2	2.6
Princi- pals	9	2	2	4	4	2	2	2.6
	10	—	4	4	—	6	4	3.0
	13	12	10	20	6	6	3	9.5
	18	4	2	8	8	5	6	5.5
Mean	con- trols	2	2	4	2	—	2	2.0
	prin- ci- pals	5	5	9	5	5	3	5.3

Fig. 20 Correlation between urine pH and chloride
concentration from principal No. 10



No erythrocytes were found in urinary sediment of both principals and controls.

Gross Lesions

Field cases

Cardiovascular system Cardiac dilation, primarily of the right ventricular chamber, was observed in all calves. The ventricular walls were flaccid and a yellow waxy appearance was noted throughout the myocardium, but no other alterations were found in the blood-vascular system (Fig. 21).

Digestive system Four hair balls about 5 centimeters in diameter were found within the rumen of calf D.

Edema of the proximal duodenum, involving the whole wall and connective tissue of the pancreas, was prominent in all the 4 calves.

The calves (A, B, and C) had several small white foci about 5 mm. in diameter distributed throughout the pancreas.

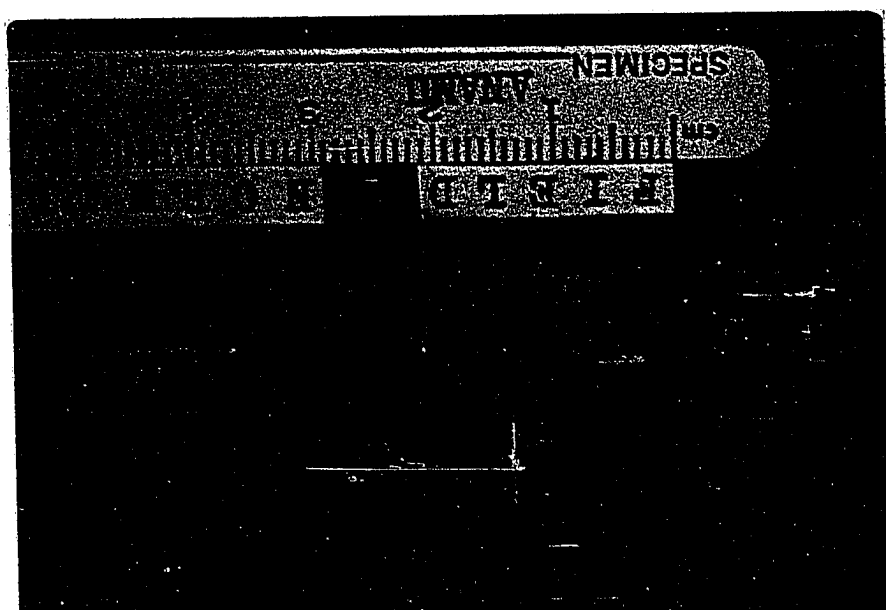
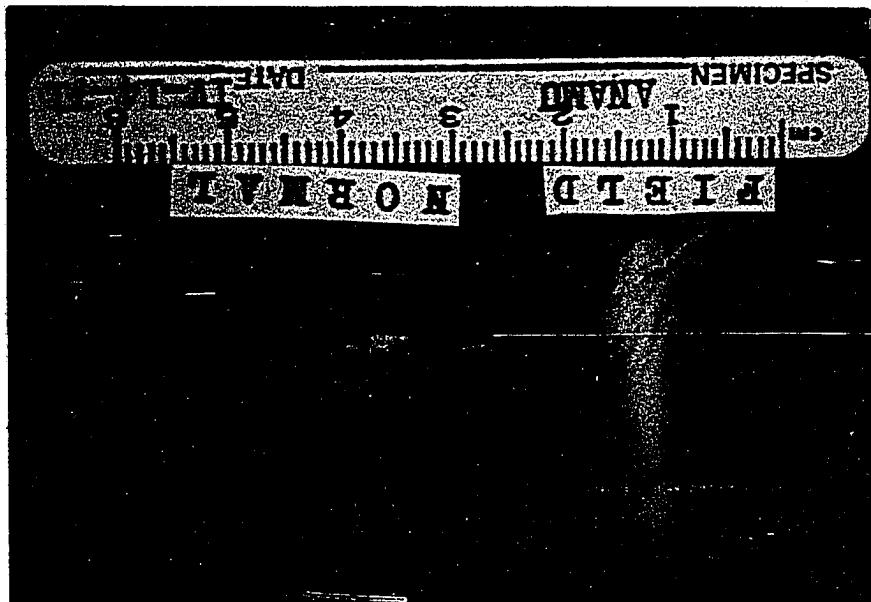
The livers of calves C and D were enlarged and congested.

Lymphatic system All the lymph nodes from these calves (A, B, C, and D) were enlarged. The spleens did not have gross lesions.

Muscular system The field cases (A, B, C, and D) had alterations in several muscles, but these lesions did not have the same distribution in all the animals.

Fig. 21 Natural case. Heart. Discoloration of
 myocardium

Fig. 22 Natural case. Vastus medialis. Skeletal
 muscle discoloration



The diaphragms of calves C and D had large irregular areas of degeneration with marked yellowish pallor, better noted on the pars costalis of the muscle. Similar lesions were found in the quadriceps femoris, especially its vastus medialis and rectus femoris heads of calves (A, B, C, and D; Fig. 22). Both psoas muscles, minor and major, of the same animals were uniformly pale and flaccid.

The same lesions were found in the laryngeal muscles of all 4 calves.

Nervous system There was marked increase of cerebro-spinal fluid, being most apparent in the cisternae. The ventricles were not dilated.

Respiratory system No gross lesions were found.

Urinary system The kidneys of all calves were slightly enlarged, very pale yellow and had dull and slightly rough surfaces (Fig. 23).

On sectioning, the kidneys were firm, especially those of calf C and they had a strong and peculiar garlic odor similar to that of the plant.

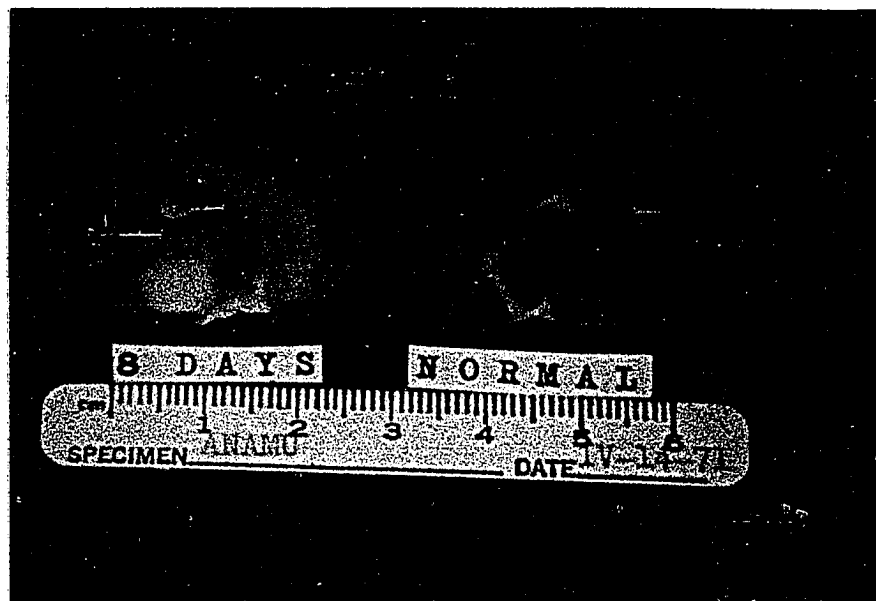
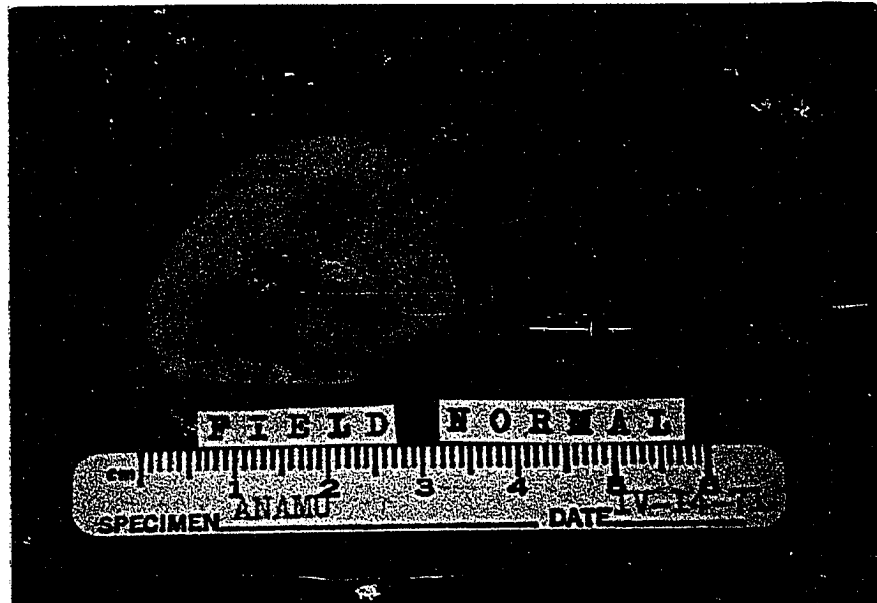
There was an apparent increase of renal cortical substance in all these field cases. This change was more remarkable in kidney sections of calf C. The medullary substance of these kidneys was greatly reduced (Fig. 23).

Experimental cases - group 1

The lesions were not very apparent during the first 8 days of this trial.

Fig. 23 Natural case. Kidney discoloration

Fig. 24 Principal No. 1, 8 days. Kidney discoloration



Cardiovascular system The myocardium was pale in principals (No. 1, 5, and 12). Principal No. 5 had an accentuated pallor of the myocardium throughout the organ. Principal No. 7 and controls (No. 4 and 15) did not have any macroscopic alteration of the heart (Fig. 25).

Digestive system There was mild edema of duodenal mucosa and serosa of all principals from this group.

Lymphatic system The lymph nodes of both principals and controls were slightly enlarged.

Muscular system All the principals (No. 1, 5, 7, and 12) had a yellowish-waxy discoloration of several muscles (Fig. 26).

Quadriceps femoris of principals (No. 1, 7, and 12) had this particular discoloration. This alteration was also noted in the biceps femoris of principal No. 1 and laryngeal muscles of principal No. 12.

The muscles from the controls appeared normal.

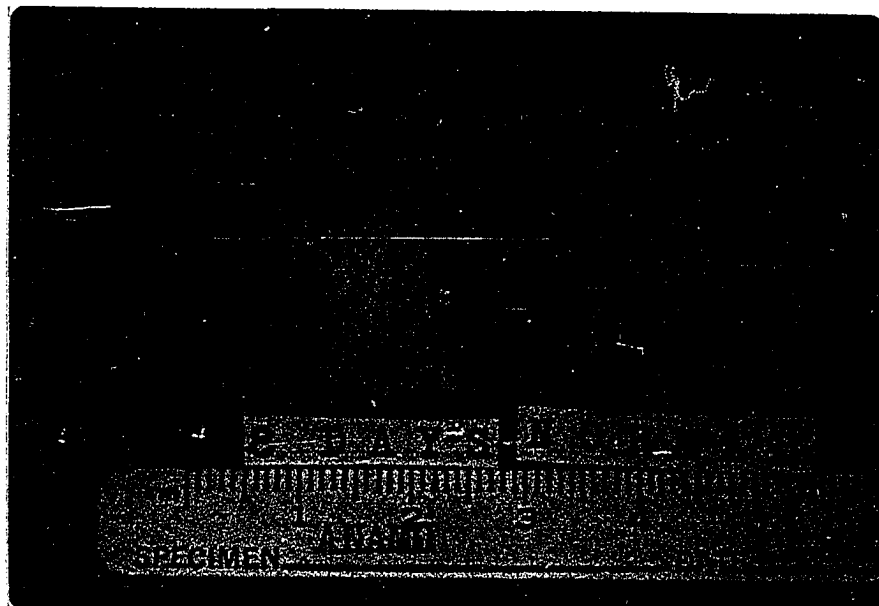
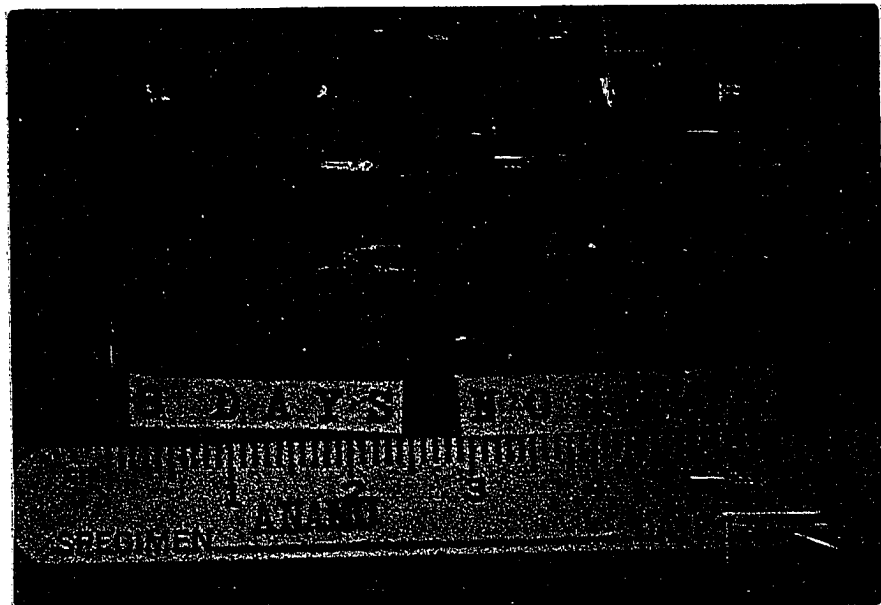
Nervous system There was an appreciable increase in the amount of cerebrospinal fluid of principals (No. 1, 5, and 12) compared to that of the controls. This accumulation was particularly prominent in the subarachnoid space and cisterna magna and pontis. The fluid was clear, colorless, and of reduced consistency.

Respiratory system No gross lesions were visible.

Urinary system The kidneys from principals (No. 1, 7, and 12) were slightly pale (Fig. 24). On sectioning,

Fig. 25 Principal No. 5, 8 days. Heart. Myocardium
discoloration

Fig. 26 Principal No. 12, 8 days. Vastus medialis.
Skeletal muscle discoloration



they had a special garlic odor similar to that noted in field cases.

Experimental cases - group 2

The principals from group 2 (No. 3, 6, 11, and 14) had similar pathologic alterations as noted in principals of group 1, but the lesions were more marked.

Cardiovascular system Marked cardiac dilation, particularly of right ventricle, was found in principals (No. 6 and 14). The myocardium was atonic and it had a characteristic light yellowish-waxy appearance. The hearts from the other principals (No. 3 and 11) only had the yellowish waxy appearance of their cardiac muscle.

The hearts from the controls (No. 2 and 17) did not have visible alterations.

Digestive system Edema of the entire duodenum was noted in principals (No. 6, 11, and 14). It was extended to ileum and jejunum in principal No. 11.

Lymphatic system The lymph nodes of both principals and controls were slightly enlarged.

Muscular system Quadriceps femoris, semimembranosus, semitendinosus and psoas major muscles of principal No. 14, were mottled by large irregular yellowish discolorations.

The principals (No. 3, 6, and 11) had a diffuse yellowish degeneration of the muscle fibers of quadriceps femoris, biceps femoris, diaphragm and some intercostal muscles. All of the affected muscles were very flaccid,

especially those of principal No. 6. Muscles from the controls appeared normal.

Nervous system All the principals had an increased amount of cerebrospinal fluid, similar to that found in field cases and principals of group 1.

Respiratory system No gross lesions were found.

Urinary system The kidneys from all the principals had a diffuse yellow discoloration, and the kidneys of principal No. 14 were also enlarged. On sectioning all had the garlic odor of anamú.

The renal cortex of principal No. 14 was visibly swollen.

No changes were noted in kidneys of the controls.

Experimental cases - group 3

The principals of this group (No. 9, 10, 13, and 18) all had better defined lesions when killed 60 days after initiation of the trial.

Cardiovascular system All the principals had cardiac dilation and discoloration of the myocardium similar to those of the principals from the 2 previously discussed groups, but the myocardiums of these principals were markedly flaccid.

Digestive system Edema of the duodenum was not as marked as in principals of previous groups, but it was present in principals (No. 10 and 18).

Lymphatic system The lymph nodes of both principals and controls were slightly enlarged.

Muscular system Similar lesions to those noted in other principals were observed in the principals of group 3. However, more muscles were involved, such as quadriceps femoris, biceps femoris, semimembranosus, semitendinosus, gastrocnemius, both psoas, intercostals, diaphragm and longissimus dorsi. The principals (No. 9 and 10) had a diffuse white-yellowish discoloration of primarily the psoas, biceps femoris and quadriceps (Fig. 27). Others had a mottled appearance, caused by partial yellowish discoloration, such as gastrocnemius, semimembranosus, and intercostals in principals (No. 13 and 18). All these muscles were flaccid. The gastrocnemius, semitendinosus, and semimembranosus muscles of principals (No. 9 and 18) were atrophic.

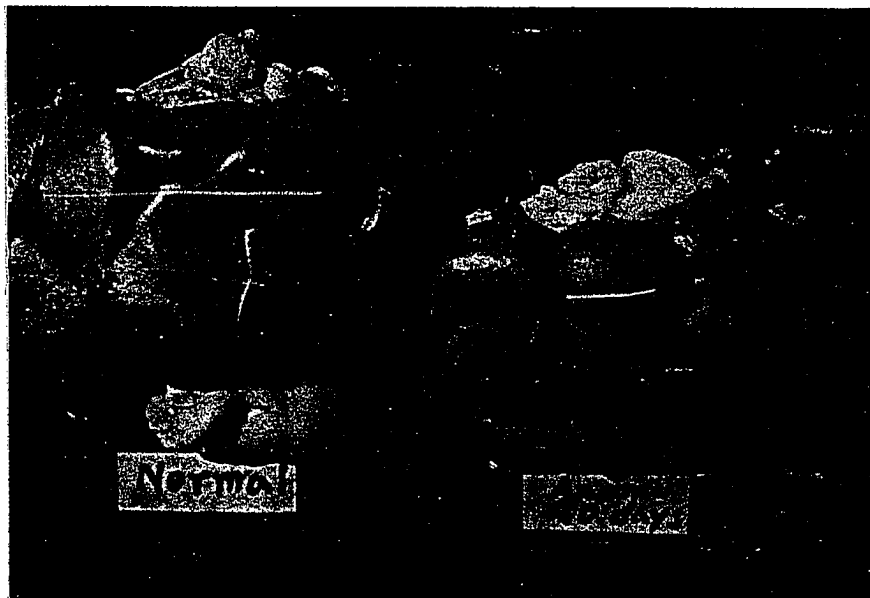
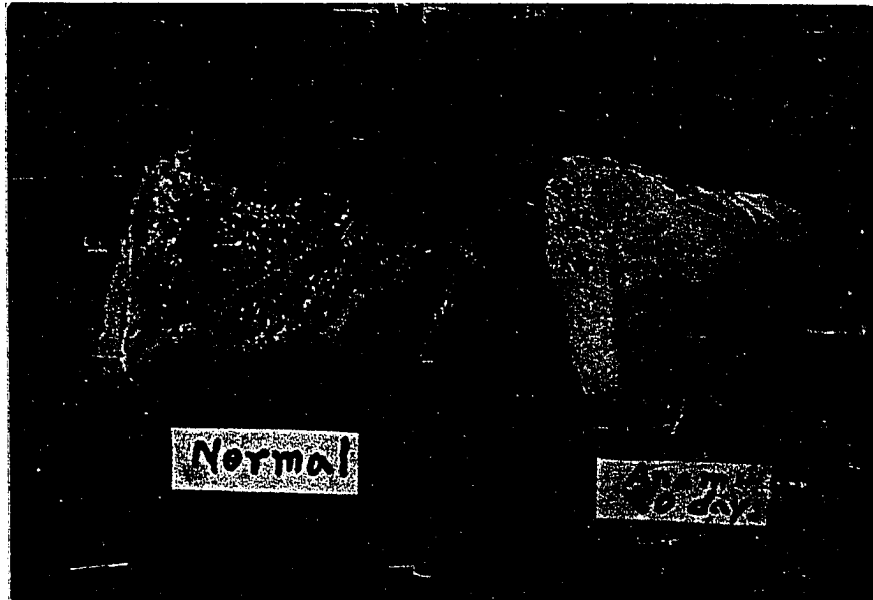
No muscular lesions were present in the controls (No. 8 and 16).

Nervous system Increased cerebrospinal fluid was the common finding in the principals of this group.

The cortical surface of the brain had an edematous appearance in principals (No. 13 and 18). There was a perineural edema along the sciatic nerves of principals (No. 9 and 10). No alteration was found in the nervous system of the controls.

Fig. 27 Principal No. 9, 60 days. Vastus medialis.
Skeletal muscle discoloration

Fig. 28 Principal No. 10, 60 days. Kidney discoloration



Respiratory system The lungs from principals (No. 9, 13 and 18) had a cyanotic appearance. Emphysematous foci of about 5 to 7 centimeters were found throughout the diaphragmatic lobes of principal No. 13.

Urinary system The kidneys from all the principals, were swollen, discolored, dull and rough surfaced. On sectioning they had the peculiar garlic odor of anamú (Fig. 28).

A summary of the gross lesions and their frequency of presentation within the population of experimental cases is shown in Table 18.

Histological Lesions

Field cases

Cardiovascular system Severe myocardial atrophy was found in field cases (A, B, C, and D). The muscle fibers were widely separated from each other with strands of connective tissue apparently from the endomysium. Definite reduction in size of muscle fibers was found in field case B. Furthermore, field case D had an increased number of nuclei per muscle fiber.

Several isolated muscle fibers were hyalinized and necrotic with small pyknotic nuclei or even, karyolytic. Other myocardial fibers had granular degeneration and many had large vesicular nuclei.

Table 18 Summary of gross lesions

Animal No.		Heart degeneration, whitish discoloration	Duodenal edema	Muscle degeneration, whitish discoloration	CSF increase	Lung cyanosis and emphy- sema	Kidney degenera- tion, swollen, whitish discolor- ation
8 day	con- trols	4 - ^a	-	-	-	-	-
	15	-	-	-	-	-	-
	prin- ci- pals	1 + ^b	+	+	+	-	+
	5	+	+	+	+	-	-
30 day	7	-	+	+	-	-	+
	12	+	+	+	+	-	+
	con- trols	2 -	-	-	-	-	-
	17	-	-	-	-	-	-
60 day	prin- ci- pals	3 +	-	+	+	-	+
	6	+	+	+	+	-	+
	11	+	+	+	+	-	+
	14	+	+	+	+	-	+
60 day	con- trols	8 -	-	-	-	-	-
	16	-	-	-	-	-	-
	prin- ci- pals	9 +	-	+	+	+	+
	10	+	+	+	+	-	+
	13	+	-	+	+	+	+
	18	+	+	+	+	+	+

Total %	controls	0%	0%	0%	0%	0%	0%
	principals	93.3%	75.0%	100.0%	93.3%	25.0%	93.3%

^aNo lesion.

^bLesion present.

Digestive system Tongue sections from field cases (A and B) had several hyalinized muscle fibers and some atrophic changes.

Dilation of Brunner's glands was noted in the duodenum of field cases (B, C, and D).

The livers were apparently normal, except for scattered hematopoietic foci throughout the parenchyma.

The pancreas from field cases (A, B, and C) had enlarged hyperplastic islets of Langerhans.

Muscular system Lesions of different types were found in several muscles.

The diaphragm was the most regularly affected muscle; the lesion was characterized by a diffuse granular degeneration in every field case.

Many muscle fibers of the diaphragm of field cases (A, B, and C) had a striking type of lesion, a central core of necrosis, which appeared as a homogeneous amorphous eosinophilic mass (Fig. 29). The necrotic fibers had swollen vesicular and some pyknotic nuclei (Fig. 30). Some of the muscle fibers were fragmented with complete myolysis of segments. The areas of myolysis were surrounded by histiocytes and neutrophils (Fig. 32). Other fibers were completely necrotic and hyalinized. The endomysial connective tissue was prominent around some muscle fibers. Longitudinal sections had segments of a wavy necrotic core, sometimes fragmented, with interruptions of surrounding cross

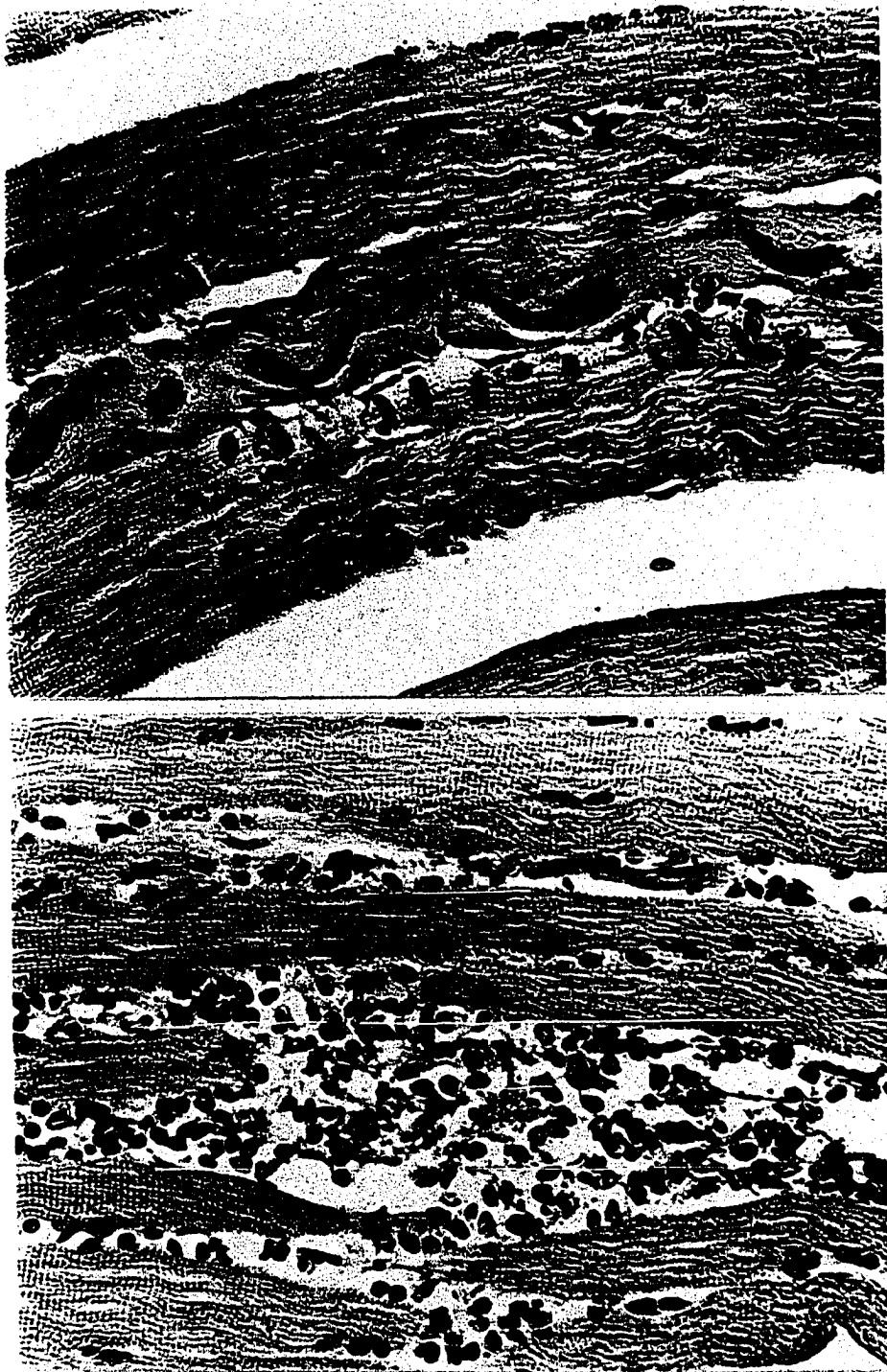
Fig. 29 Natural case. Diaphragm. Cross section with
central core necrosis of muscle fibers
H & E stain x 64

Fig. 30 Natural case. Diaphragm. Higher magnification
of Fig. 29 showing central core necrosis
H & E stain x 400



Fig. 31 Natural case. Diaphragm. Longitudinal section
with segments of a wavy necrotic core
H & E stain x 400

Fig. 32 Natural case. Diaphragm. Longitudinal section
with focus of myolysis surrounded by macrophages
and neutrophils
H & E stain x 340



striations (Fig. 31).

There was some separation of muscle fibers by edema or proliferating fibrous connective tissue.

Cricoarytenoideous dorsalis muscles had several hyalinized muscle fibers with some fragmentation and an increase of endomysial connective tissue.

The intercostal muscles from field cases (A, B, and D) had large numbers of hyalinized muscle fibers, some atrophic fibers, and endomysial connective tissue proliferation.

Lesions in quadriceps muscles were only found in field cases A and D and consisted of hyalinized fibers with pyknotic nuclei and a mild increase of endomysial connective tissue.

The tendomuscular junction of the gastrocnemius had some atrophic and hyalinized muscle fibers.

Nervous system No pathologic alterations were found in the central nervous system but changes were noted in motor end plates of affected muscles to be described later in the text.

Respiratory system No lesions were observed in the lungs, trachea and turbinates.

Urinary system Nephritis was found in all field cases, but all nephrons were not equally affected. The lesions were pronounced in the renal cortex. Areas of

collapsed tubules and altered glomeruli could be noted contrasting with areas of dilated tubules and enlarged glomerular tufts, which completely filled Bowman's spaces. This pattern indicated involvement of the whole nephron (Fig. 33). The enlarged glomeruli had a marked hypercellularity, chiefly of mesangial cells. Some glomeruli were shrunken, and at various stages of sclerosis. The Bowman's capsules of the atrophic glomeruli were thickened and hyalinized and a wide zone of periglomerular and intertubular fibrosis was present (Fig. 34). There was a marked adventitial fibrosis of the arteries (Fig. 35). The tubular epithelium had degenerative changes: vacuolation and cloudy swelling. Necrotic changes, such as pyknosis, karyorrhexis, karyolysis, and cellular fragmentation were observed in epithelium of both proximal and distal convoluted tubules, but these changes were more prominent in the former.

Hyaline and cellular casts were present within lumens of distal and proximal tubules. Cellular casts were composed mainly of neutrophils. Foci of lymphocytes and scattered neutrophils were present in the interstitial tissue.

Experimental cases

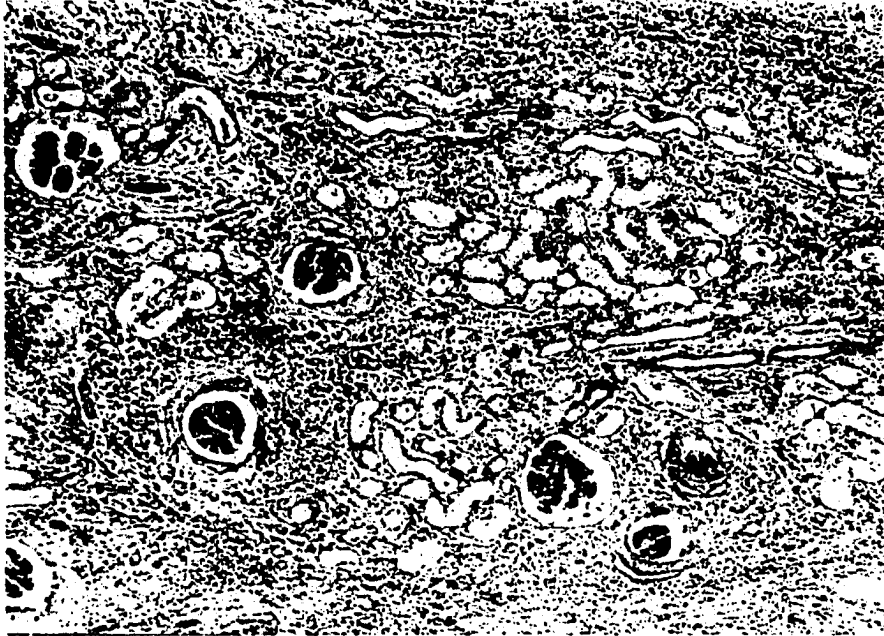
Cardiovascular system Principals from group 1 (No. 1, 5, 7, and 12) had no visible lesions of the myocardium.

Myocardial fibers were notably irregular in size in all principals of group 2. Cross sections of the myocardium

Fig. 33 Natural case. Kidney. There are dilated and collapsed tubules, interstitial fibrosis and inflammation
H & E stain x 64

Fig. 34 Natural case. Kidney.
Scarring of the glomeruli
H & E stain x 400

Fig. 35 Natural case.
Kidney.
Periarteriolar fibrosis.
Cellular casts
H & E stain
x 400



revealed bundles of shrunken fibers, with pyknotic and karyolytic nuclei. The fibers were widely separated from each other.

The myocardium sections of principal No. 6 had a large number of nuclei in relation to the number and size of the muscle fibers.

The principals from group 3, had more advanced myocardial changes than the 2 previous groups. The myocardial fibers were extremely atrophic, particularly those of principals No. 13 and 18, and the spaces between muscle fibers were occupied by immature connective tissue fibrosis (Fig. 36). Scattered bundles of fibers were necrotic and hyalinized (Fig. 37).

Digestive system Mild atrophy of lingual muscle fibers was noted in some principals at 8, 30, and 60 days of the trial. No lesions were found in the duodenum of principals from groups 1 and 2, but principals No. 9 and 18, after 60 days had cystic dilation of Brunner's glands (Fig. 38).

Hepatic changes other than hematopoietic foci were not found in the controls and principals.

The pancreatic acini of controls and principals were normal. The islets of Langerhans appeared normal in all group 1 animals. Hyperplastic islets were found in principals (No. 6 and 11) of group 2 and No. 9 and 13 of group 3, but principal No. 6 had extremely enlarged islets (Fig. 39).

Muscular system Granular degeneration and a slight increase of endomysial connective tissue were noted in the

Fig. 36 Principal No. 10. Heart. Marked increase of
connective tissue between muscle bundles
H & E stain x 64

Fig. 37 Principal No. 18. Heart. Fragmentation and
necrosis of muscle fibers and infiltration of
macrophages
H & E stain x 400

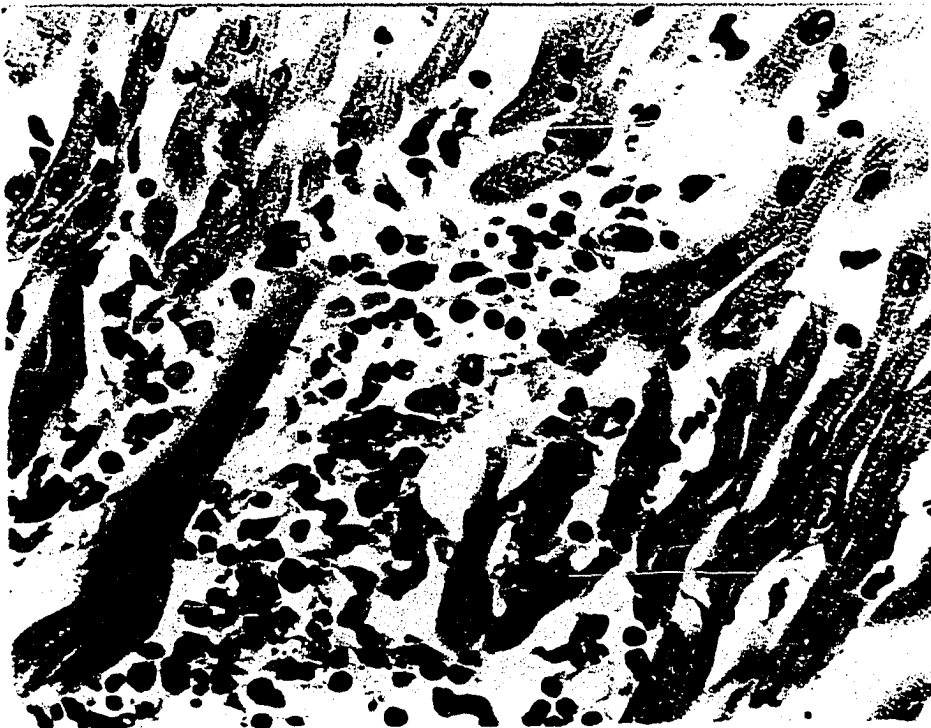
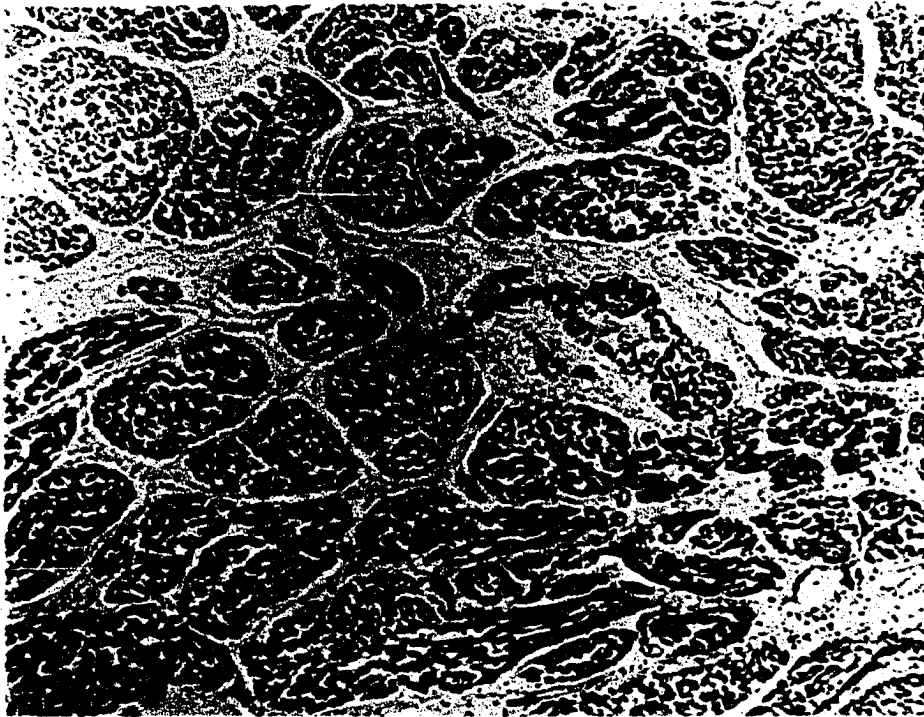
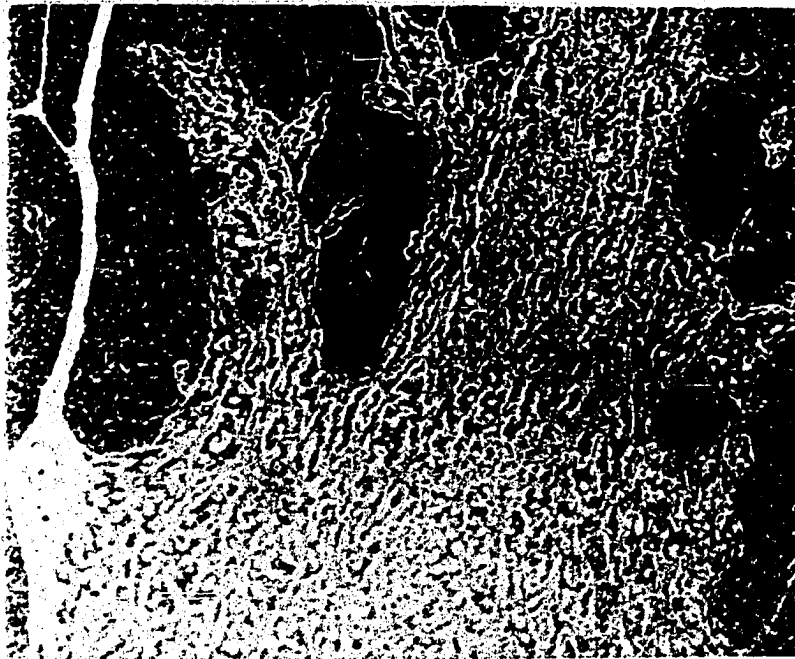


Fig. 38 Principal No. 18. Duodenum. Cystic dilation of
Brunner's glands
H & E stain x 64

Fig. 39 Principal No. 6. Pancreas. Hyperplastic islets
of Langerhans
H & E stain x 64



diaphragms of all group 1 principals. Some swollen muscle fibers were seen in principals No. 7 and 12.

In group 2, the lesions were more prominent with several bundles of hyalinized muscle fibers in the diaphragms of all principals. Some of the muscle fibers had a central core of hyalinization similar to that noted in field cases. This characteristic lesion was better defined in principal No. 3. Some muscle fibers had segments of myolysis surrounded by an inflammatory exudate of histiocytes and neutrophils.

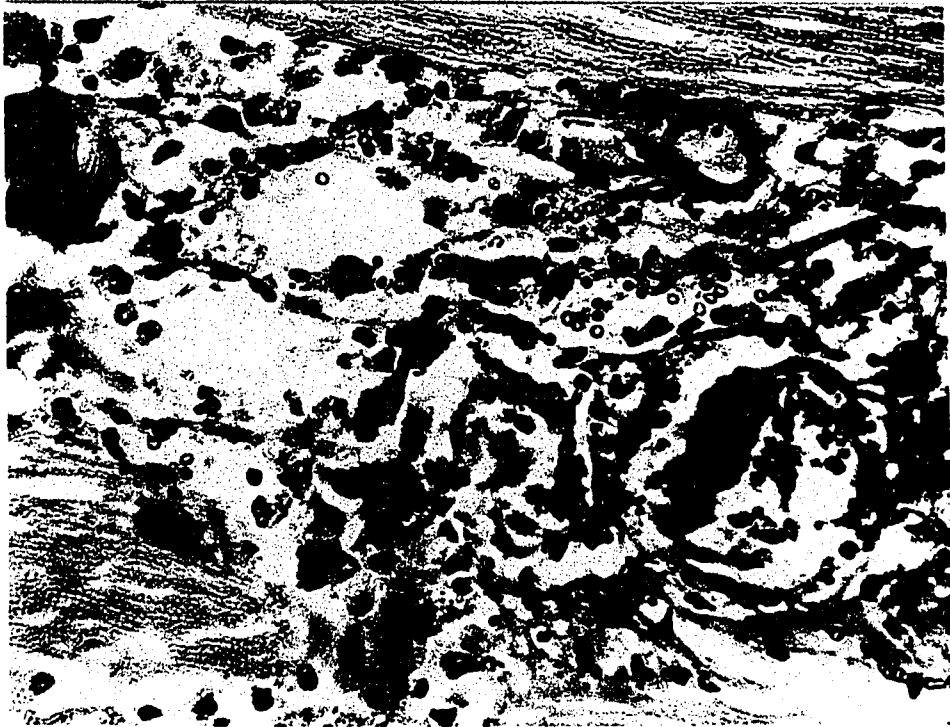
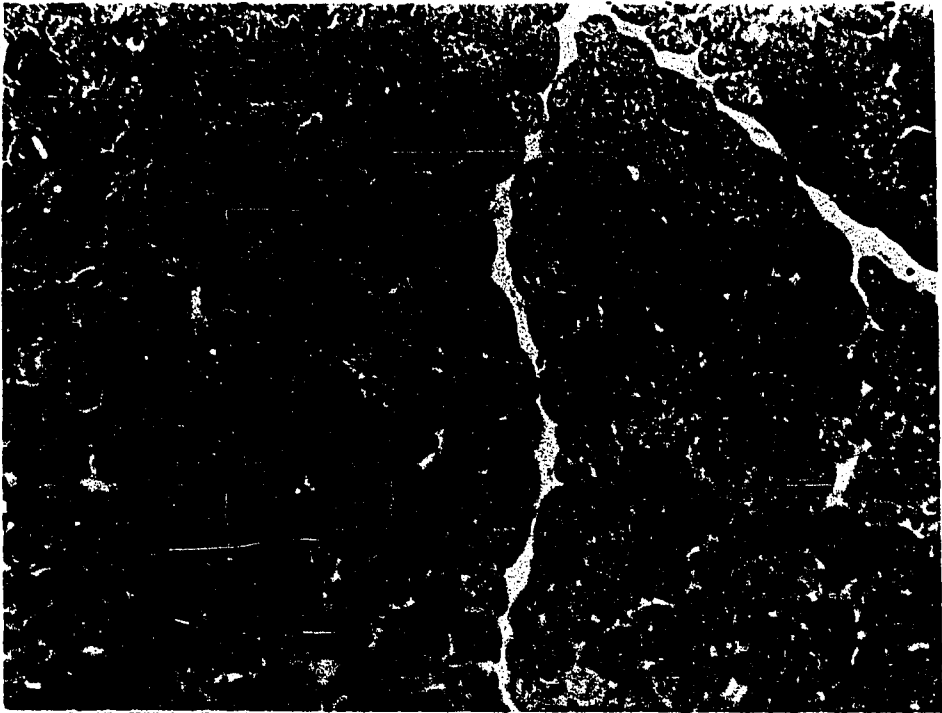
In group 3, the principals had extensive hyalinization and necrosis of diaphragmatic muscle fibers. Many of the muscle fibers of principals No. 9 and 10 had the central core necrosis as previously described for some field cases and some principals of group 2 (Fig. 40). There was an appreciable increase in endomysial connective tissue and segmental myolysis.

An early indication of a pathologic alteration in the gastrocnemius muscle at its tendomuscular junction of principals in group 1 was an intense eosinophilic staining of scattered muscle fibers (Fig. 42). All the principals of group 2 had lesions similar to those described for principals of group 1.

There was marked atrophy of muscle at the tendomuscular junction of gastrocnemius in principals of group 3, particularly in principals No. 9 and 18. This was characterized

Fig. 40 Principal No. 10. Diaphragm. Cross section with central core necrosis and some angulated fibers. H & E stain x 64

Fig. 41 Principal No. 11. Intercostal. Longitudinal section with myolysis and cellular infiltration H & E stain x 400



by irregular size of muscle fibers, centralization of nuclei and basophilia of the fibers. The nuclei were large and vesicular and there was proliferation of endomysial connective tissue. Other muscle fibers were hyalinized, but their nuclei were still at the periphery of the fiber. Occasional central core necrosis was found (Fig. 43). The above changes appeared in different gradations in bundles of muscle fibers.

The other muscles, intercostals, vastus medialis and cricoarytenoideus dorsalis were not altered as much as diaphragm and gastrocnemius. In principals of group 1 there were only scattered muscle fibers with granular degeneration. The principals of group 2 had several segments of muscle fibers with hyalinization and myolysis (Fig. 41). There was slight muscle atrophy in principals (No. 3, 6, and 14) particularly in intercostal muscles. In group 3 there was atrophy of a few bundles of muscle fibers especially of intercostals and cricoarytenoideus dorsalis muscles.

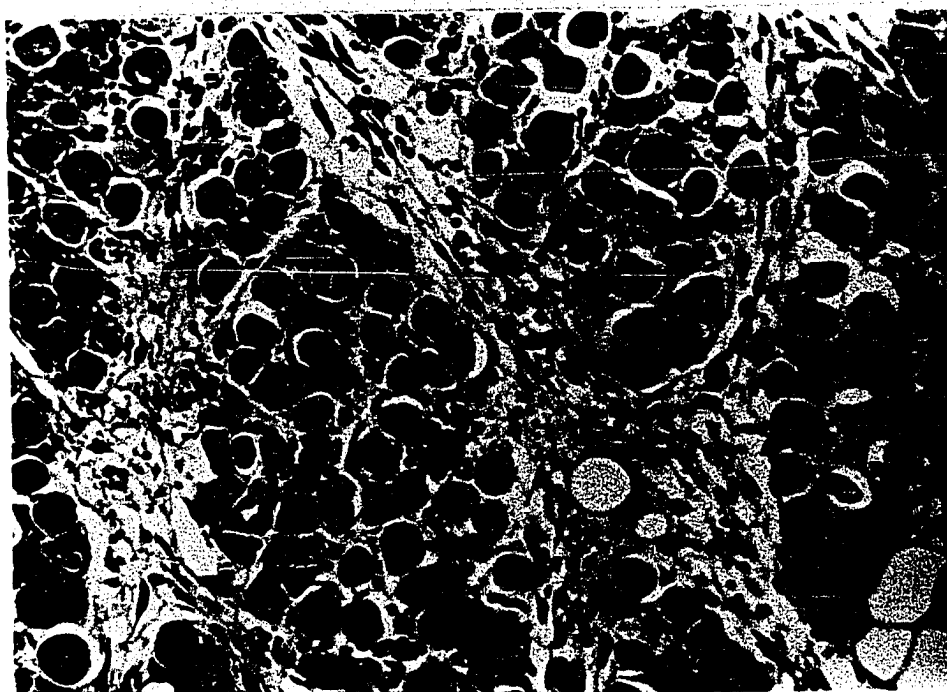
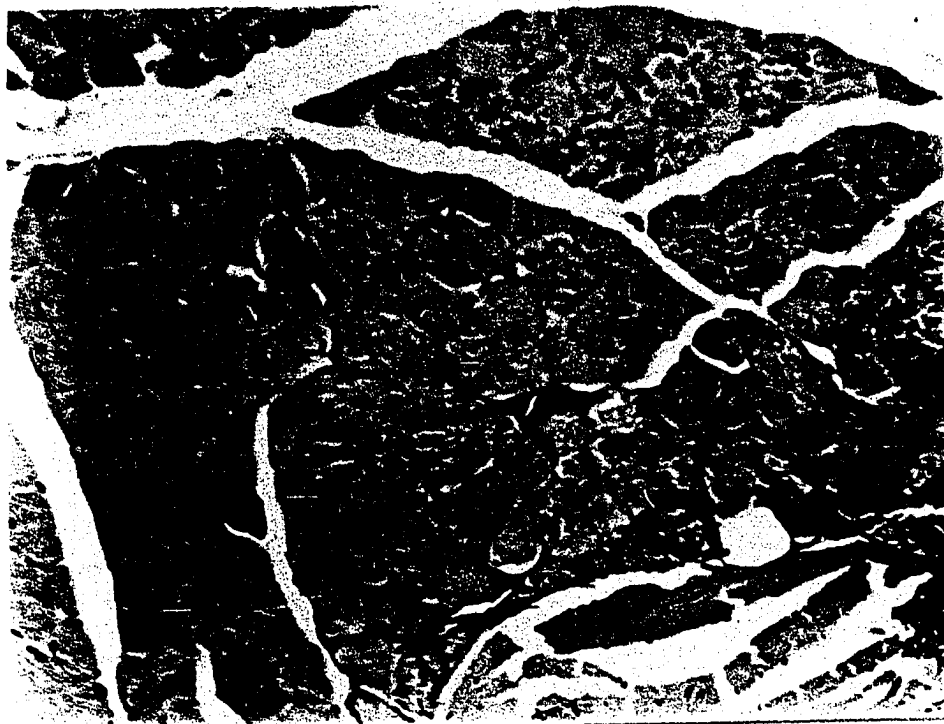
Nervous system No lesions were found.

Respiratory system No lesion that could be ascribed to this toxic plant was noted.

Urinary system Varying degrees of nephritis were found in each of the principals. Eosinophilic amorphous proteinaceous material had accumulated within Bowman's spaces and tubular lumens of principals (No. 1, 5, and 12) of group 1. Some glomeruli were enlarged, occupying most of Bowman's

Fig. 42 Principal No. 3. Gastrocnemius. Scattered muscle bundles appear homogeneous and stained intensely with eosin. H & E stain x 64

Fig. 43 Principal No. 18. Gastrocnemius. Irregular size and basophilia of muscle fibers and centralization of nuclei. H & E stain x 160



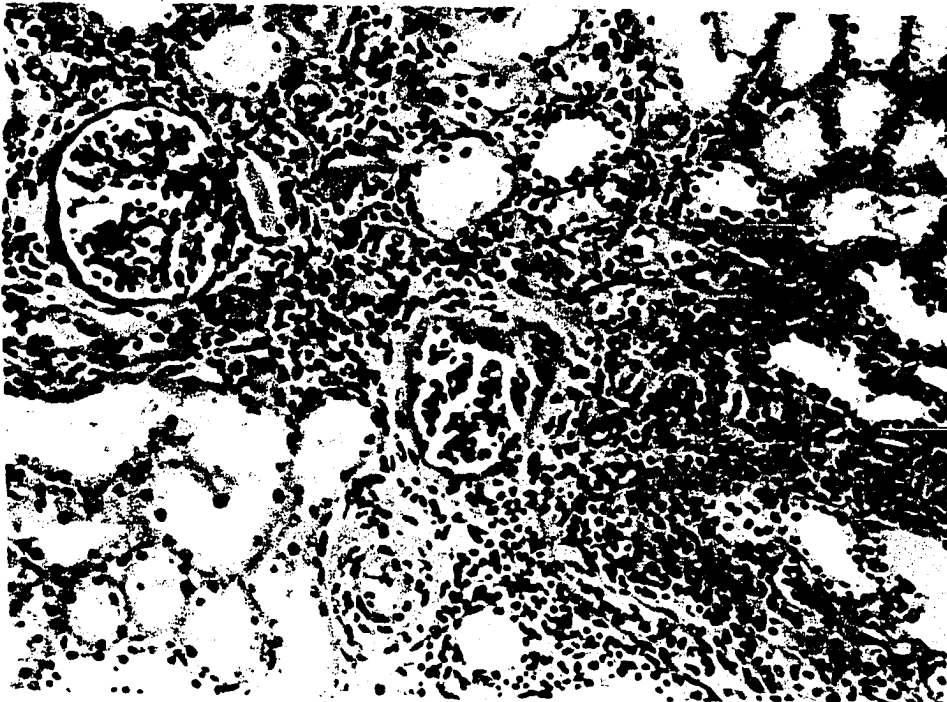
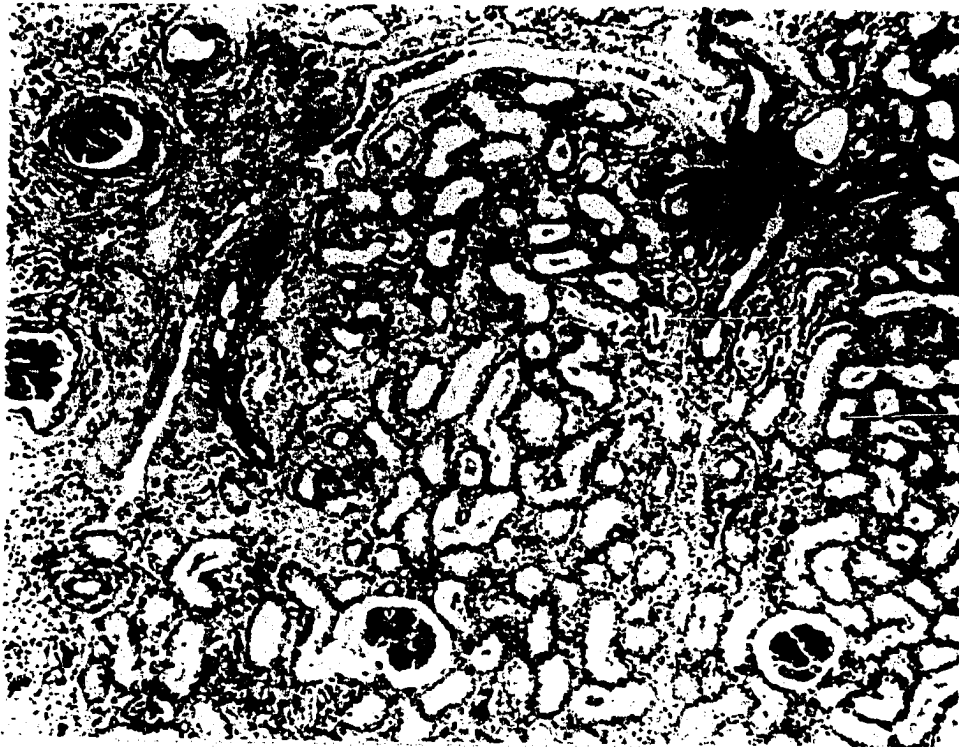
space. Bowman's capsules were mildly thickened.

Three principals of group 2 (No. 3, 6, and 14) had a particular pattern of the lesions in the kidney. There were areas of slightly dilated tubules and enlarged glomerular tufts proximal to areas of collapsed tubules and shrunken degenerated renal corpuscles. An appreciable increase of mesangial cells was noted within the glomerular tufts of swollen glomeruli. The Bowman's capsules were very thick and hyalinized. Degenerative vacuolar and necrotic changes were present in tubular epithelium. There were a few hyaline and cellular casts.

The kidneys of principals of group 3 had more severe lesions. The renal corpuscles were shrunken and scarred and also had thickened Bowman's capsules surrounded by increased reticulin fibers (Fig. 45). Other glomeruli were swollen, with marked mesangial cell hyperplasia and vacuolation of the glomerular tufts. Some tubular dilation, mainly of proximal convoluted tubules was present. Varying degenerative changes of the tubular cells were noted with many cells being necrotic. Some tubular basement membranes were thickened. Interstitial fibrosis was marked around the collapsed nephrons and several mononuclear cells, lymphocytes and plasma cells, were in the area as well as a few neutrophils. These lesions were best defined in principals (No. 9, 10, and 13).

Fig. 44 Principal No. 10. Kidney. Dilated tubules (right) collapsed tubules and periglomerular fibrosis (left). H & E stain x 64

Fig. 45 Principal No. 10. Kidney. Bowman's capsules are thickened. Periglomerular fibrosis. Note hyaline casts adjacent to glomerulus (upper left). x 160



The principals No. 7 of group 1 and 11 of group 2 and the controls had no microscopic alteration of the kidneys.

Histochemical studies

Heart No unsaturated lipids were found in myocardial sections of field cases, principals or controls. The glycogen content was definitely decreased in field cases and principals compared to that in controls. Loss and irregular thickness of striations in atrophic fibers was demonstrated by PTAH stain in the myocardium of field cases B and C and principals (No. 3, 6, 9, 13, and 18; Fig. 46).

Skeletal muscle Unsaturated lipids were not detected in muscle fibers of field cases, principals and controls by Oil red O stain.

No differences were found in glycogen content of field cases, principals and controls, as determined by PAS stain.

PTAH stain stained the necrotic cores of the muscle fibers dense homogeneous dark blue (Fig. 47). A wavy dark blue core was noted along segments of muscle fibers in longitudinal sections and the striations around the necrotic core were indistinct.

The atrophic muscles, stained with Cajal's stain, had elongated and thickened synaptic plates (Fig. 49). Some terminal axons were extremely elongated with irregular swollen knobs (Fig. 50). Degenerative swelling and dark staining of the filaments was also noted (Fig. 51).

Fig. 46 Principal No. 13. Heart. Loss and irregular thickening of striations in myocardial fibers. PTAH stain x 160

Fig. 47 Natural case. Diaphragm. Dense homogeneous core which stained blue but appears red-brown in the illustration. Note indistinct striations in some muscle fibers. PTAH stain x 400

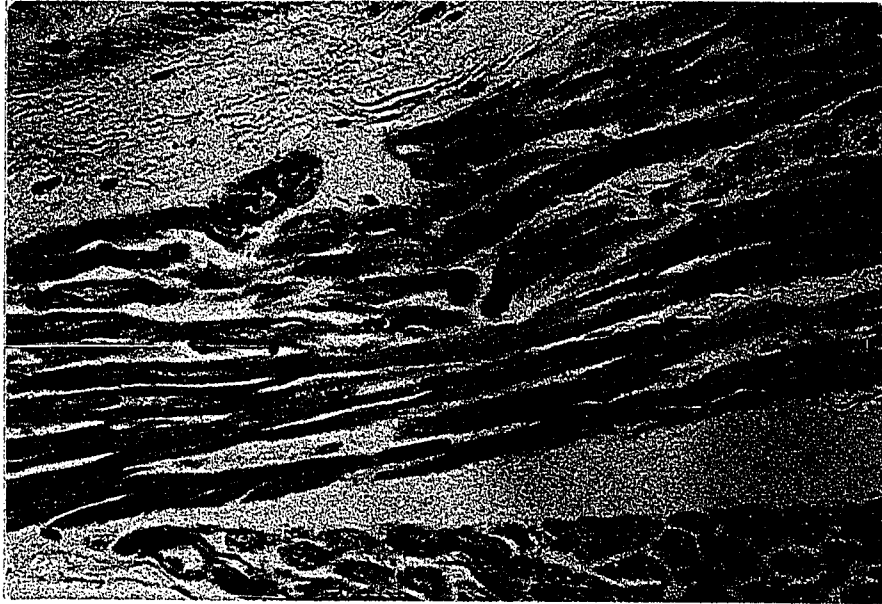


Fig. 48 Control. Motor end
plate. Normal
Cajal's stain x 500

Fig. 49 Principal No. 18.
Motor end plate.
Thickened and
elongated synaptic
plate. Cajal's
stain x 500

Fig. 50 Principal No. 18.
Motor end plate.
Terminal axons
extremely elongated
with irregular
swollen knobs.
Cajal's stain x 500

Fig. 51 Principal No. 18.
Motor end plate.
Terminal axonic
filaments are
swollen and
fragmented.
Cajal's stain
x 500



Liver No differences of glycogen content were found in liver sections of field cases, principals, and controls, as determined by PAS stain.

Pancreas A larger number of β -cells was found in islets of Langerhans of principals No. 6 and 11 (group 2), 9 and 13 (group 3), and field cases B and C, than in those of controls. The enlarged islets of principal No. 6 did not have visible α -cells (Fig. 52 and 53).

Spinal cord and ischiatic nerves The myelinated tracts were normal in all field cases, principals and controls as evaluated by Marchi's stain.

Kidney A marked increase of reticulin fibers around blood vessels, glomeruli and adjacent to altered nephrons was demonstrated by Jones' kidney stain (Fig. 54 and 55). This particular alteration was found in field cases (A, B, and C) and principals No. 3 and 6 (group 2) and No. 9, 10, 13, and 18 (group 3).

Thickened Bowman's capsules were stained black with Jones' kidney stain and deep red with PAS stain (Fig. 57). The thickened basement membranes were better appreciated with PAS stain (Fig. 57). There was a direct correlation between the increase of thickness of Bowman's capsules and basement membranes and the increase of the number of reticulin fibers.

The hyaline casts were strongly PAS positive.

Fig. 52 Control. Islet of Langerhans. Normal. Note content of α -cells (pink). Gomori's pancreatic islet cell stain x 350

Fig. 53 Principal No. 6. Islet of Langerhans. Hyperplastic islet. Note absence of α -cells. Gomori's pancreatic islet cell stain x 350

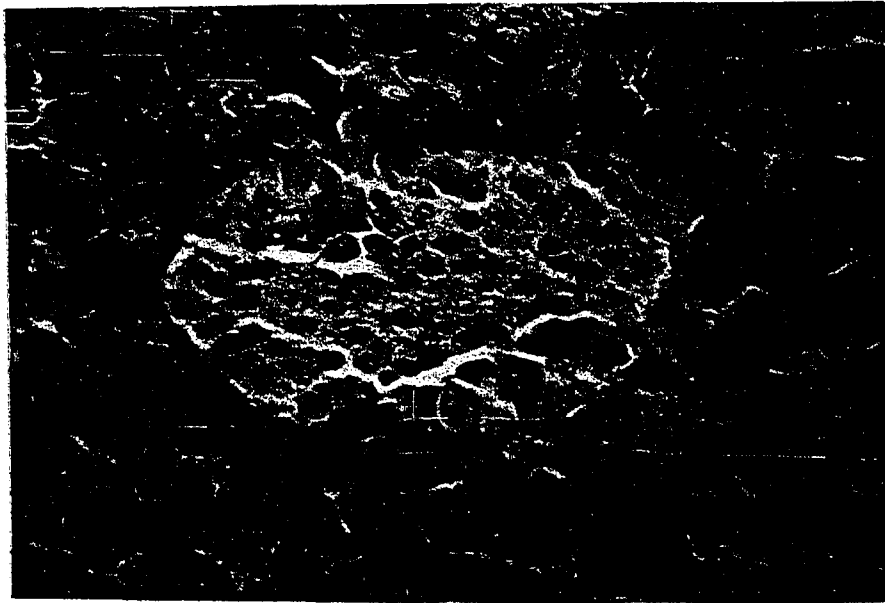


Fig. 54 Control. Kidney. Normal. Jones' Kidney stain
x 160

Fig. 55 Principal No. 3. Kidney. Dark black staining
material around glomeruli and between tubules is
composed of reticulum fibers. Jones' Kidney
stain x 160

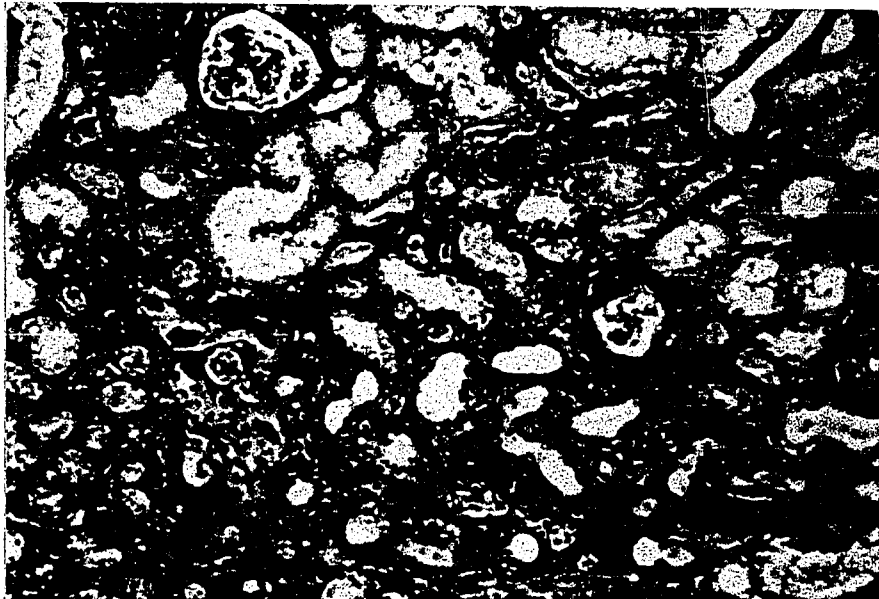
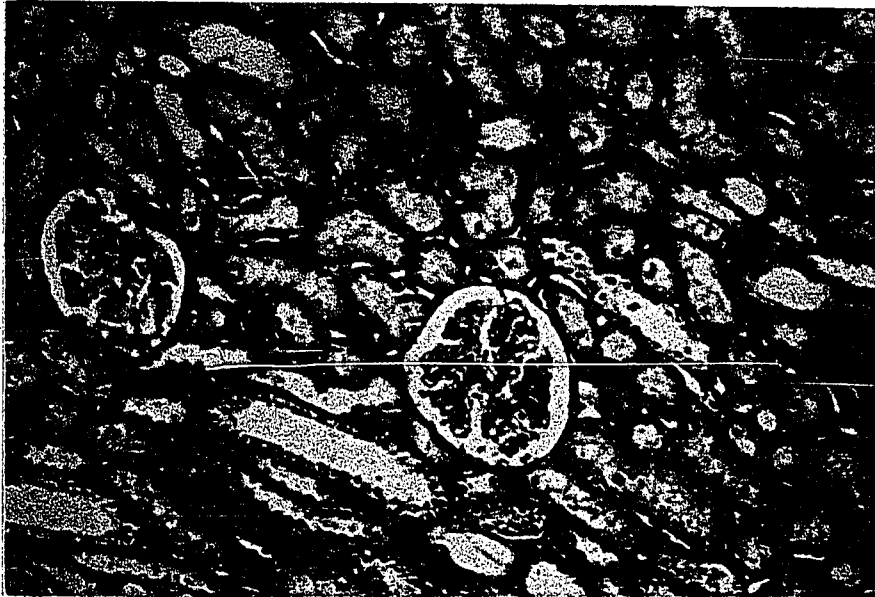
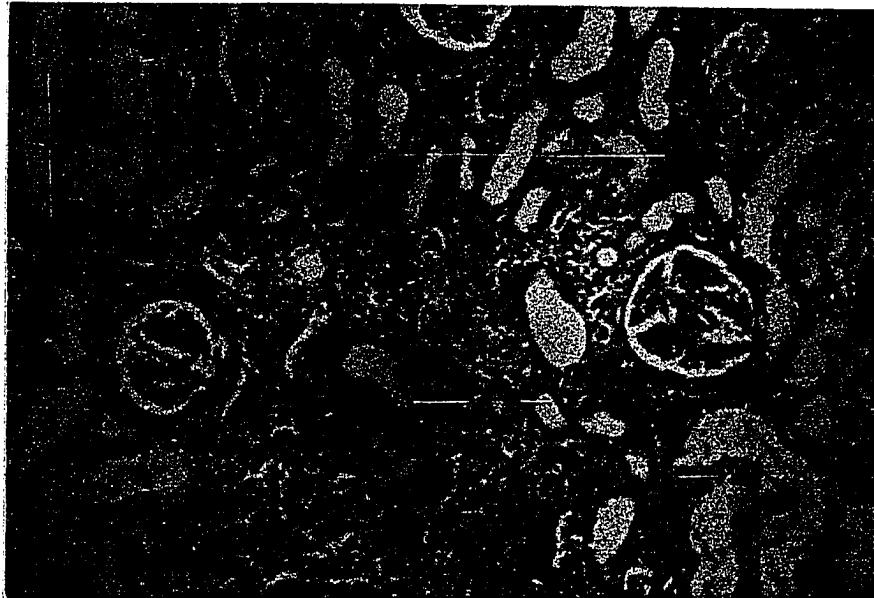
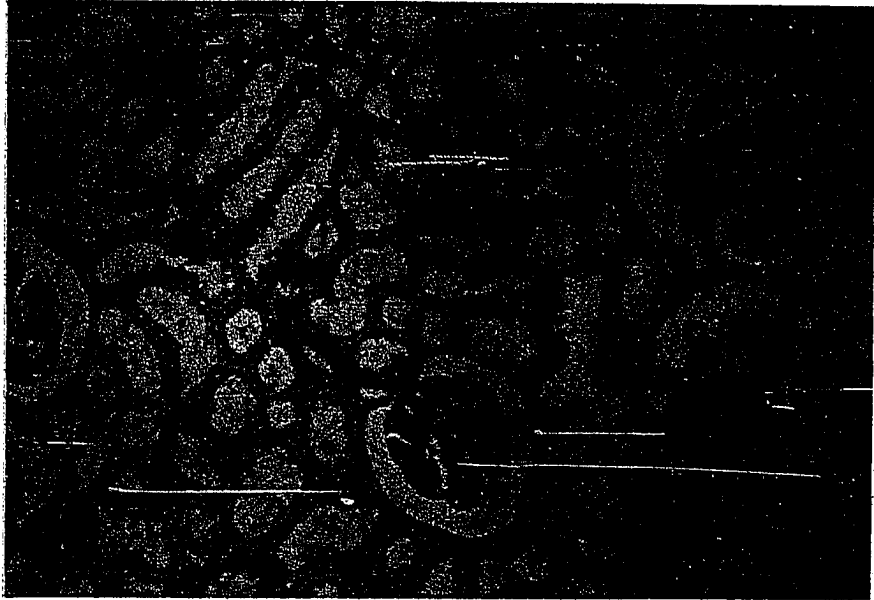


Fig. 56 Control. Kidney. Normal. PAS stain x 160

Fig. 57 Principal No. 10. Kidney. Note deposits of PAS positive material in Bowman's capsules and basement membranes of tubules. PAS stain x 160



Electron Microscopy Studies

Field cases

Heart There was an apparent increase of the cytoplasmic space between organelles within myocardial cells.

The mitochondria were swollen, distorted and some outgrowths of the outer membrane were present whereas the inner membrane clumped inside the matrix. In some cells the mitochondrial membranes were disrupted. The cristae were found in different stages of fragmentation and dissolution (Fig. 59). Many electron dense bodies were present within mitochondria and some were located outside of mitochondria. These bodies were amorphous, finely granular and had a major electron dense peripheral zone. Some of these bodies were surrounded by a double membrane (Fig. 60).

There was a marked decrease in numbers of glycogen granules. The smooth endoplasmic reticulum (SER), particularly the fenestrated collar, was slightly dilated (Fig. 61), especially close to the areas where the electron dense bodies were present.

The sarcolemma, constituted by the plasma membrane of the cardiac fiber and an extracellular basement membrane, was distended in several foci where collagen fibers were present in large number.

Skeletal muscle All the muscles of field cases saved for electron microscopic studies had similar ultrastructural

Fig. 58 Control. Normal muscle

- A. Mitochondria
- B. Glycogen
- C. Myofibrils
- D. Z line
- E. A band
- F. I band
- G. H band
- H. Sarcoplasmic reticulum
- M. M-line x 64,000

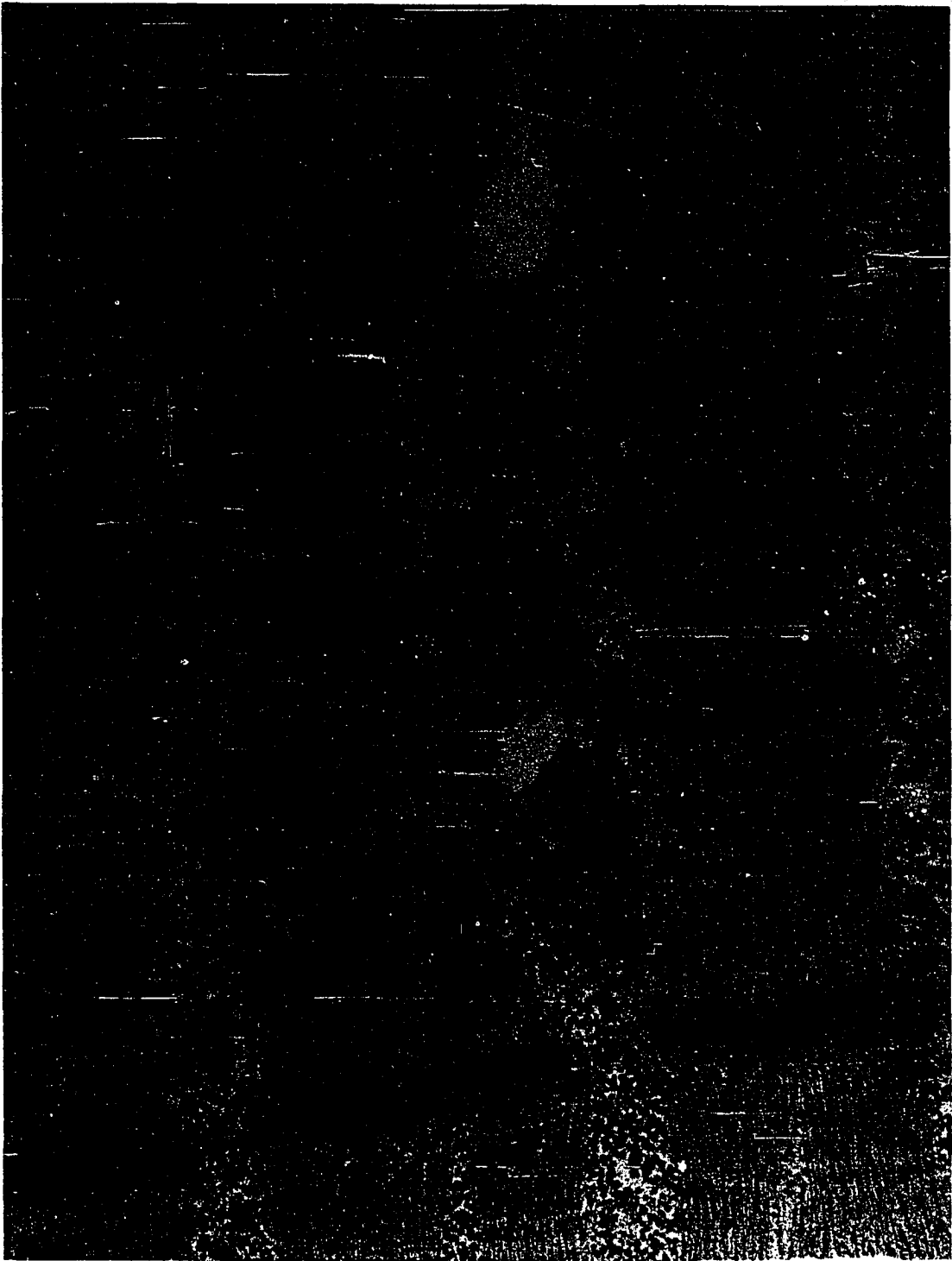


Fig. 59 Natural case. Heart. Massive mitochondria
damage: swelling, loss of crystae, outpocketing
of outer membranes (arrows), glycogen depletion
x 60,000

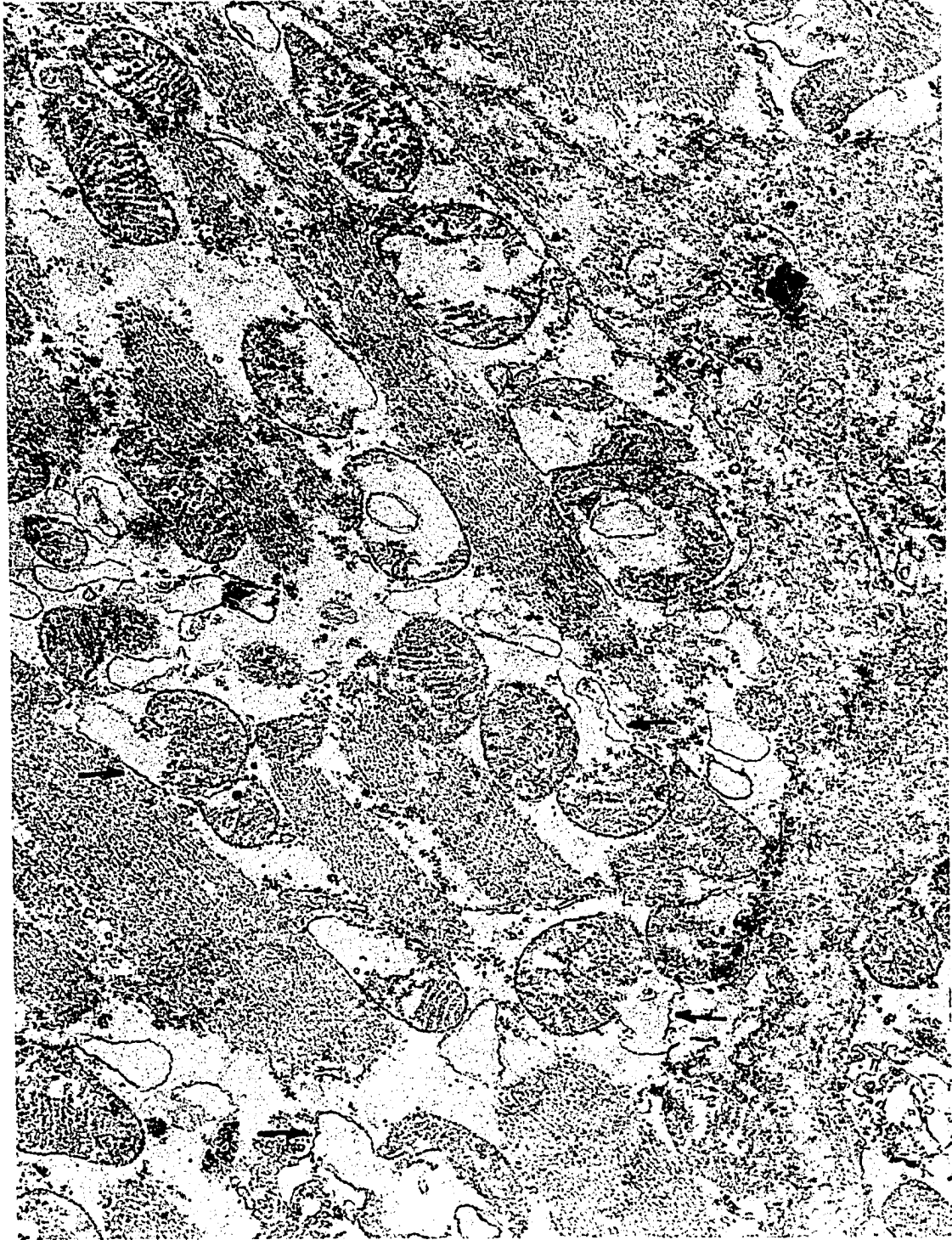
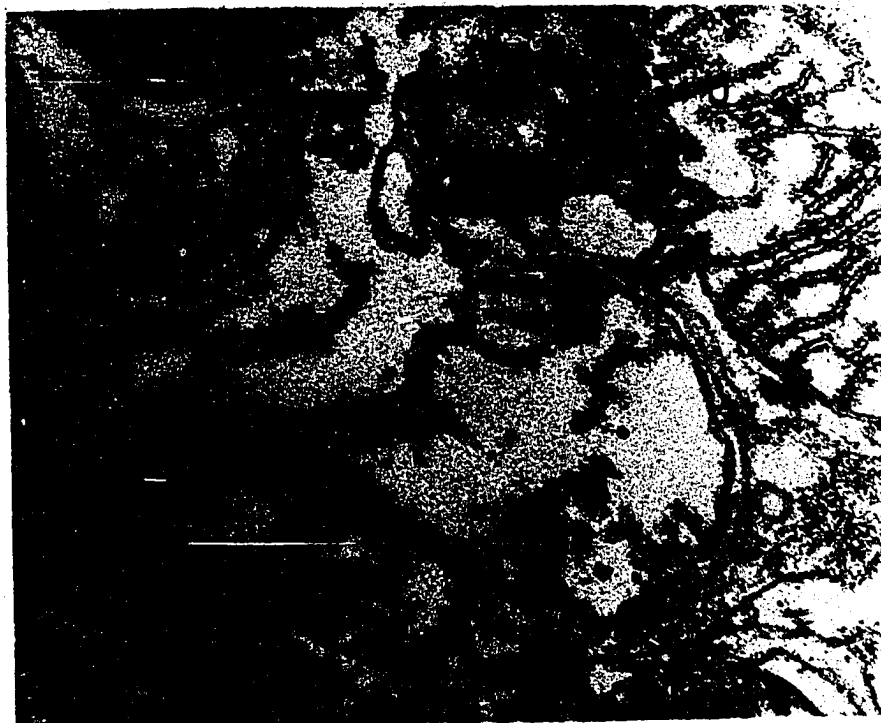


Fig. 60 Natural case. Heart. Amorphous, finely granular osmiophilic bodies surrounded by a double membrane, resembling the mitochondrial involution toward lysosomes x 60,000



Fig. 61 Natural case. Heart. Slight dilation of fenestrated collar (FC) close to osmiophilic body (OB) x 68,400

Fig. 62 Principal No. 6. Heart. Lysis of mitochondrial crystae. Rupture of mitochondrial membranes (arrow) and osmiophilic (O) finely granular material inside and outside of mitochondria x 70,000



changes.

Mitochondria were swollen and their cristae disrupted and lysed. Complete disruption was seen in some mitochondria. When both membranes, outer and inner, were ruptured glycogen granules were present in the mitochondrial matrix.

Osmiophilic electron dense bodies, similar to those found in myocardial cells, were found within and outside of mitochondria.

Distended vesicles of longitudinal and transverse (T-system) smooth endoplasmic reticulum (SER) were prominent in quadriceps muscle cells. Glycogen depletion from muscle cells was also noted. Strictures of several myofilaments were noted at the Z line (Fig. 65).

Kidney The lamina densa of the glomeruli and the basement membranes of proximal and convoluted tubules were irregularly thickened. Splitting and mottling of some basement membranes of convoluted tubules, were commonly found. Occasionally large vacuoles were found within cytoplasm of the proximal convoluted tubular epithelial cells.

Experimental cases

Heart Only minimal ultrastructural changes were present in group 1 principals. There was an irregular distribution of glycogen within the cytoplasm. Some large glycogen accumulations were present at one side of the cell, while at other sides the glycogen granules were absent.

Slight mitochondrial cristae fragmentation was noted.

In group 2 principals there was some cellular glycogen depletion. The mitochondrial cristae were fragmented and electron dense granular material was present within some altered mitochondria (Fig. 62). The nuclear membranes of some nuclei were irregularly folded imparting a shrunken appearance. The chromatin in these nuclei was condensed and distributed around the peripheries with occasional clumps in the center (Fig. 63). The myocardiums of group 3 principals had similar cellular glycogen depletion as that observed in principals of group 2. The mitochondria were markedly swollen. There were fragmentation and lysis of the cristae, rupture of outer membranes and condensation of finely granular osmiophilic material within and outside of mitochondria (Fig. 64). The intercellular spaces were distended by an extremely large amount of collagen fibers especially in principal No. 13 (Fig. 66).

Skeletal muscle Skeletal muscles of principals from group 1 had no ultrastructural lesions. Skeletal muscles from all principals of group 2 had several pathologic alterations. There was glycogen depletion. The mitochondria were swollen with loss and some fragmentation of cristae and clumping of membranes within cytoplasmic matrix. Some finely granular electron dense bodies were noted within and outside of mitochondria.

Fig. 63 Principal No. 6. Heart. Irregular folding of nuclear membrane and condensation and margination of chromatin x 30,000

Fig. 64 Principal No. 14. Heart. Osmiophilic bodies (OB) within and outside of mitochondria x 14,400

143-144

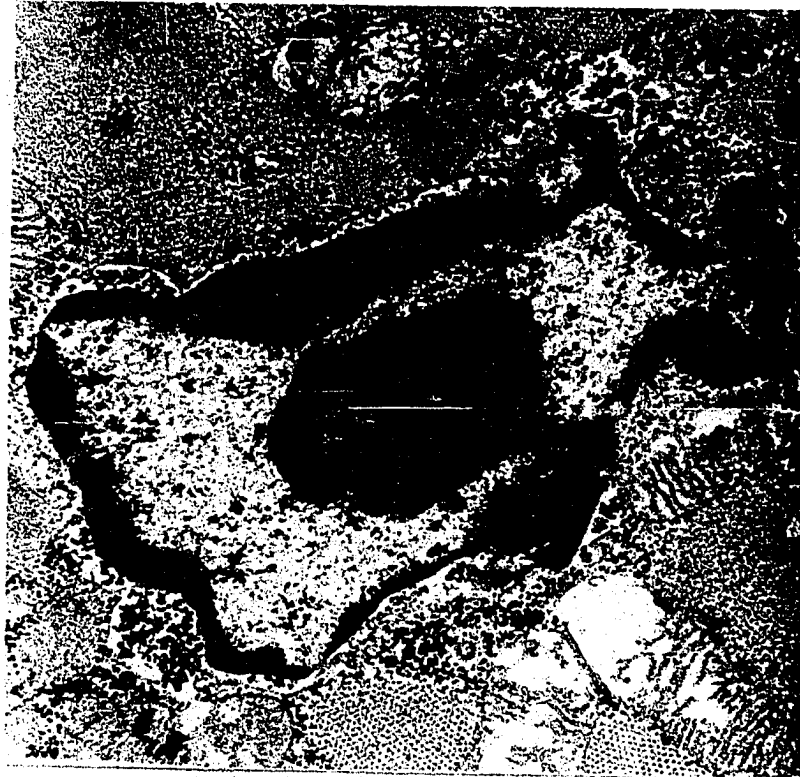


Fig. 65 Natural case. Diaphragm. Strictures (S) of myofibrils at the Z line. Glycogen (G) penetration into mitochondria x 36,000

Fig. 66 Principal No. 13. Heart. Collagen fibers (C) in distended intercellular spaces x 19,500



Large numbers of collagen fibers were found in the intercellular spaces.

Smooth endoplasmic reticulum (SER) was dilated and few coarse glycogen granules were noted around it.

The ultrastructural changes in skeletal muscles of principals of group 3 were more prominent. The mitochondria were swollen, with loss of cristae and outer membrane out-pocketings (Fig. 68). Some infoldings of the membranes were noted (Fig. 70). Many mitochondria were found completely disrupted with only a finely granular osmiophilic material persisting within poorly preserved membranes (Fig. 69). Some membranes were agglutinated and myelin figures appeared to have developed from them.

The glycogen was depleted.

Collagen fibers and a few lymphocytes were found within intercellular spaces.

Kidney Kidney sections from principals of group 1 did not have ultrastructural alterations. After 30 days, the lesions became apparent in principals of group 2 (Fig. 72). There was irregular thickening of the glomerular capillary basement membranes at points of apposition to the mesangial cells. The mesangial cells were increased in number. The mesangial matrix was abundant and separated the endothelium from the basement membrane. Osmiophilic condensed inclusions were present in the cytoplasm of mesangial cells (Fig. 73). The lamina densa also had some irregular thickenings with

Fig. 67 Principal No. 6. Vastus medialis. Distorted and swollen mitochondria. Fragmentation of crystae, glycogen penetration and osmiophilic body within x 48,600

Fig. 68 Principal No. 9. Vastus medialis. Outpocketing of outer membrane of mitochondria x 30,000

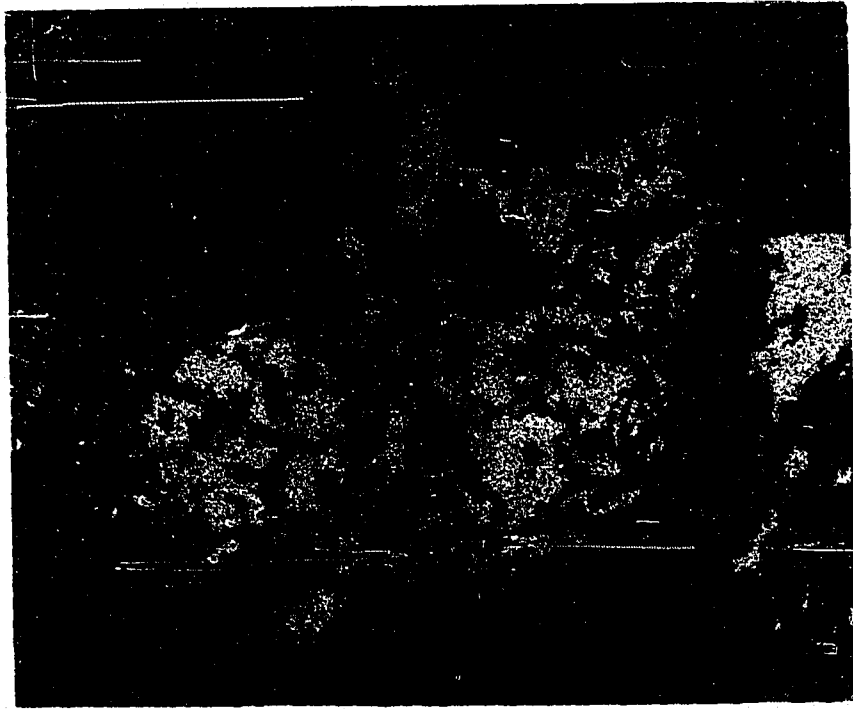


Fig. 69 Principal No. 13. Vastus medialis. Lysed
mitochondria with poorly preserved membranes
x 38,400

Fig. 70 Principal No. 10. Vastus medialis. Infolding
of mitochondrial membranes (arrow) x 28,800

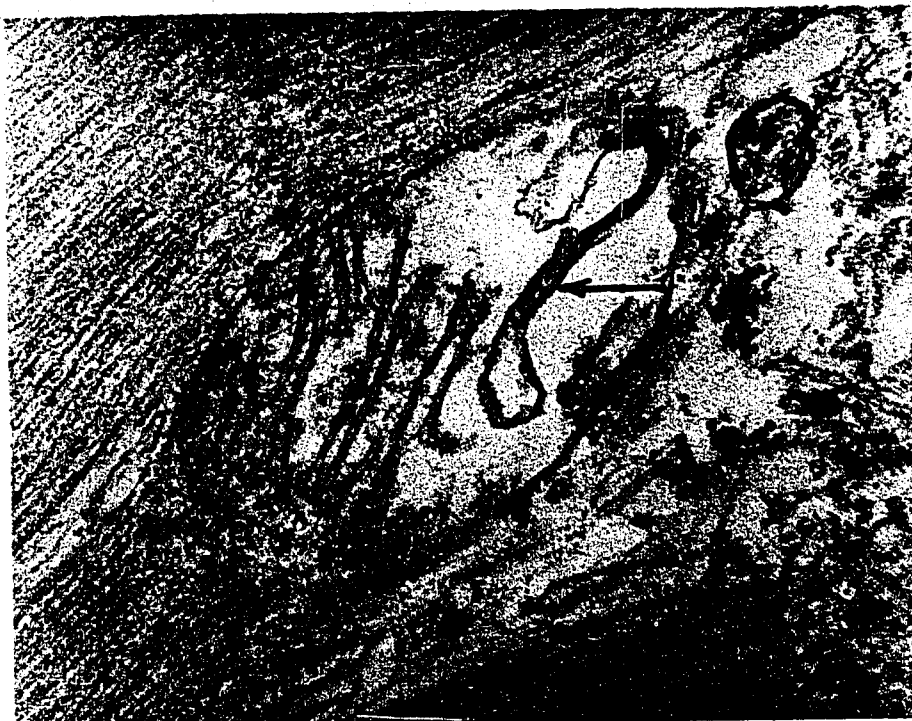
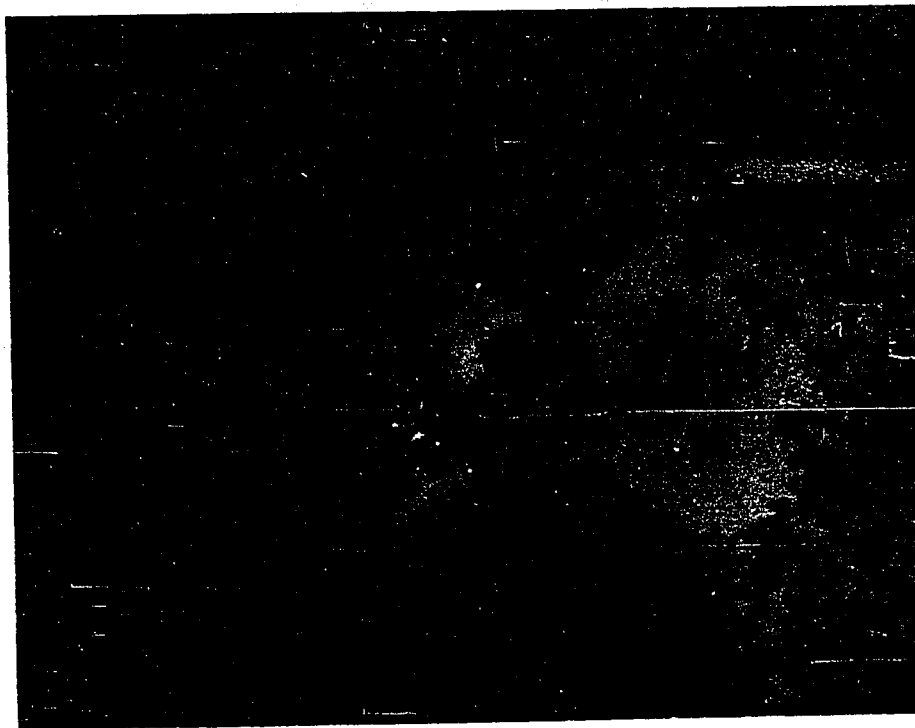


Fig. 71 Principal No. 10. Diaphragm. Disarrangement of myofilaments (arrows). Swollen (A) and lytic (B) mitochondria x 109,200

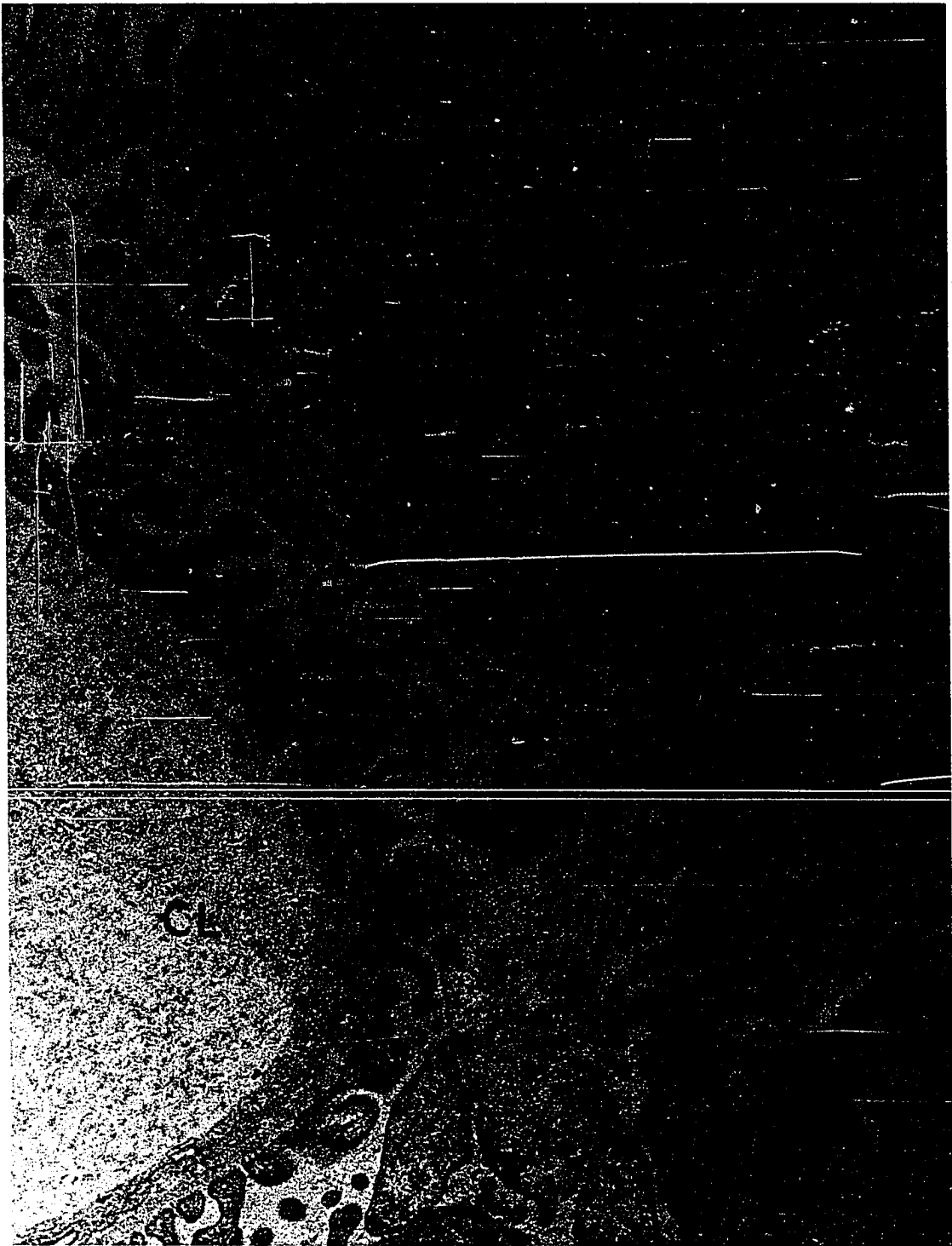


Fig. 72 Control. Glomerulus. Normal.

CL Capillary lumen
BS Bowman's space
E Epithelial cell
P Podocytes of epithelial cells
M Capillary basement membrane
arrows Fenestrated endothelium
x 30,000



Fig. 73 Principal No. 10. Glomerulus. Increased number of mesangial cells (M) with increase deposition of mesangial matrix (m) and cytoplasmic inclusions (I) x 52,900



some infoldings to the epithelial podocytes and mottling (Fig. 74).

The fenestrated endothelial cytoplasm that lined the entire capillary lumen, had villous proliferations (Fig. 74).

The basement membranes of proximal and distal convoluted tubules were very irregular in thickness, especially in proximal convoluted tubules. Splitting and mottling of these basement membranes were also noted (Fig. 75).

The cells from proximal convoluted tubules had notable focal cytoplasmic degradation characterized by the presence of large autophagic vacuoles (cytosegresomes) containing masses of granular material and entrapped organelles (Fig. 76). Numerous crystalloid structures with the same size and density as mitochondria, but with angular outlines were seen in proximal convoluted tubular cells of principals (No. 6 and 14) of group 2. The limiting membranes of these structures were more electron dense than mitochondrial membranes (Fig. 77 and 78). Some mitochondria were swollen and some had dense osmiophilic bodies within the matrix. Several cytosomes were also present in the cytoplasm of cells of both proximal and convoluted tubules.

A thick layer of homogeneous electron dense material was found on the surface of the proximal convoluted tubular cells. This appeared to have caused bending and atrophy of the microvilli of brush borders (Fig. 79).

Fig. 74 Principal No. 13. Glomerulus. Villous
proliferation of fenestrated endothelium (arrow).
Infolding of lamina densa to filtration space
(L) x 36,000

Fig. 75 Principal No. 18. Distal convoluted tubules.
Splitting of basement membrane (BM) x 20,000

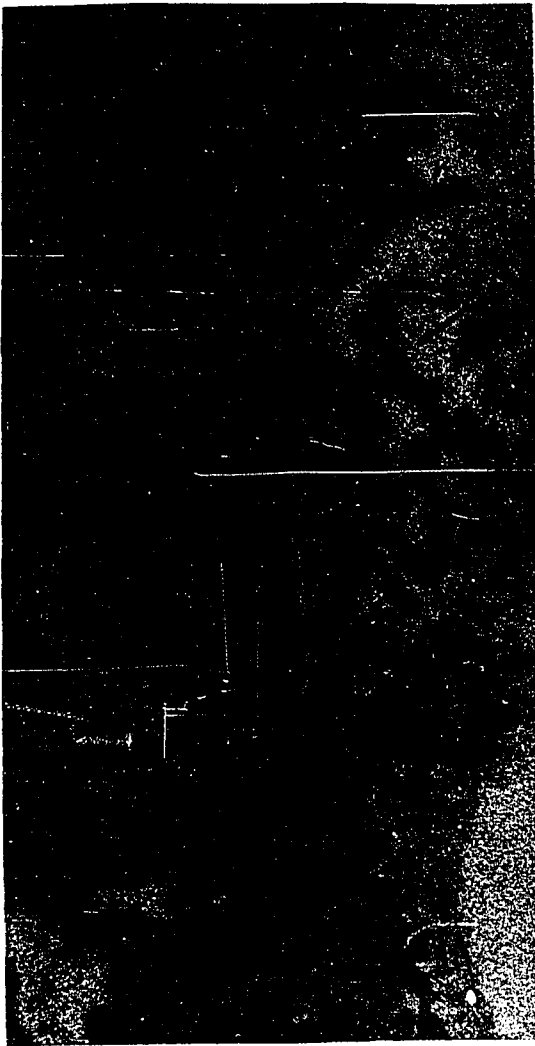


Fig. 76 Principal No. 10. Proximal convoluted tubule. Irregular thickness and mottling (arrow) of basement membrane. Focal degradation of cytoplasmic components with formation of cytosegresomes (C) x 52,900



Fig. 77 Principal No. 14. Proximal convoluted tubule.
Crystalloid structures in cytoplasm (arrows)
x 31,500

Fig. 78 Principal No. 6. Proximal convoluted tubule.
Crystalloid structure in cytoplasm (arrow)
Cytosome (C) x 27,800

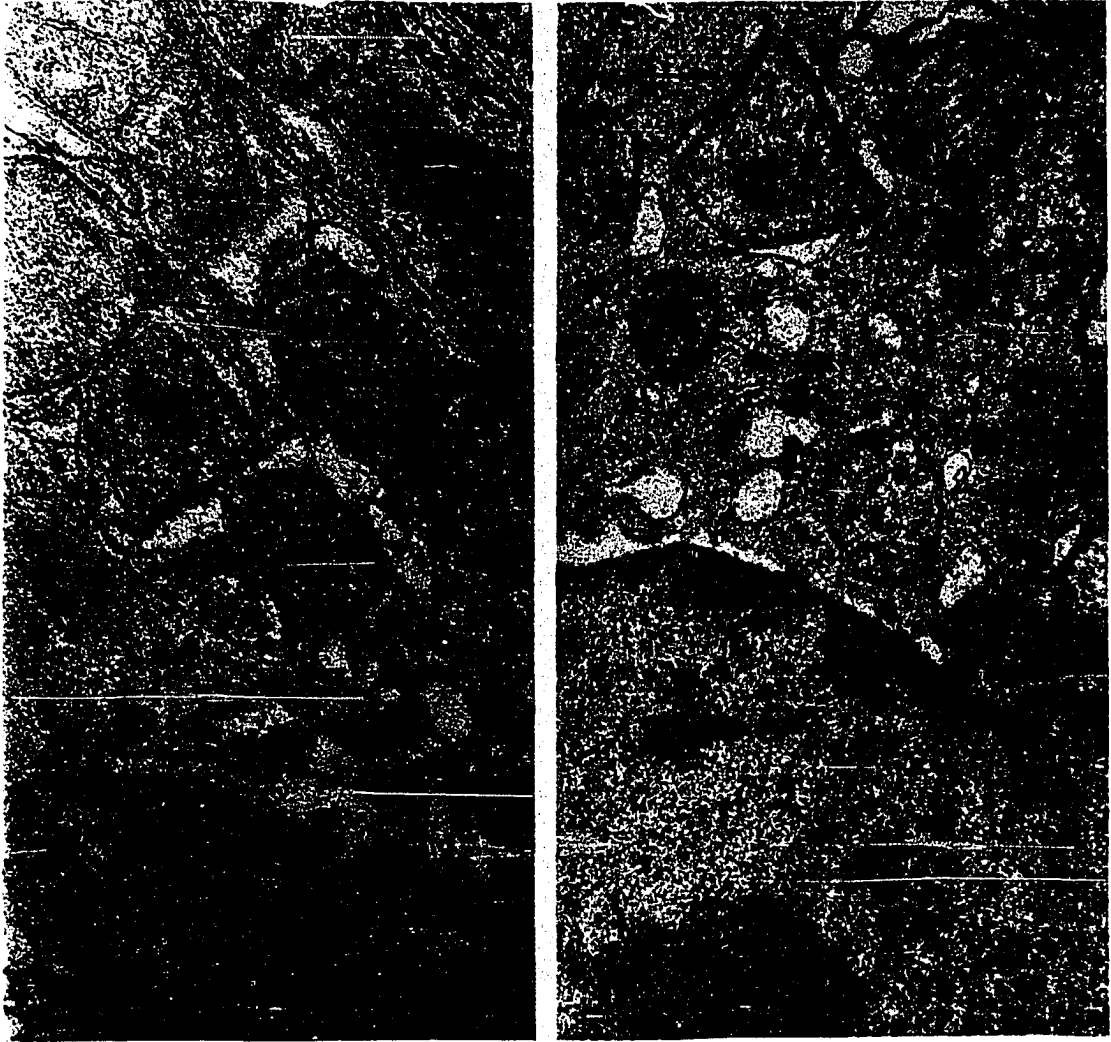


Fig. 79 Principal No. 13. Proximal convoluted tubule.
Finely granular electron dense material (E)
on luminal surface causing atrophy to
microvilli (M). Swollen mitochondria with
lysis of cristae (SM). Tubular lumen (TL)
x 16,800



Collagen fibers were present in large numbers in inter-cellular spaces of the proximal and distal convoluted tubules.

The ultrastructural changes found in the kidneys of principals after 60 days of the trial (group 3) resembled those described for field cases and principals of group 2, but at this time the thickness and splitting of basement membranes were more pronounced and the collagen fibers between the cells were more numerous. Crystalloid structures were also observed in principals (No. 10, 13, and 18). Furthermore, loss of mitochondria, disruption of infolded cytoplasmic membranes and agglutination of membrane structures were noted in necrotic proximal convoluted tubules.

Plant Chemistry

Qualitative chemical analysis of dried parts of anamú, made in the laboratory of toxicology of ICA, Colombia¹, revealed a strong positive reaction for alkaloids in roots and slightly positive in the stems, as determined by Mayer, Valser and Bouchardat methods for alkaloid determination.

The amino acid composition of anamú is shown in table 19.

Table 19 Amino acid composition of anamú

Lysine	.043 μ M./mg.	Proline	.076 μ M./mg.
Histidine	.018 μ M./mg.	Glycine	.093 μ M./mg.

¹G. Rivera, ICA, Bogotá, Colombia. Private communication, 1971.

Table 19 (continued)

Ammonia	.169 $\mu\text{M.}/\text{mg.}$	Alanine	.080 $\mu\text{M.}/\text{mg.}$
Arginine	.037 $\mu\text{M.}/\text{mg.}$	Half cystine	—
A Imino	.067 $\mu\text{M.}/\text{mg.}$	Valine	.057 $\mu\text{M.}/\text{mg.}$
B Imino	.009 $\mu\text{M.}/\text{mg.}$	Methionine	.014 $\mu\text{M.}/\text{mg.}$
Aspartic Acid	.081 $\mu\text{M.}/\text{mg.}$	Isoleucine	.039 $\mu\text{M.}/\text{mg.}$
Threonine	.047 $\mu\text{M.}/\text{mg.}$	Leucine	.076 $\mu\text{M.}/\text{mg.}$
Serine	.056 $\mu\text{M.}/\text{mg.}$	Tyrosine	.026 $\mu\text{M.}/\text{mg.}$
Glutamic Acid	.084 $\mu\text{M.}/\text{mg.}$	Phenylalanine	.037 $\mu\text{M.}/\text{mg.}$

In Fig. 80, 2 new undefined neutral amino acids were present between arginine and aspartic acid peaks. The test was repeated to evaluate these findings. The absorbance of these new amino acids was comparable to that of proline, therefore they were called A Imino and B Imino amino acids, and their quantitation determined as is shown in table 19.

However, in screening chemical analyses of anamú in the Department of Chemistry at Iowa State University alkaloids and saponins were not detected, but a new interesting compound was found. Studies to elucidate the chemical structure of this substance are still in progress in the above department. However, Wildman and Richardson (1972) suggest an approximate structure.

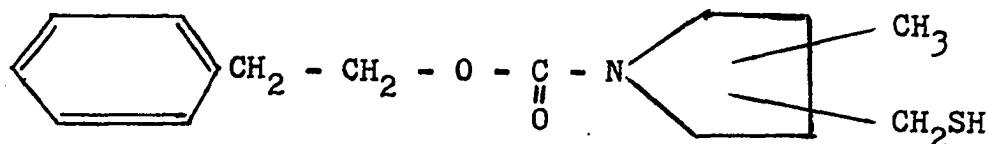
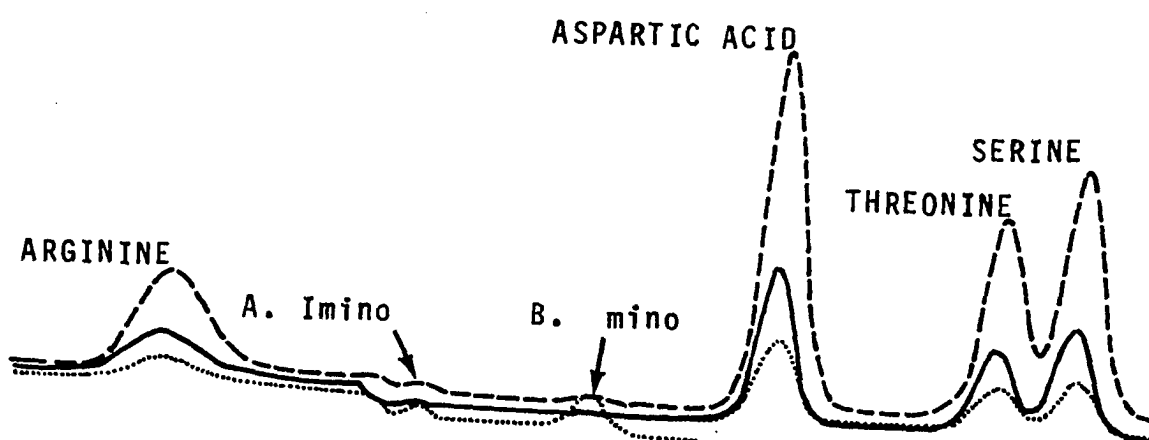
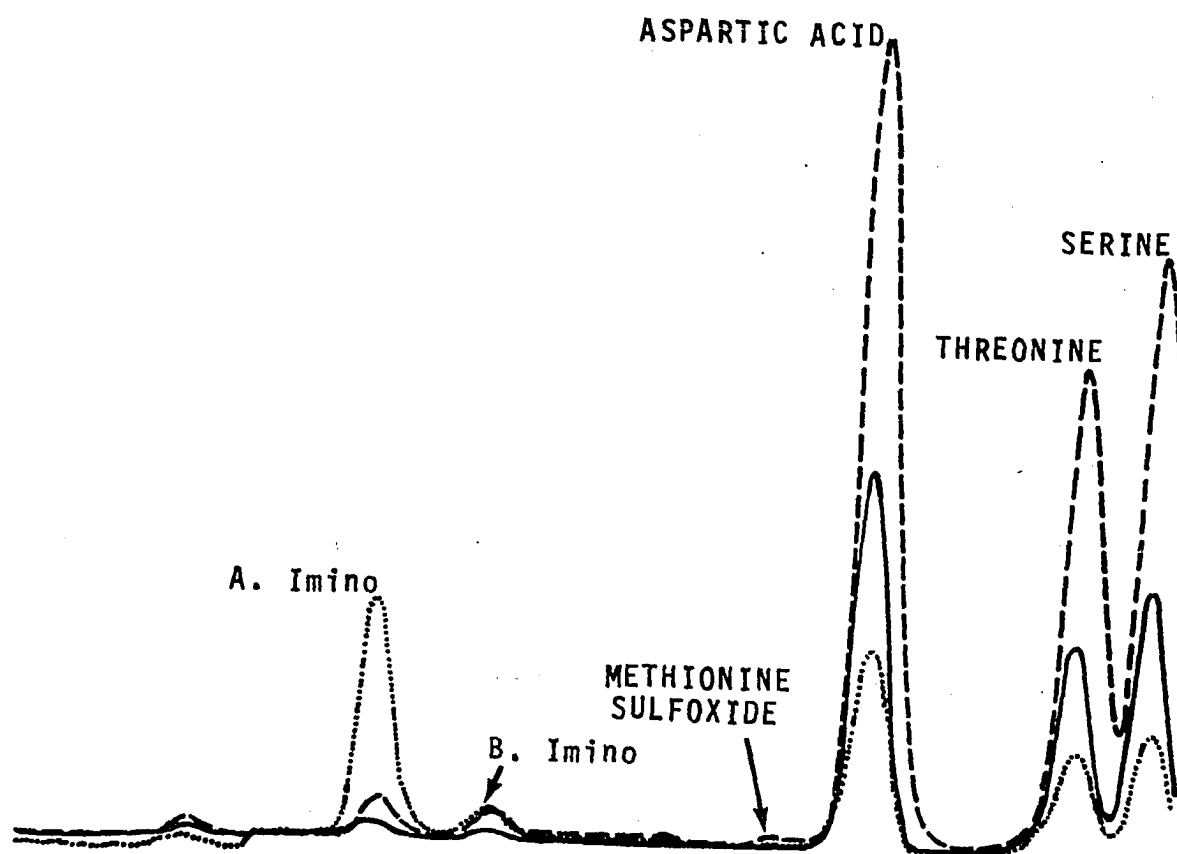


Fig. 80 Two new Imino amino acids (A and B) were recorded in the analysis of anamú



DISCUSSION

Mascagnia concinna (cansaviejo) and Petiveria alliacea (anamú) were initially incriminated as causative agents of a toxicity for cattle in the north coast area of Colombia, but results obtained from preliminary trials (Ruiz and Roberts, 1971) indicated that anamú was primarily responsible for the toxicity in calves. Furthermore, Pinzon¹ informed the author of a similar clinical disease syndrome in calves from Magdalena medio cattle region, where large amounts of anamú were identified but no cansaviejo was found.

Physiopathology of Digestion

Anamú toxicity caused some bloat and frequent eructation under experimental conditions. The normal reflex of eructation is stimulated when there is an increase of intraruminal pressure (Hill, 1966). The frequency of eructations is dependent on the increase of intraruminal pressure (Hill, 1966).

Chemical studies of anamú (Wildman and Richardson, 1972) demonstrated a volatile substance as one of the main chemical components of the plant. This substance actually could be released by the mechanical and physical processes occurring

¹F. Pinzon, ICA, Bogotá, Colombia. Private communication, 1971.

in rumination and in this way it could contribute to increased intraruminal pressure that stimulated the multiple reflex eructation in the principals of the trial.

The improper functioning of the eructation reflex results in bloat (Blood and Henderson, 1963). One of the determining factors in bloat is frothiness. The foaming properties of proteins, saponins and rumen liquor have been studied by Mangan (1959). He found that cytoplasmic proteins appeared to be the principal foaming agent in causing bloat in ruminants. Several other factors have been implicated to explain bloat in cattle (Mullenax et al., 1965). In anamu toxicity, frothiness is probably caused by soluble proteins contained in the plant, since saponins were not found in chemical analyses.

Anamu caused profuse lacrimation in principals, whenever eructation was present. The supposition was that the volatile substances reached the lacrimal gland via the nasolacrimal duct, or had direct irritant effect on conjunctival mucosae or reached the lacrimal gland via the blood stream. The last seems to be more acceptable, because the serous ocular efflux was seen even 3 hours after feeding the plant to the principals, and lacrimation was absent in the controls that were in the same isolation unit. These observations are supported by the work of Dougherty and Cook (1962) who studied the routes of eructated gas expulsion in cattle. They found that more than half of the eructated gases passed

into the trachea. The rumen gases that reached the lungs would be absorbed into the blood stream and this was completely demonstrated by Dougherty et al. (1964) by using C^{14} -labeled gases and taking continuous samples from the carotid artery, parotid salivary gland and milk. They found that C^{14} -labeled gas appeared quite rapidly in carotid artery and parotid salivary gland. In the milk of goats the gas was found in about 3 minutes after eructation. It is postulated that the volatile substance of anamú probably enters the blood stream via the pulmonary route although absorption into the blood stream through the intestinal wall can not be ruled out.

Other evidence supporting the blood stream circulation of the toxic substance is the off flavor and garlic odor similar to that of anamú in the milk of lactating cows (Blohm, 1962; Pérez, 1956; Standley, 1931). Dougherty et al. (1962), by mixing onion slurry with rumen ingesta and placing this mixture in an aerator to bubble air to the lungs of a cow, demonstrated that the pronounced off flavor of the milk was absorbed in the lungs and distributed through the blood stream. Cystic dilation of Brunner's glands was found in field cases (B, C, and D) and principals (No. 9 and 18) after 60 days of anamú feeding. This may result from an excessive secretion of the glands caused by the irritating effects of anamú compounds coming through the blood stream, similar to that in the lacrimal glands.

Renal Dysfunction

All principals had renal lesions related in extent and severity with the length of duration in the trial. The fact that the principals of group 1, in the first 8 days of the trial, had more significant tissue changes in the kidney (swelling of some glomerular tufts and tubular degeneration) than in other tissues, indicated that these organs were the first and important targets of anamú toxicity. During this time the blood urea nitrogen (BUN) was increased in the principals while that in the controls was almost unchanged (Table 12 and Fig. 17).

The lesions became more severe in group 2 principals and extensive glomerular damage with cellular hyperplasia and later fibrous scarring were noted along with tubular degeneration and necrosis.

Definite increase of BUN was noted by 15 days in the principals; this increase later (around the 24th day) returned to the normal level. This irregularity of BUN levels during this time indicated that the damage in the kidneys was not extensive enough to cause constant high levels of BUN and that the functions of the altered nephrons were being compensated by unaffected nephrons.

The finding of these particular kidney lesions in the principals of group 2, directed the interest of the author to take urine samples from all the animals of group 3 during

the last 30 days of the trial, in order to make a better evaluation of the renal pathologic syndrome.

The renal lesions were more pronounced in the principals of group 3 with fibrous scarring of renal corpuscles, more pronounced thickening of Bowman's capsules and tubular basement membranes, and more apparent tubular necrosis. The interstitial fibrosis was most obvious around the numerous affected nephrons. Clinical signs and laboratory findings of polyuria and low specific gravity urine observed in the principals of group 2, at about 30 days, were related to the extensive renal tubular damage.

The intermittent positive findings of glucose and protein in the urine of principals of group 3 accompany the nephron alterations noted in acute and chronic stages of nephritis. At the time of these clinical findings there were areas of complete occlusion of nephrons, fibrous scarring of glomeruli and extensive tubular damage contrasting with areas of swollen glomeruli, dilated tubules and less marked tubular degeneration and necrosis. The particular pattern of anamú toxicity lesions was associated with the physio-anatomical bases of kidney circulation, explained by Corcoran and Weller (1967), by which some deep glomeruli are plasma rich and some peripheral are plasma poor. Then, it is understandable that some glomeruli had more contact with anamú substances than others. Therefore, it was supposed that those plasma rich glomeruli were first and more severely affected than the

plasma poor glomeruli that later assumed the function of the affected ones. Continued ingestion of anamú would be followed by more altered nephrons.

Necrosis and sloughing of the renal tubular epithelium noted on histopathological examination supported the findings of cellular and hyaline casts in urinary sediment.

Loss of chloride anions was related with low urine pH in principals from group 3 (Fig. 19) during the last 30 days and suggested that an improper acid-base balance (leading to acidosis) was produced as a result of anamú toxicity. This acidosis could be related with the behavioral changes of temperament noted in the principals during the last 45 days of the trial.

Electron microscopic studies of the kidneys from the principals supported all the clinical and morphological changes previously discussed.

Thickening and splitting of the basement membranes of glomeruli and tubules were related with the intermittence of proteinuria. Squire et al. (1962) observed that the escape of large-sized molecules (alpha-globulin, beta-lipoprotein) was more obvious through damaged basement membranes. However, Churg (1968) pointed out that in progressive basement membrane damage, proteinuria may eventually decrease or disappear and even small molecules such as nitrogenous waste products, may be retained.

The increased cellularity of the glomeruli observed by light microscopy was delineated by electron microscopy as a hyperplasia of mesangial cells, with large increase of mesangial matrix (Fig. 73) that was composed of fibrils probably collagen, embedded in mucopolysaccharide-rich ground substance (Churg, 1968) and react positively with PAS stain (Trump and Bulger, 1968). In the present study, a large amount of PAS-positive material was observed within glomerular tufts. This material was identified as mesangial matrix and thickened basement membranes by electron microscopy. The submicroscopic alterations of the tubular epithelial cells were similar to the light microscopic findings of the kidneys from principals.

Degenerative changes were characterized by cytoplasmic edema (rarefaction of cytoplasm) which apparently started with small surface vesicles which later fused forming large vacuoles close to the basilar infoldings of the cell membrane. A similar alteration has been found in kaliopenic nephropathy in man (Biava et al., 1963). The significant mitochondrial swelling, primarily of proximal convoluted tubules, found in kidneys of principals of group 3, also is considered a degenerative change of tubular cells (Churg, 1968). The necrotic changes were identified by the presence of focal cytoplasmic sequestration (cytosegresomes) found in a large number of tubular cells of the principals. The osmiophilic

material on the surface of proximal convoluted tubules that was causing bending and atrophy of brush border was interpreted as a condensed abnormal substance, probably anamú compound. The effect of this material on the surface of the tubule, other than mechanical, could have interfered with normal reabsorption of electrolytes and water.

Myopathic Syndrome

The muscles are contractile organs specialized for the maintenance of posture and locomotion. A continuous state of partial contraction of the skeletal muscles in the conscious animal is known as muscle tone, and this tone is responsible for the normal posture (Breazile, 1970). Movement is initiated by interrelated and organized contraction of groups of skeletal muscles.

Incrimination of anamú in this myopathic syndrome, on clinical bases, could be questioned, since a muscular sign is common to many diseases. Therefore, it is necessary to explain some basic concepts used by the author to elucidate the involvement of anamú in causing clinical myopathy in cattle. Some muscular disorders in young humans and animals have been described as congenital in origin (Adams et al., 1967; Gardner-Medwin and Walton, 1969; Morton et al., 1963; Benthlem, 1970). However, no familial occurrence of muscle disease could be determined in the principals of the trial. The

4 animals with the natural disease were taken from a herd of 60 from which 35 calves were showing muscle weakness and hypotonicity. Also similar outbreaks were observed in 3 other herds located in 3 different departments, Cesar, Cundinamarca, and Sucre. Furthermore, the syndrome appeared simultaneously in many calves of different maternal origin, and the signs did not appear before 4 months of age. In this way a congenital origin of the myopathic syndrome was ruled out.

Systemic bacterial infections as causes of this syndrome were also discarded because the temperature was normal and no aerobic or anaerobic bacteria were cultured from the natural or experimental cases. The most significant observation, involving anamú as causative agent of this myopathy, was the constant finding of the plant on the farms where the outbreaks were noted and the fact that removal of the animals from the pastures with anamú was followed by apparent recovery.

Anamú toxicity caused weakness and hypotonicity of skeletal and cardiac muscles in naturally and experimentally diseased animals. All the signs observed in the field cases could be reproduced in experimental calves, except the particular "roaring" noise after expiratory respiration. This noise was assumed to be produced by an excessive flaccidity of the muscles of the larynx.

It is of particular interest that the active muscles of the myocardium, diaphragm, and intercostals were consistently

and severely affected by anamú toxicity. By the nature of the physiological activity of these muscles they would receive a greater exposure of the toxicologic factors of anamú than others in the body, since the blood supply to muscles in activity is larger than that in resting muscles as observed by Krogh (1918).

The poisonous or toxic factor(s) of anamú is cumulative as the first evidence of disease was not noted until about the 8th day. The clinical signs paralleled the development of the lesions and the lesions and clinical signs progressed in severity with increased duration of anamú feeding. The posture of the animal was disturbed and an interference in locomotion was most apparent in animals of groups 2 and 3. It was believed that these clinical signs were primarily reflections of the previously described lesions of muscles. However, the toxic factor(s) of anamú may have some effect on neural conduction especially at the motor end plate as it was noted that this structure had irregular and swollen knobs on terminal axons with a thickened synaptic plate (Fig. 50). The lesions of the motor end plates could be secondary to the atrophy of muscle fibers.

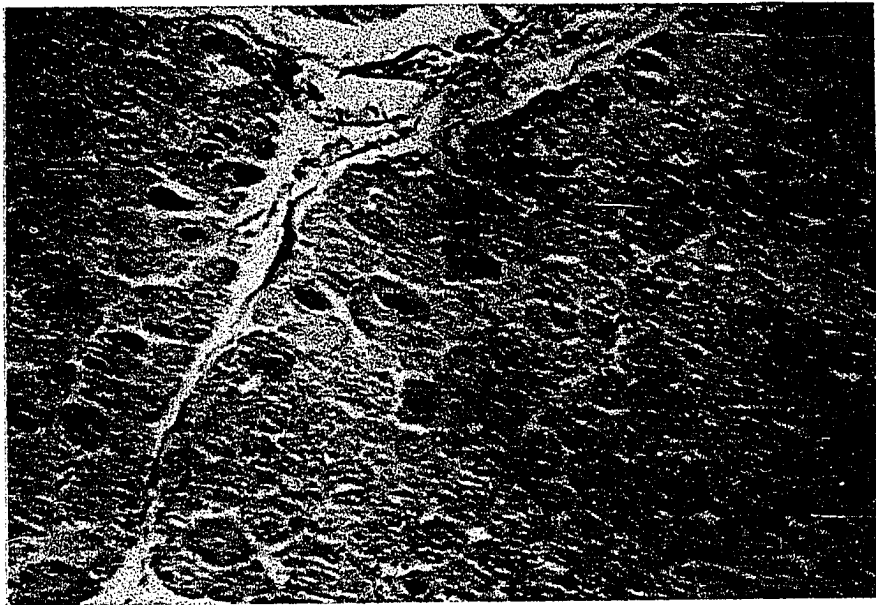
More definitive work should be done to properly assess the significance of degenerative changes of the motor end plates in this disease as a cause of improper functioning and/or atrophy of muscle fibers. Furthermore the ganaderos

noted that some animals that had recovered from anamú toxicity fatigued readily when they walked long distances going to market sales. The permanent impairment of the myocardium primarily and probably to a lesser extent the atrophy of skeletal muscle followed by replacement fibrosis probably are responsible for this fatigue.

An important economic aspect of extensive atrophy of all voluntary and involuntary striated muscles followed by replacement fibrosis (chronic myositis and myocarditis) is the decreased value of carcass quality. The extensive chronic nephritis in many kidneys would decrease their value as edible organs.

The particular central core necrosis of muscle fibers in diaphragms of field cases (A, B, and C), and principals (No. 3 of group 2 and 9, 10, and 18 of group 3) was comparable with the lesions of central core myopathy of humans (Shy and Magee, 1959). The central cores were stained intensely with PTAH and with Gomori's trichrome stain they stained bluish while the rest of the fibers stained red (Fig. 81) as was noted by Engel et al. (1961). However, some differences were found. In anamú toxicity, the central cores were interrupted and did not extend throughout the entire length of the muscle fibers as noted by Engel et al. (1961), Gonatas et al. (1965), and Shy and Magee (1959). In anamú toxicity, the fibers having central cores were scattered throughout the tissue, but not

Fig. 81 Natural case. Diaphragm. Central core necrosis
Gomori's trichrome stain x 160



in a generalized pattern as described by Engel et al. (1961) in central core disease.

Swelling of mitochondria and lysis of the crystae were common findings in both entities. Disarrangement of myofilaments and some irregularity of the Z lines within the sarcomere as described in central core disease by Mrozek et al. (1970) were found in anamú toxicity. No report of central core necrosis in the unpaired muscle of the diaphragm in domestic animals as recorded in this research was found in the literature.

Dilation of cardiac chambers with flabbiness of the myocardium noted in the principals of groups 2 and 3 contributed to the rapid fatigue during forced exercise. The extensive endomysial fibrosis would prevent the compensatory cardiac effort in reestablishing the normal cardiac output leading to a generalized hypoxia which has been referred as the main sign of "cattle fall" in Colombia. The marked atrophy of myofibers with the extensive endomysial fibrosis may be responsible for some of the cardiac arrhythmia noted in principals (No. 6, 10, 11, 13, 14, and 18) after 30 days, since the contractile ability of some myofibers would be completely abolished in some areas or simply decreased or even normal in other areas of the myocardium. The contraction of groups of fibers would be performed with large irregularity that could produce one or more extra sounds and under exercise the extra sounds could be confused within the result-

ant tachycardia which contrasted with spontaneous normal beating that was audible as bradycardia.

Ultrastructural studies of the muscles complemented the clinical, laboratory and light microscopy findings. Irregular size of myofilaments, depletion of cytoplasmic organelles, constrictures of the Z lines of many muscle fibers and deposition of large numbers of collagen fibers were ultrastructural changes related with the atrophy observed in field cases and principals. The most striking alterations were in glycogen content and mitochondria which are chemically related to the muscular energetics failure noted in weakness and hypotonicity in this myopathy.

The initial energy for the contraction of the myofibrils is dependent on an adenosine triphosphate (ATP). ATP in the muscle is obtained from anaerobic (Gergely, 1969) or aerobic breakdown (De Robertis et al., 1970) of glucose. The main fuel of these processes is glycogen that is broken down according to the energy requirement of the muscle (De Robertis et al., 1970; Gergely, 1969; Searcy, 1969). The end product lactic acid, in anaerobic glycolysis, may be carried by the blood stream to the liver where it could be partly oxidized and partly resynthesized to glycogen (Cori, 1941). The lactic acid also may accumulate in the muscle causing fatigue or may be transformed to pyruvic acid to enter the Krebs's cycle in mitochondria (De Robertis et al., 1970). In the body there are muscles with slow and steady activity in

which the aerobic pathway takes place and muscles where sudden demands of energy originate and the anaerobic pathway is more predominant (Gergely, 1969).

In experimental and natural anamu toxicity, the mitochondrial damage found was more extensive and severe in the muscles of the myocardium and diaphragm, they also had the most prominent lesions noted by light microscopy. These muscles are constantly active and their mitochondria content is larger because of the oxidative utilization of glucose. Ultrastructural studies of muscles in principals and field cases showed swelling, breakdown of cristae and outpocketing of mitochondrial membranes indicating severe damage of membrane permeability. Suppression of energy had to be expected because of influx of calcium ions through altered membranes, when calcium and other ions replace the electron transport system (Rosse and Lehninger, 1963) mitochondria no longer phosphorylate ADP to ATP. Relaxation should be the result of massive mitochondrial damage since oxidative phosphorylation occurring in mitochondria is the most important source of ATP for myofibril contraction. This explains not only a biochemical lesion in a relatively low number of myofibrils but also may explain the partial functioning of the myocardium and skeletal muscle that results in irregular contraction of myofibers, since they are receiving different gradients of energy.

The osmiophilic bodies found within and outside of altered mitochondria, also indicating mitochondrial damage, have been considered intermediate stages of mitochondrial involution and incorporation into lysosomes that started with lysis of the cristae. Pavel et al. (1971) described similar changes of liver mitochondria in viral hepatitis.

Muscular glycogen depletion correlated with serum glucose levels. It was more marked in animals with lower serum glucose levels such as principals No. 6 and 9 (Table 11). Furthermore, these principals also had enlarged islets of Langerhans and α -cells were reduced in number or absent (Fig. 53). The changes in the pancreas may explain the low glucose levels and the decrease of muscle glycogen, since they are interrelated. It appears that a toxic factor induced an accelerated glycogen breakdown which depleted muscular depots. Serum glucose, through insulin mediation, was incorporated into the cells to supply the energy demand. However the cells could not utilize the glucose completely because of mitochondrial damage. The way the toxic factor induced the glycogen breakdown in muscle tissues remains questionable.

The level of glutamic oxaloacetic transaminase in serum increased with muscle degeneration and necrosis. Although the determination of SGOT is not as specific test of muscular damage as creatine phosphokinase (Coodley, 1970), it was

used because of its availability. The increase of SGOT level (from 71.26 to 99.37) noted in group 1 at about 8 days indicated that the cellular disruption started at about that time. However, the SGOT level continued increasing and reached values twice the pre-trial ones in groups 2 and 3. Some principals in group 3 had SGOT values approximately 3 times the pre-trial ones. The controls, in contrast, had more constant values that oscillated between 83 and 69 International units as noted in Table 10 and Fig. 17.

Coodley (1970), Searcy (1969), and Wroblewski (1959), demonstrated that SGOT levels in humans were not specific for muscle disease because many tissues had transaminase activity however, elevations of SGOT levels have been found related to many muscular conditions: surgical trauma (Craver et al., 1957; D'Abbicco and Tullio, 1959; Nickell and Allbritten, 1957; Lemley-Stone, 1955), acute myositis, polymyositis, and dermatomyositis (Coodley, 1970), and neuromuscular disease (Murphy and Cherniak, 1958).

In veterinary medicine, SGOT values of more than 400 units have been found in white muscle disease of lambs and Swingle et al. (1959), Kuttler and Marble (1958) reported values as high as 2000-3000 units per ml. in lambs and 300 to 890 units per ml. in calves. These values were 3 to 20 times larger than control animals. Buck et al. (1961) found increased levels of SGOT in lambs fed carbon tetrachloride,

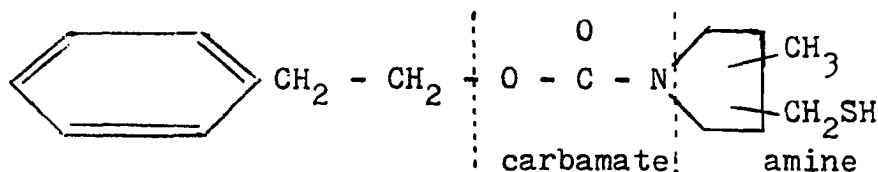
lead acetate, potassium arsenate, and plant poisonings: Psathyrotes annua, Helenium hoopesii, and a mix of Astragalus pubentissimus and A. thompsonae. Lambs receiving carbon tetrachloride had values higher than 2000 units, the lead acetate and potassium arsenate fed lambs had practically no elevations and the lambs fed the plants (Astragalus pubentissimus and A. thompsonae mix) had moderate to very high levels of SGOT (Psathyrotes annua). Dollahite and Henson (1965) found that SGOT values increased 10-18 times in goats poisoned with Karwinskia humboltiana (coyotillo) and a 33-fold increase in goats poisoned with Cassia occidentalis. These results were confirmed later by O'Hara et al. (1969) feeding calves with dried beans of Cassia occidentalis.

Comparing the lesions in the above syndromes with those of anamú, it is possible to deduce that the SGOT values are related with the type and extent of the muscular damage (Cornelius et al., 1959; Coodley, 1970; Kuttler and Marble, 1958; Swingle et al., 1959). In most of the above-mentioned conditions there is massive necrosis of the muscle bundles while in anamú toxicity the myolysis is limited to individual fibers of the muscle bundle. This finding is also related to the ability of the muscle to regenerate and explains the temporary relief of signs once the intoxicated animals are removed from the source of anamú.

Plant Chemistry

No specific toxic principles of anamú had been identified by chemical analysis until Wildman and Richardson (1972) isolated a volatile substance by steam distillation. The chemical structure of this substance, suggested by preliminary spectroscopic studies by Wildman and Richardson (1972) does provide an explanation of the toxicologic effects of anamú. The assumptions made here are speculative.

Essentially the structure resembles a carbamate and hydrolysis of it may give rise to the actively toxic fractions: carbamate and/or amine.



Carbamates have a parasympathomimetic action by inhibiting cholinesterase (Radeleff, 1970). This inhibition, according to Koelle (1970), is accomplished by being a competitive substrate of cholinesterase. Thus acetylcholine would be present in excess in the myoneural junction. A small excess of acetylcholine at the myoneural junction causes an abnormal increase in muscle contractions, whereas a large excess produces an abnormal decrease in muscular activity. In anamú toxicity weakness preceded by stiffness in some principals was the prominent muscular sign and the above pharmacological action could have played a role in

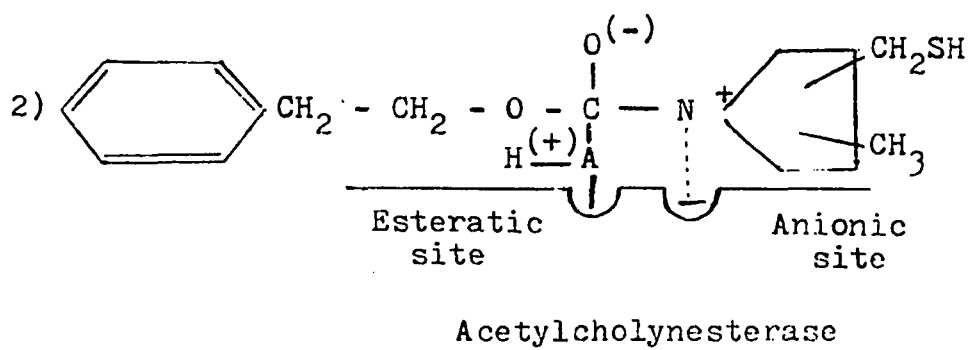
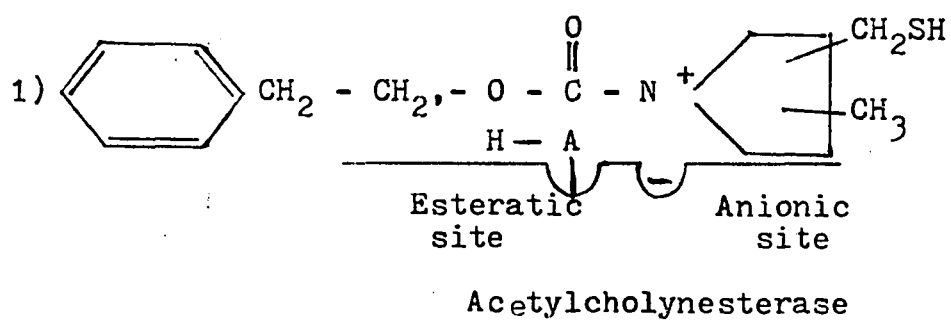
these signs. Furthermore the extensive mitochondrial damage noted in anamú toxicity has also been described as one of the muscular lesions of Carbaryl (1 - naphthyl methyl carbamate; Radeleff, 1970). This damage could be a determinant factor of the lack of muscular energy that leads to relaxation and weakness of the muscles.

The role of the amine nucleus in the toxic syndrome is not as well understood as that of the carbamate, unless the amine had a role in the interaction of the whole molecule with acetylcholinesterase. It is possible that the initial electrostatic attraction is accomplished by the anionic site of the enzyme and the N^+ atom of the amine nucleus. This first chemical interaction probably is followed by the electrophilic attraction between the protonated acidic group (-A-H) of the esteratic site of cholinesterase and the C atom of the carboxyl group of the anamú volatile substance (Fig. 82). Similar interaction has been explained to occur between amino-carbamy⁻ esters and acetylcholinesterase (Koelle, 1970).

The positive alkaloid reaction found by Rivera (1971) possibly was caused by the amine fraction of the volatile substance of anamú.

However the multiplicity of unrelated signs and lesions found in anamú toxicity suggests the presence of additional toxic principals in the plant.

Fig. 82 Suggested chemical interaction between the anamú
volatile substance and acctylcholinesterase at
the myoneural junction
1. pre-interaction stage
2. interaction stage



The Effects of Anamú on Laboratory Personnel

Anamú also had some harmful effects on human beings during handling or processing. Every time that the plant was cut or chopped, it emitted a strong garlic odor that, upon inhalation by the workers, caused irritation of nasal and ocular mucosae, leading to a serous nasal discharge and lacrimation, respectively. These signs remained for about 1 hour, even after breathing fresh air.

Many of the workers developed a headache some 15 minutes after inhalation and it persisted for about 3 hours. This headache was localized in the frontal-paranasal region.

Two of the people, after many contacts with anamú, developed an urticariform dermatitis on hands and arms which disappeared approximately 10 minutes after washing them with soap and water.

SUMMARY AND CONCLUSIONS

Evaluation of the toxic effects of anamú, Petiveria alliacea, a common weed of the cattle raising areas of Colombia, was made by clinical, laboratory and pathologic studies in natural and experimental diseased calves.

Eighteen experimental calves were separated into 3 groups. Two controls and 4 principals were selected randomly in each group. Each principal received 3 gm./kg./day of anamú components. Clinical observations were made daily and blood was sampled twice a week for hematologic, BUN, SGOT and serum glucose determinations. Urinalyses were made from the animals during the last 30 days of the trial.

Calves from each group were electrocuted and a thorough necropsy performed at 8, 30, and 60 days post-anamú feeding time. Approximately 30 tissue specimens were saved from each animal and pathologic studies were made by histochemical methods, light, and electron microscopy.

Physiopathology of Digestion

Shortly after eating the animals had mild bloat and frequent eructation of garlic smelling gases, followed by lacrimal secretion. Intermittent diarrhea was noted throughout the trial. The only lesion in the digestive tract was cystic dilation of Brunner's glands noted in principals of group 3 (60 days). The toxic principals of anamú passed

into the blood through intestinal absorption and/or lung capillary absorption, since volatile compounds of anamú reach the lungs with the eructated ruminal gases. The circulating toxic principles stimulated glandular secretion that was related with lacrimation and the cystic dilation of Brunner's glands.

Renal Dysfunction

The renal disease was first clinically evidenced at about 15 days by frequent micturition of a small amount of urine having the strong garlic odor of anamú. At 30-35 days the principals had polyuria and urinalysis demonstrated: low specific gravity (1.002 to 1.010), acid pH (5.0 to 7.0), intermittent positive findings of protein and glucose, and a progressive increase of chloride concentration. Large numbers of leukocytes, epithelial cells, and granular and hyaline casts were noted in urinary sediment. At necropsy, the kidneys of all principals had yellowish waxy discoloration. The kidneys of principals of groups 2 and 3 were also enlarged and had the peculiar garlic odor of anamú. The glomerular swelling and hypercellularity noted in group 1 principals was progressively developing toward a typical glomerulonephritis in group 2 and 3 principals. Lesions were characterized by thickening of Bowman's capsules and tubular basement membranes, fibrous scarring of renal corpuscles, tubular degeneration and necrosis, and interstitial fibrosis

around the most altered nephrons. Other nephrons had less severe changes.

The ultrastructural studies confirmed the glomerulonephritis with increased numbers of mesangial cells, large depositions of mesangial matrix, irregular thickenings, splitting, and protuding formations of basement membranes of glomerular capillaries, and epithelial tubular cells. The toxic principles possibly concentrated in tubular lumens since an abnormal osmiophilic, finely granular material was found on the epithelial surface causing atrophy of microvilli and impaired tubular reabsorption. Many tubular epithelial cells had focal sequestrations of the cytoplasmic organelles into cytosegresomes and the proximal convoluted tubular cells had crystalloid structures in the cytoplasm of the same size and electron density as mitochondria.

The renal lesions, the low pH, and the excretion of large amounts of chloride in the urine were suggestive of a general electrolyte imbalance and acidosis that was causing some behavioral changes and probably an increased pressure of the cerebrospinal fluid. At necropsy the principals appeared to have increased amounts of cerebrospinal fluid.

The Myopathic Syndrome

The first signs of muscular weakness appeared at about 8-12 days and were characterized by flexion of tarsal joints, abnormal lateral rotation of tarsal joints when walking,

stiffness in some animals, and incoordination in walking. With time the symptoms progressed and the animals fatigued quickly and usually spread their hind legs to assist in their support. Slight pressure on their back caused them to flex their joints. The muscles were flabby and atrophic after 30 days. Forced exercise caused the animals to fall down in about 30 seconds to 1 minute while the controls ran for 4 to 5 minutes. The lesions were grossly characterized by yellowish waxy discoloration and hypotonicity of muscles such as myocardium, diaphragm, intercostals, cricoarytenoideus dorsalis and vastus medialis.

Histologically there was only granular degeneration, hyalinization, and myolysis of segments of muscle fibers in group 1 principals (8 days). A central core necrosis was found in diaphragms of 3 natural and 4 experimental cases. This central core necrosis resembled the central core disease of humans, considered to be a congenital myopathy.

The limited muscular necrosis was related to a slight increase of SGOT levels (71 International units pre-trial, 215 International units post-trial).

The lesions in group 3 principals were more related to atrophy than necrosis.

The electron microscopic studies were compatible with the muscular weakness. Massive mitochondrial swelling with lysis of cristae and involution toward lysosomes indicated the impairment of energy throughout the muscle. Furthermore,

glycogen depletion was noted. The depletion of muscular glycogen correlated a slight decrease of serum glucose which was probably caused by excess insulin secretion from hyperplastic islets of Langerhans. Deficient numbers or complete absence of α -cells in the islets was noted in 2 natural and 4 experimental cases. This subject should receive more emphasis in the future to elucidate the specific action of anamú compounds in the endocrine pancreas. Chemical analysis of the plant showed the approximate structure of a volatile substance that resembled a carbamate containing an amine nucleus at one end and a phenethyl at the other. If this chemical structure is completely elucidated, the clinical signs and lesions can probably be explained on the basis of its pharmacologic action.

This study definitely demonstrated the capability of anamú to produce a disease in cattle that was unsuspected for many generations because of its silent development. It also demonstrated the great economic importance that anamú is playing in Central and South America where this weed is grazed. The toxicologic syndrome produced by anamú will affect the carcass quality of cattle by the development of extensive muscular atrophy and replacement fibrosis. Furthermore, anamú can be considered one of the main causes of the "cattle fall" syndrome of the north coast cattle area of Colombia.

This investigation provided a new model to study such human diseases as central core disease of muscles, glomerulonephritis and hormonal disorders related with hyperplastic islets of Langerhans. The chemical studies of anamú gave new chemical compounds that, when used properly, can be a valuable tool for therapeutic medicine and research.

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