AMINOSTILBAMIDINE—A FUNGISTATIC AGENT

In Vitro Sensitivity Studies, Tissue Fluorescence—Distribution, and Therapeutic Trials in Coccidioidomycosis*

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Recent demonstration of the therapeutic efficacy of stilbamidine and 2-hydroxystilbamidine in blastomycosis (1–9) has stimulated investigation of other diamidine derivatives as fungistatic agents. Hydroxystilbamidine has been shown to have some suppressive influence on the course of coccidioidomycosis (10–12). Substitution of an amino for the hydroxy group results in a highly soluble orange crystalline compound, designated as aminostilbamidine. This newest stilbamidine derivative effectively inhibits the *in vitro* growth of *C. immitis*. The present study was directed toward establishing the *in vitro* spectrum of sensitivity of the important "systemic" fungi, evaluation of therapeutic efficacy in five patients with coccidioidomycosis, and observations of the human tissue concentration and distribution of aminostilbamidine.

In Vitro Sensitivity Studies

Inhibition of *Blastomyces dermatitidis* and *Coccidioides immitis* occurred at concentrations of 25 mcg/ml of aminostilbamidine (Table I), a level similar to that required for complete inhibition of blastomyces by other stilbamidine compounds (13). No effective inhibition of cryptococcus, nocardia or candida was observed.

Toxicity Studies (14)

Aminostilbamidine injected intravenously into mice has approximately the same acute toxicity as hydroxystilbamidine. Its chronic toxicity in guinea pigs is also roughly similar to that of hydroxystilbamidine. Minimal fatty liver degeneration occurred at the dosage level of 10 mg/kg.

Therapeutic Trials

Case 1. (Previously reported (12).) A 28 year old Air Force sergeant, previously stationed in Texas and Wyoming, was found on routine examination in December, 1951 to have a solitary pulmonary nodule in the right upper lobe. A wedge resection was performed in January, 1952 and a histologic diagnosis of coccidioidal granuloma was made. Postoperative empyema required repeated drainage and excision of sinus tracts containing *Coccidioides immitis*. Meningitis developed in July, 1952 with symptoms of headache, photophobia, findings of nuchal rigidity, papilledema, and fever. Cerebrospinal fluid showed increased pressure (160 mm.), 2000 leukocytes, protein 147 mg., sugar 52 mg., chloride 690

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Received for publication July 13, 1956.

TABLE I

Comparison of in vitro sensitivity to Aminostilbamidine of C. immitis with that of other fungi

Organism	No. of Strains Studied	No. of Strains Inhibited	Drug Concentration mcg./ml.
Blastomyces dermatitidis*	12	1	1
		9	5
		2	10
Nocardia asteroides	3	0	100
Cryptococcus neoformans	4	1	250
		3	500
Candida albicans	2	0	250
Coccidioides immitis†	2	2	25

* Yeast phase.

† Tissue phase. Spherule-rich pus, aspirated from subcutaneous abscesses, was used as inoculum.

mg./100 cc., and spherules of *C. immitis*. Right empyema persisted, and clubbing and splenomegaly were noted. Serum globulin was 4.23 gm./100 cc. and ESR was 26 mm./hr.

Considerable clinical improvement occurred in the months following treatment with hydroxystilbamidine, in total dosage of 23 gm. However, in June, 1954, while asymptomatic and working as a truck driver, evidence of residual low grade infection persisted. Spinal fluid contained 160 cells/cu. mm., glucose was lowered to 36 mg./100 cc., and protein was elevated to 200 mg./100 cc., although spinal fluid now remained sterile. Serum and spinal fluid complement fixation titers remained unchanged (1:64 and 1:16 respectively).

From June 23 to July 9 a course of treatment with aminostilbamidine was given. Daily injections of 150 mg were given in 200 cc. of 5 per cent dextrose in distilled water to a total of 1850 mg. There were no side effects and the patient was discharged asymptomatic.

For one and a half years he remained well, daily driving a milk truck a distance of 120 miles. He was asymptomatic until November, 1955 when severe headache, slight diplopia and vomiting developed. His general condition was excellent. Findings included nuchal rigidity and bilateral papilledema. Examination of spinal fluid revealed pressure of 305 mm, 460 cells/cu. mm., glucose 18 mg. and protein 187 mg./100 cc. All cultures were negative. After two spinal taps the meningeal signs disappeared and aminostilbamidine therapy was resumed. The drug was discontinued, after a total dosage of 5.25 gm. had been given, because of the appearance of tremor of the extremities. Roentgenogram of the chest was normal. The number of cells of the spinal fluid decreased to about 200/cu. mm. The decreased glucose and increased albumin content remained stationary. Titer of serum complement fixation test was unchanged (1:64). Hepatic and renal function remained normal.

Although the papilledema had receded and neurologic examination was negative, the headaches recurred frequently. The headache was always relieved after a spinal tap but within a week the pain recurred to such a degree that repeat spinal tap was necessary. After a few weeks urination became difficult and a cord bladder with complete loss of control of bladder function developed. It was, therefore, decided to try to decrease the pressure of the spinal fluid by making a communication between the spinal canal and the marrow of the fifth lumbar vertebra.

After this operation had been performed on February 28, 1956, the bladder control returned. Rather severe pains in the left thigh appeared, possibly connected with pressure of the catheter introduced in the spinal canal on one of the lumbar roots.

Case No. 2 (Previously reported (12).) A 22 year old Negro farm laborer became ill in August, 1951, while working as a farm laborer in the San Joaquin valley, with complaints of fever, cough, weight loss, chest pain, purulent sputum, and weakness. During the en-

suing months, recurrent tender subcutaneous masses were incised or drained spontaneously, forming persistent draining sinuses on the chest wall, forearms, and in the presternal area. In April, 1952, roentgenograms showed right upper lobe pneumonitis, and periostitis and destruction of right fifth and tenth ribs. *Coccidioides immitis* was recovered both from sputum and from drainage of subcutaneous sinuses. Serum complement fixation and precipitin tests were positive (1:32 and 1:40 respectively).

In October 1952 a course of stilbamidine therapy totalling 1.65 gm. was given, with some symptomatic improvement. In March, 1953 the patient was admitted to Cook County Hospital with persistent complaints. He was emaciated and febrile, with multiple chronic draining skin ulcerations and sinuses, right supraclavicular abscess, and localized rib tenderness. Blood globulin level ranged from 4.0 to 5.5 gm./100 ml. Roentgenograms again showed pneumonitis and rib destruction. Sputum and sinus drainage contained *C. immitis*. Coccidioidin skin test was positive (1:100). Hepatic and renal function were normal.

Treatment with 2-hydroxystilbamidine totalling 20 gm. from April 1, 1953 to July 24, 1953 resulted in diminution in size and in drainage from skin ulcers and sinuses, but general condition remained unchanged. Subsequently the patient developed a purulent arthritis involving both ankles. A six week course of isonicotinic acid hydrazide (300 mg./day) effected no change. Recurrent crops of new abscesses appeared on the chest wall, upper extremities and femoral areas. In January, 1954 a left psoas abscess appeared, manifested by flank pain, psoas spasm, and an enlarging flank mass.

Treatment with aminostilbamidine was instituted January 9, 1953; the drug was given in dosage of 150 mg./day, dissolved in 200 cc. of 5 per cent dextrose in distilled water and administered by slow direct intravenous injection. The injections were usually accompanied by mild generalized pruritis which was controlled by reducing the rate of injection. After a total of 3.35 gm. the daily dose was increased to 250 mg.; at a total dose of 4.25 gm. nausea appeared and persisted; at 6.6 gm. (2/18/54) therapy was temporarily discontinued because of persistent vomiting and weight loss. Treatment with 150 mg./day was resumed on 3/6/54. Yellow discoloration of the skin was noted after a total dose of 7 gm. had been given. Hepatic and renal function remained normal. The left flank abscess gradually subsided. However, low grade fever ranging to 100° persisted and the patient's general condition progressively deteriorated. Aminostilbamidine administration was discontinued 4/3/54 after a total dose of 10 gm. had been given. Serum complement fixation tests showed a progressive rise in titer to 1:512. Sacral decubitus ulcers and oral moniliasis developed. On 4/9/54 the patient expired after a total duration of illness of three years.

At necropsy extensive osteomyelitis of the lumbar spine was found which had led to bilateral psoas abscesses from which *C. immitis* was easily cultured. Coccidioidal granulomas were found histologically in the spleen, lungs, lymph nodes, and bone. In addition, pure cultures of *Candida albicans* were obtained from non-caseating granulomas of lung and lymph node. Apparently a terminal moniliasis complicated the progressive coccidioidomycosis.

Comment

The disseminated coccodioidal infection pursued a relentless, progressive course unaffected by all therapy including aminostilbamidine. The patient's general condition was grave prior to therapy and the drug was poorly tolerated.

*Case No. 3.** A 26 year old white male welder became ill following a visit to El Paso, Texas in January 1953 with onset of complaints in March 1953 of chest pain, cough, expectoration of purulent and blood streaked sputum, weakness, headache, vomiting and fever. Initial examination revealed nuchal rigidity, increased cerebrospinal fluid pressure, pleo-

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* Clinical and pathologic observations of this patient were permitted through the cooperation of Dr. Craig Borden, Chief of Medicine, and Dr. Henry Telo, Pathologist, Veterans Administration Research Hospital, Chicago, Ill.

cytosis (1100 cells), increased protein (125 mg./100 ml.) and decreased sugar (12.5 mg./100 ml.). Chest roentgenogram showed thin-walled multilocular cavities in the right upper lobe. Coccidioidin skin test was negative. Culture of spinal fluid was sterile but *C. immitis* was grown from sputum.

On transfer to the V.A. Research Hospital (Chicago, Ill.) the patient's only complaints were headache, nausea, and occasional vomiting. Pertinent physical signs included slight nuchal rigidity, positive Brudzinski sign, bilateral papilledema, and palpable spleen tip. Sedimentation rate was 16 mm./hr.; hemogram and urinalysis were normal. Serum albumin was 4.0 gm., globulin 4.0 gm. per 100 ml. Cephalin and thymol flocculation tests were normal. Phenosulfonphthalein excretion was 48 per cent in 15 minutes. Examination of spinal fluid revealed pressure of 280 mm. of water, 428 cells (95 per cent lymphocytes), protein 240 mg., sugar 38 mg./100 ml. and chloride 100 mEq/L, but no organisms were seen or grown. Sputum culture again showed *C. immitis* and coccidioidin skin test remained negative. Serologic tests (performed by Dr. Charles E. Smith) showed (on 6/29/54) blood complement fixation titer of 1:32, and in spinal fluid complement fixation 1:256, precipitin 1:40, confirming disseminated coccidioidal infection with meningitis.

Treatment with aminostilbamidine was instituted on 7/2/54. The drug was diluted in 200 cc of normal saline and given by rapid intravenous drip. Initially 150 mg. was given daily for three days, then 200 mg./day was given to a total dosage of 10 grams, completed 9/21/54. The drug was well tolerated without side effects.

Two to three weeks after institution of therapy the patient became completely asymptomatic. A beginning yellowish discoloration of the skin was noted. The results of hepatic and renal function tests remained normal. Serum globulin fell to 2.5 gm./100 ml. Low grade fever ranging to 100° persisted during July and August; during September and October temperature remained normal. Serial examinations of spinal fluid showed return of pressure to normal, some decrease of pleocytosis (to 116 cells), rise of sugar (to 60 mg./100 ml.), but progressive rise of protein (280 mg., 840 mg., 1800 mg./100 ml.). Serum complement fixation titers persisted unchanged.

However, the patient remained free of symptoms only for several weeks. He then began to complain of midscapular pain and numbness and tingling of the lower trunk and both lower extremities. In September, 1954 weakness of both legs appeared and rapidly progressed. He was readmitted with paresis and hyper-reflexia of both lower extremities, bilateral Babinski signs and tenderness over the third dorsal spine. Papilledema had persisted. A sensory level was found at the fourth dorsal segment. Lumbar puncture (10/13/54) showed xanthochromic fluid, protein 1800 mg., sugar 29 mg./100 ml., and 86 cells. Spine films were normal. Myelogram showed a block at the level of D-10. Thoracic laminectomy on 10/14 revealed an extensive thickened arachnoid granulation tissue surrounding the spinal cord; biopsy revealed *C. immitis* in the granulation tissue. The patient expired on 10/16/54.

Necropsy diagnoses included: (1) Chronic and acute purulent meningitis due to *C. immitis*; bronchopneumonia, right upper and lower lobes.

Comment

Coincident with administration of aminostilbamidine the patient became free of symptoms, temperature became normal, serum globulin returned to normal, and an apparent improvement in the spinal fluid findings was manifested by decrease of pressure and cell count and rise of glucose level. However, as the meningitis subsided, a localized granuloma caused partial cord transection and typical cerebrospinal fluid findings of spinal block. Although laminectomy was imperative, it undoubtedly contributed to renewed generalization of meningitis. Although it is possible that the meningitis was suppressed by aminostilbamidine treatment, sufficient activity remained for the formation of localized granu lation tissue at the site of the block.

Case No. 4: A 21 year old white male became ill in August, 1950 with the first of several recurrent episodes of bilateral spontaneous pneumothorax. Hospitalization from August,

1951 to January, 1952 failed to define the etiology of a right upper lobe cavity measuring 1.5 x 1.0 cm. Following pneumoperitoneum in May, 1952 the cavity decreased in size and surrounding reaction lessened. Bronchial and gastric cultures were negative for acid fast bacilli. Skin tests showed Mantoux to be positive (PPD #1) and histoplasmin and cocci-

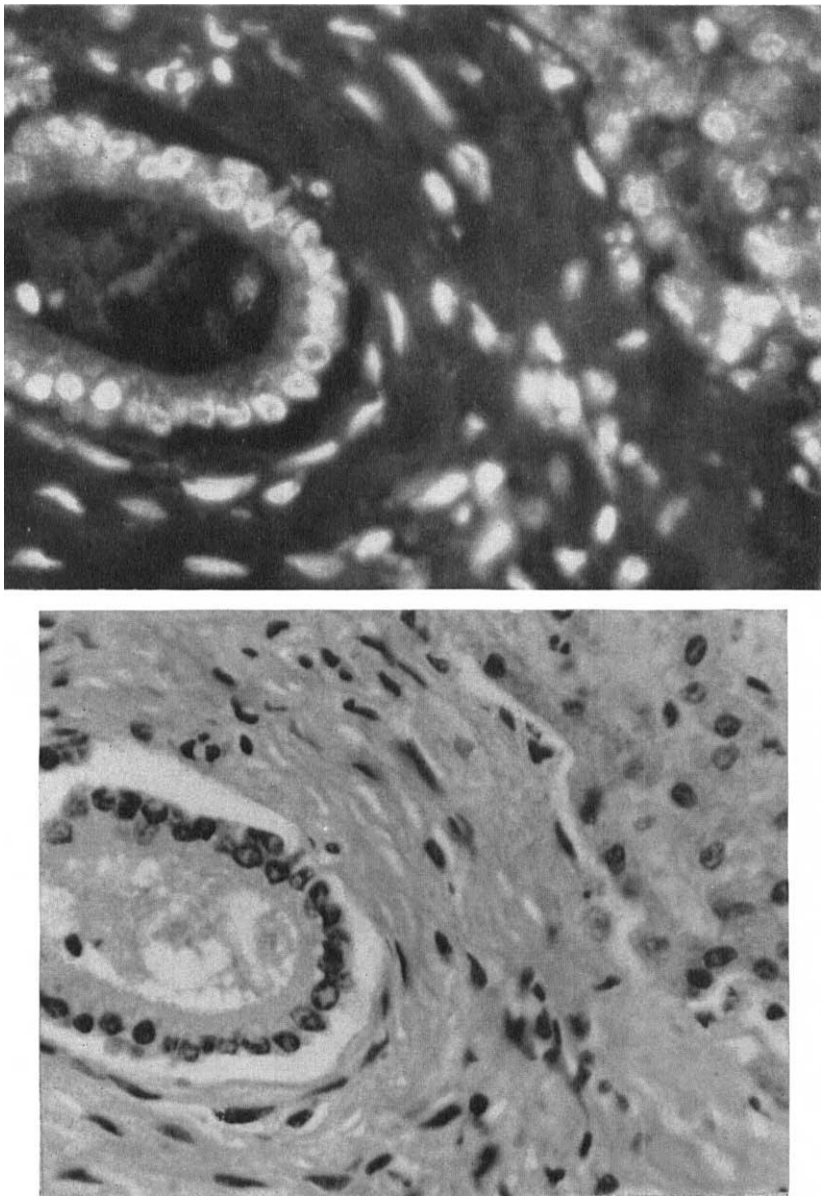


FIG. 1. Bile duct and hepatic parenchymal cells (Case 2) after 5 months in unstained glycerol mount (above) showing intense nuclear and only slight cytoplasmic fluorescence. The same section following H & E stain (below) permits comparison of individual cellular fluorescence and staining qualities (210 ×).

dioidin doubtful. Following a fifth pneumothorax, segmental resection of the posterior segment of the right upper lobe was performed on 10/20/52. The histologic diagnosis of the resected specimen was coccidioidomycosis which was confirmed by culture. Coccidioidin skin test was now positive and complement fixation titer was 1:80 (blastomyces 1:5). Postoperatively bronchopleural fistula and empyema developed, requiring a second decortication procedure on 12/18/52, and on 5/12/53 a thoracoplasty was performed. Pleural cultures were consistently positive for *C. immitis* until the bronchopleural fistula closed two months later. Hepatic and renal functions and blood proteins were normal in July, 1953. From

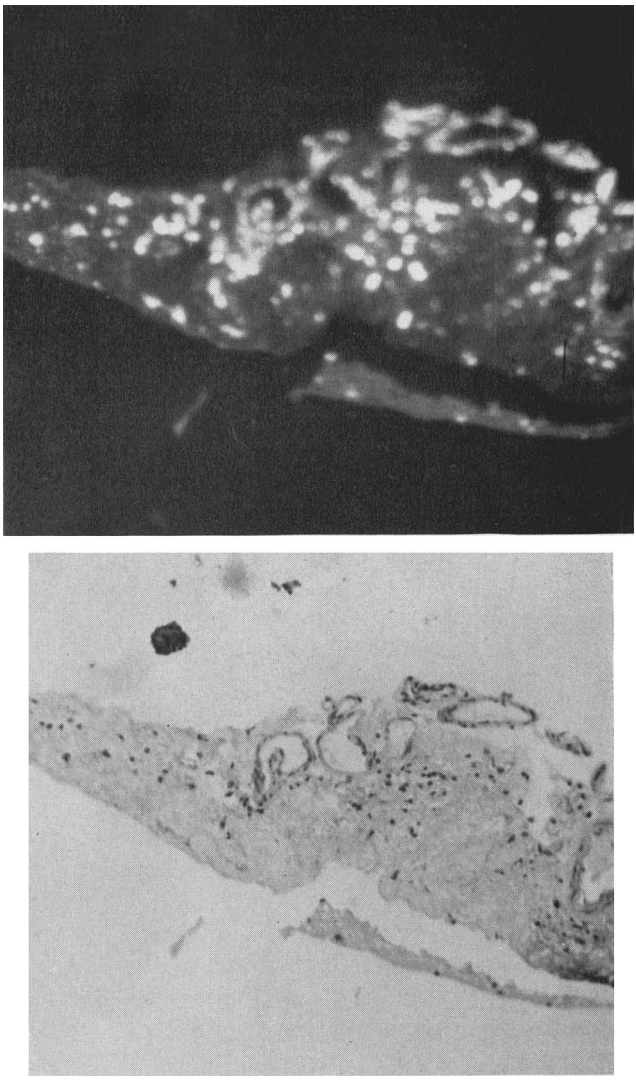
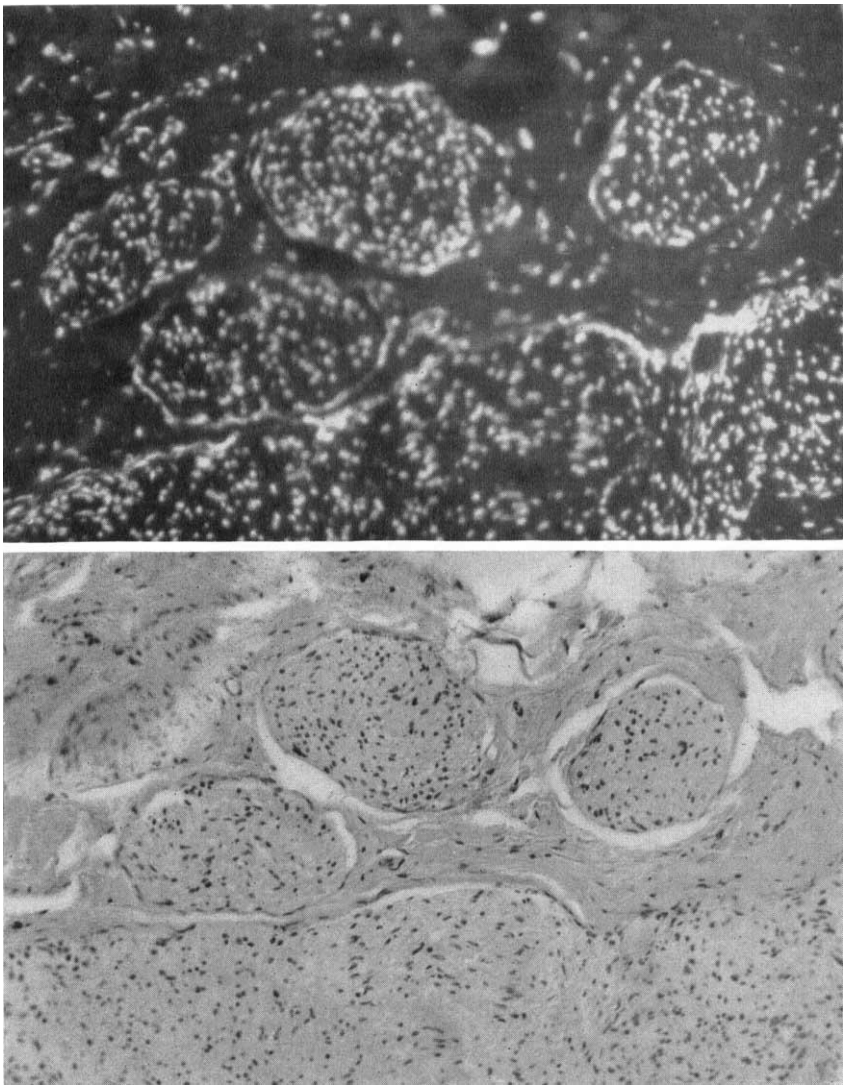


FIG. 2. A. Moderate fluorescence (above) of unstained normal meninges (Case 2) compared with H & E stain (below) indicating deposition of stilbamidine derivative in apparently normal meninges (50 ×). B. Moderate fluorescence (above) of nuclei of neurolemma in ganglion and nerve fibers (Case 2), compared with H & E stain (below) (50 ×).



(FIG. 2B)

7/20/53 to 10/9/53 a total of 16 gms. of hydroxystilbamidine was given with some anorexia and nausea. Cultures of gastric washings, which were persistently positive during treatment, were reported negative in October, and only dubiously positive in November. Complement fixation titer was 1:16. The patient was asymptomatic except for failure to gain weight. ESR was 2-4 mm./hr.

Treatment with aminostilbamidine was begun 12/10/53 with daily intravenous injections of 225 mg. continued to a total dosage of 2.3 gm. Side effects were limited to mild nausea (controlled by 100 mg. of Dramamine® daily) and lemon-yellow discoloration of the skin. Thereafter seven cultures of gastric washings were negative. The patient gained 4 pounds and general condition appeared improved.

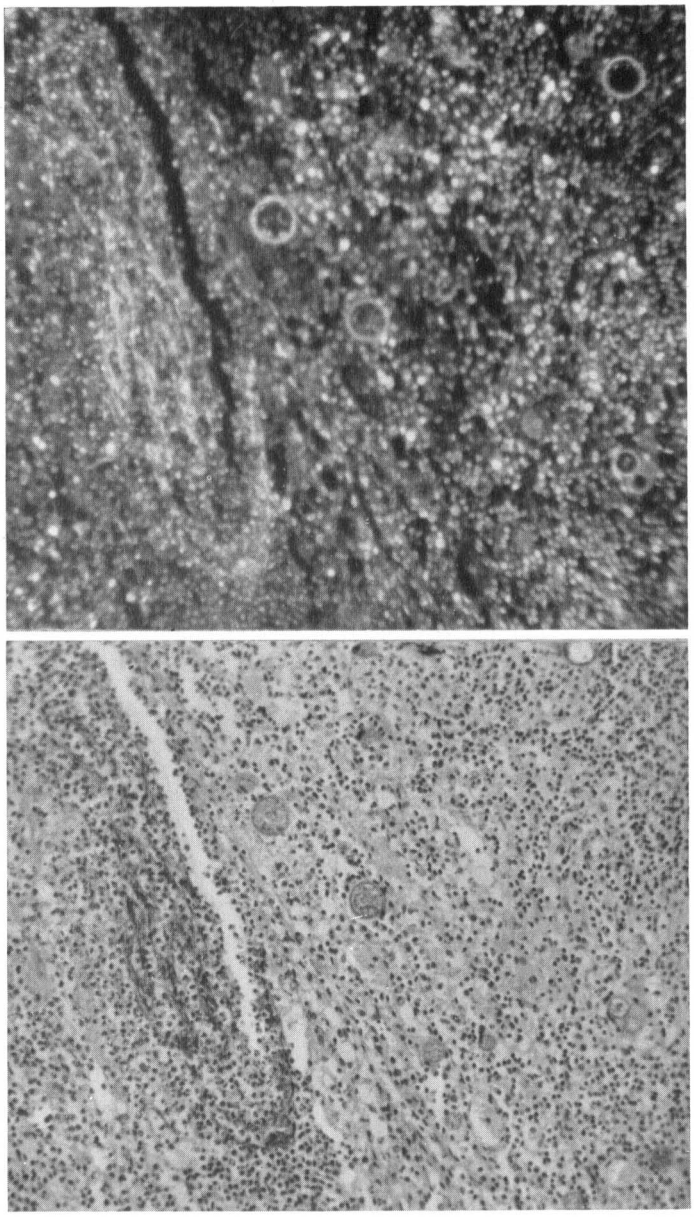
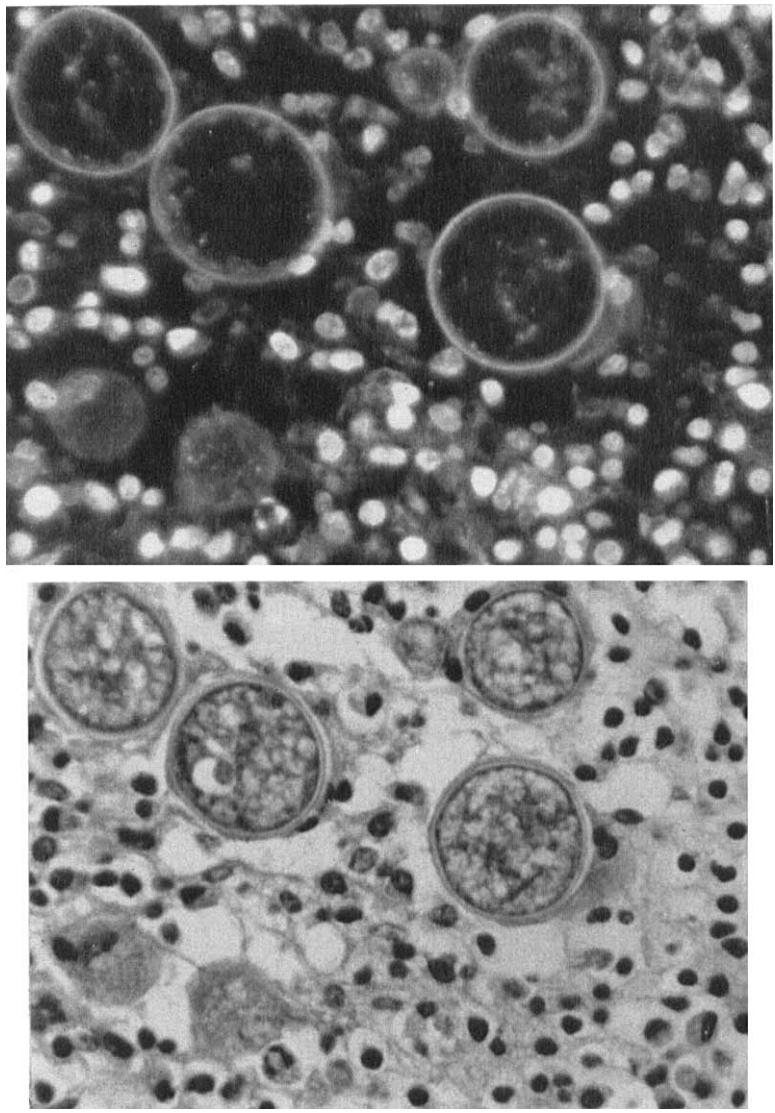


FIG. 3. A. Peribronchial lymph node granuloma (Case 2) showing strong fluorescence (above) of scattered inflammatory cells and of several spherules of *C. immitis*. Spherules can be identified in H & E stain (below) (50 ×). B. Group of spherules of *C. immitis* in lymph node granuloma (Case 2) showing (above) strong fluorescence of envelope or capsule and some fluorescence outlining endospores within, compared with H & E stain (below) (210 ×).



(FIG. 3B)

Case No. 5: A 28 year old white male had onset of illness in June 1954 with right chest pain, hemoptysis, and a large (2 cm.) cavity in the right lower lobe. All skin tests were positive. *Coccidioides immitis* was cultured from sputum. Agglutination was positive in a low titer.

Because of bleeding, segmental resection was performed on 10/7/54. Postoperatively a course of treatment with 2-aminostilbamidine was given for 12 days, in total dosage of 2.75 gm., with no apparent side effects. Sputum diminished in volume and the patient became asymptomatic and afebrile. On 10/26 sputum culture was still positive and one of three gastric washings in November and December was positive.

Studies of Tissue Fluorescence

Distribution of fluorescent drug in tissues was examined in two cases by the technics previously applied to stilbamidine and hydroxystilbamidine tissue fluorescence (15). Unstained rehydrated histologic sections from the various organs were examined microscopically in glycerol mounts, with an ultraviolet

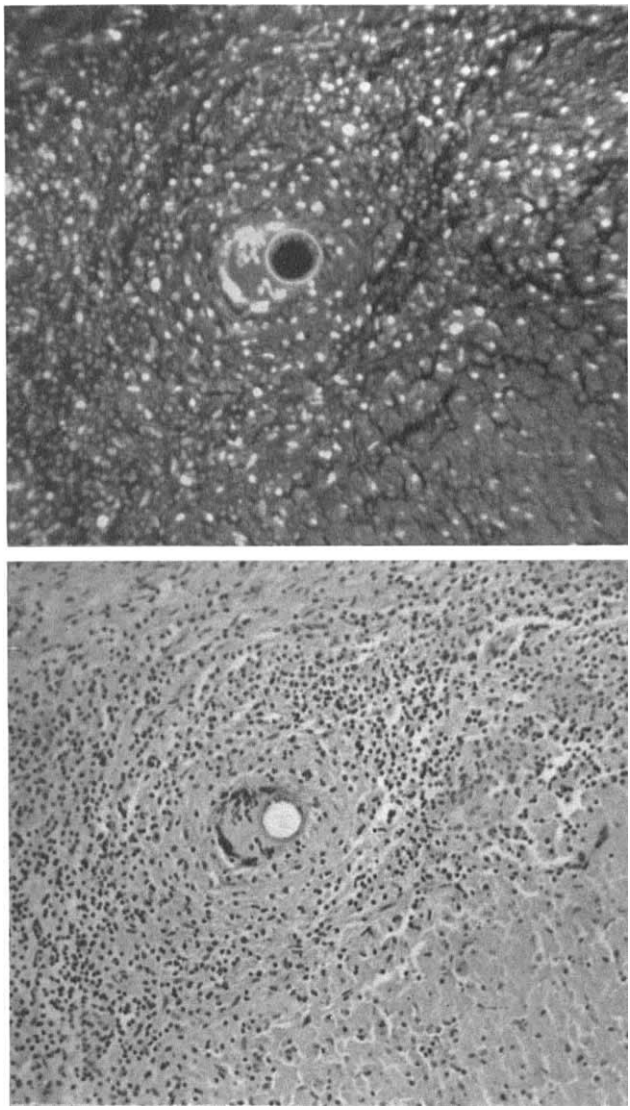
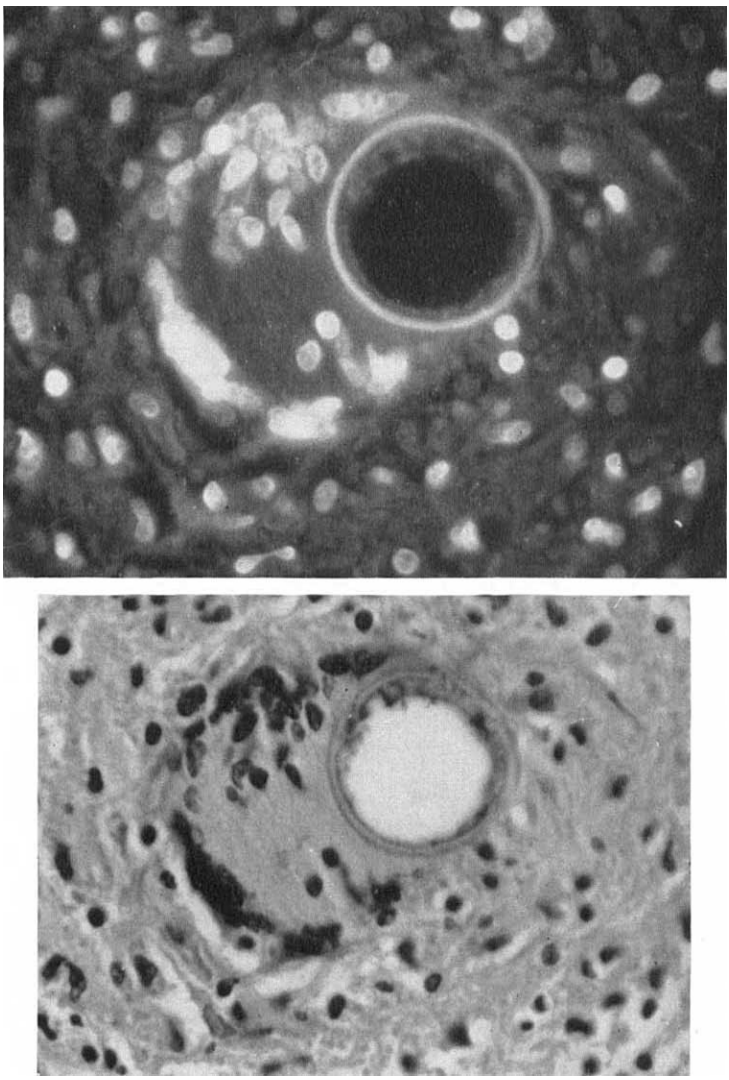


FIG. 4. A. Splenic granuloma (Case 2) showing fluorescence (above) of inflammatory cells, nuclei of giant cell and spherule of *C. immitis*, compared in H & E stain (below) (50 ×). B. High power view of same spherule within giant cell with strong fluorescence (above) compared in H & E stain (below) (210 ×).



(Fig. 4B)

light source, for the presence of fluorescent material. Photomicrographs were made of groups of fluorescent cells; following staining (with H & E) the same cells were photographed in white light for purposes of comparison.

The fluorescence of aminostilbamidine is greenish-yellow in carnoy-fixed tissues, yellow-orange in formalin-fixed material; it is apparently distinguishable from the yellow fluorescence of hydroxystilbamidine. In Case 2 the fluorescence (Figs. 1–5) resulted from a mixture of that given by deposits of stilbamidine, hydroxystilbamidine and aminostilbamidine which had been given successively. Both nuclei and cytoplasmic granules present in the various tissues were strongly

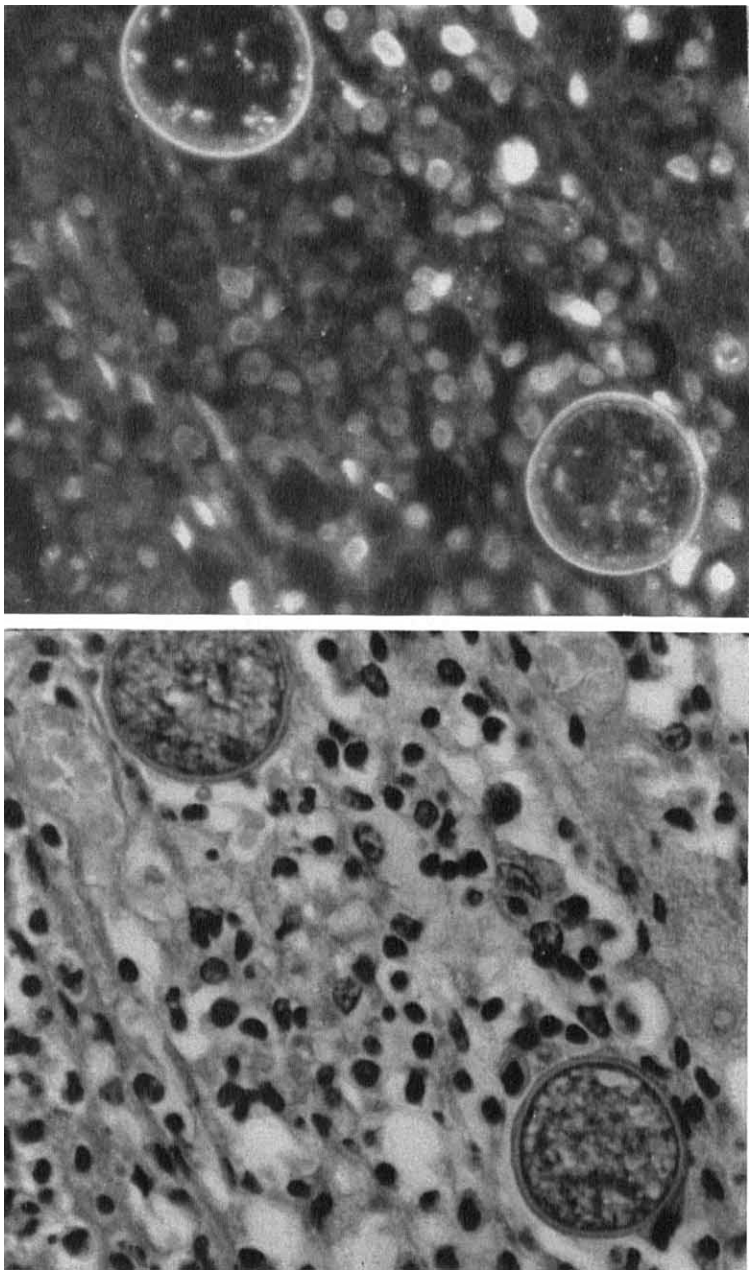


FIG. 5. Spherules of *C. immitis* (Case 2) showing strong fluorescence (above) of envelope and of granular material within spherules, identifiable in H & E stain (below) as endospores (210 ×).

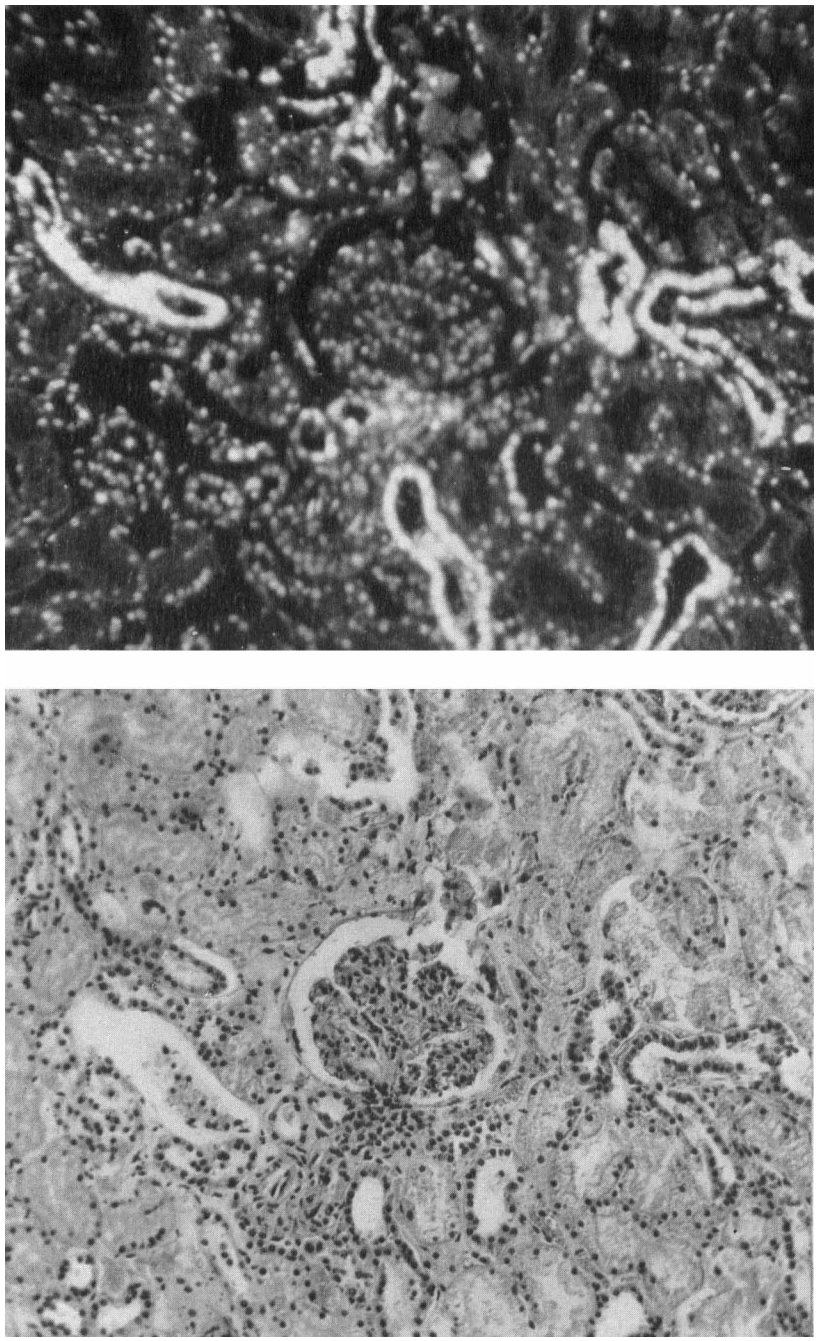


FIG. 6. Normal kidney (Case 3) showing strong fluorescence (above) of renal tubular epithelial cells and weak glomerular fluorescence, compared with H & E stain (below) (50 ×).

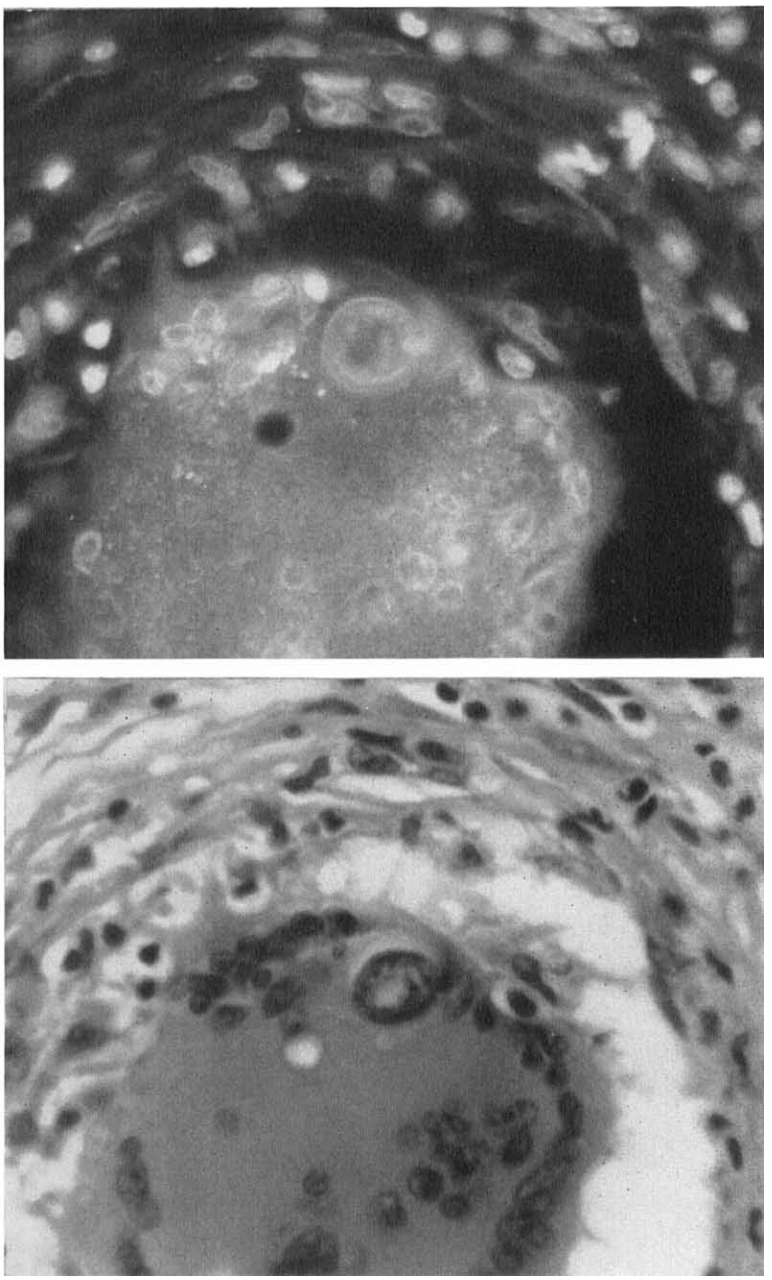
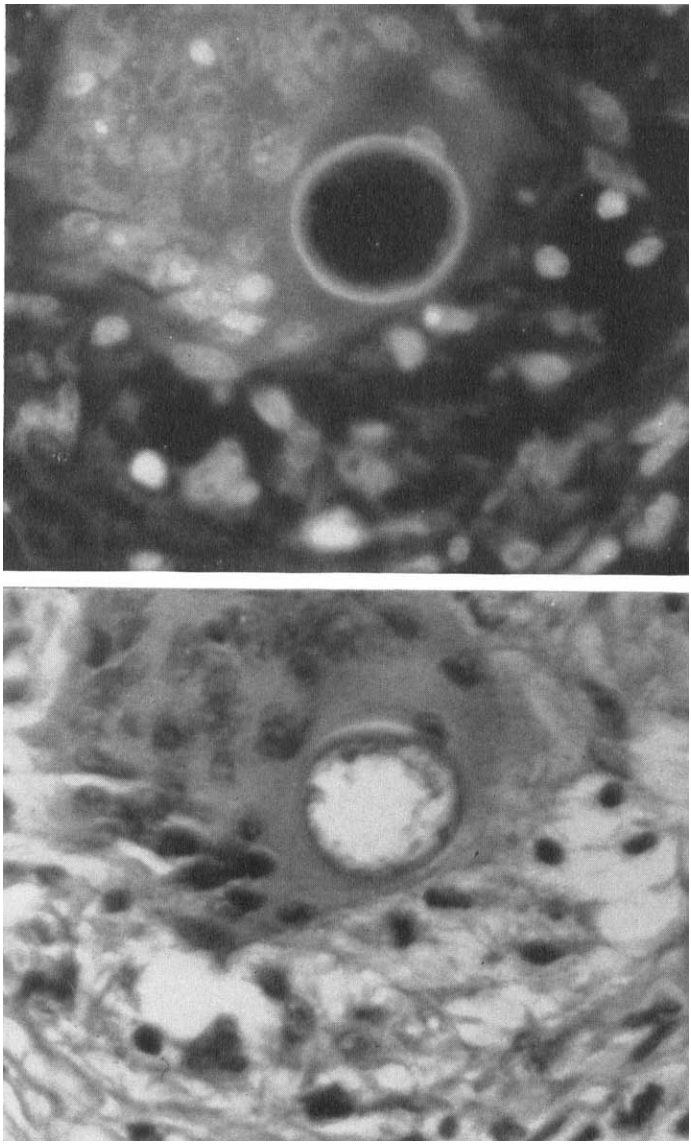


FIG. 7. A. Spherule containing giant cell in epidural granuloma, from surgical (laminectomy) biopsy in Case 3, showing fluorescence (above) of giant cell nuclei and of spherule envelope, compared with H & E stain (below) (50 ×). B. Higher magnification of same (210 ×).



(Fig. 7B)

fluorescent. The granular fluorescence faded after 12 to 48 hours, leaving brilliant nuclear fluorescence which persisted for at least five months (Fig. 1).

In Case 3 only aminostilbamidine had been administered and the fluorescence of the tissue was due only to the presence of aminostilbamidine. In both cases, the most intense fluorescence was observed in nuclei and cytoplasmic granules of liver, adrenals and kidney (Fig. 6). Less intense but nonetheless strong fluorescence was noted in bronchial epithelium, ductile epithelium generally, leuko-

cytes in inflammatory exudate, and skin. Fluorescence of nerve tissue and meninges was of lesser intensity. No fluorescence was observed in connective tissue and in striated muscle.

However, most striking was the fluorescence observed in the spherules of *Coccidioides immitis* (Fig. 3, 4, 5 and 7). The envelope of the spherules fluoresced yellow-green, indicating the specific character of the fluorescence as due to the aminostilbamidine. Moreover the endospores, when present, also were fluorescent and thus became visible in the unstained tissue (Fig. 5).

The above findings were identical in each of the two cases examined histologically.

DISCUSSION

The order of *in vitro* sensitivity of coccidioides to aminostilbamidine approximates that of both coccidioides and resistant blastomyces to hydroxystilbamidine (13). Since blastomycosis is so amenable to therapy with the stilbamidines, and some suppression, however unsatisfactory in terms of "cure," is achieved in the case of hydroxystilbamidine therapy of coccidioidomycosis, it is possible that derivative compounds such as aminostilbamidine may have a higher therapeutic index. The toxicity of aminostilbamidine is low, and in the dosage administered (maximum 10 gm.) no hepatic or renal toxicity was observed. Side effects coincident with its administration resembled those observed with the other stilbamidine compounds, namely anorexia, nausea and occasionally vomiting. As is the case with hydroxystilbamidine (12), these side effects were most pronounced in the patients who were most seriously ill prior to treatment, and were not observed in the individuals whose general condition was good prior to therapy.

The tissue distribution of aminostilbamidine is identical with that previously observed with hydroxystilbamidine (15). Unlike stilbamidine itself, both amino- and hydroxystilbamidine are contained not only in cellular nuclei but also in cytoplasmic granules. Aminostilbamidine thus appears to have the same tissue repository property as hydroxystilbamidine and could, therefore, extend its fungistatic action over a prolonged period of time following cessation of its administration.

Nevertheless the clinical response of coccidioidomycosis to aminostilbamidine therapy was disappointing. Of five patients treated, the two with chronic coccidioidal meningitis showed no effect specifically attributable to the drug. The third (Case 2), with progressive disseminated disease, had a relentless progression to a fatal outcome unaffected by any form of treatment. The two remaining patients had localized pulmonary disease; in both, symptomatic improvement followed aminostilbamidine therapy, but in only one did cultures become sterile. In each of the latter cases the total dose given was small, 2.3 gm. and 2.75 gm. It is thus possible that in non-meningitic and especially in localized coccidioidomycosis, aminostilbamidine may exert a suppressive influence on the course of the infection. However, it is in just such localized coccidioidal lesions

that evaluation of any form of therapy becomes difficult in view of the notorious indolence, chronicity and occasional spontaneous remission of such lesions.

SUMMARY

1. Aminostilbamidine was inhibitory to two strains of *Coccidioides immitis in vitro*, in concentrations of 25 micrograms/cc. of medium.

2. Five patients with chronic coccidioidomycosis were treated with aminostilbamidine in dosage ranging from 2.3 to 10 gm. No curative effect was observed in coccidioidal meningitis. Some suppression of activity possibly occurred in two cases of localized pulmonary disease. No toxicity was observed other than yellow discoloration of skin and nausea.

3. Tissue fluorescence studies in two cases showed strong fluorescence in liver, adrenal and kidney cells, in both nuclear and cytoplasmic granules. Lesser fluorescence was observed in other tissues, indicating the tissue repository character of the compound. The coccidioides spherules and their endospores were also strongly fluorescent, indicating incorporation of the compound within the organism.

Acknowledgment: This investigation was supported by a research grant, E-483, 483-C, and 483-CS, from the National Microbiological Institute of the National Institutes of Health, U. S. Public Health Service, and in part by a grant from the Drs. Leonard H. and Louis D. Weissman Medical Research Foundation.

The aminostilbamidine was generously supplied by the Wm. S. Merrell Company.

Serologic tests for coccidioidal infection were generously performed by Dr. Charles E Smith, University of California, School of Public Health.

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