EXPERIMENTAL MONILIASIS IN MICE *

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It is generally recognized that *Candida albicans* is the only species of Candida known to be pathogenic to man and to a number of laboratory animals. However, there is little available description of the pathologic changes encountered in experimental moniliasis in animals. In the past, inflammatory reaction to the organism has been attributed either to direct tissue irritation or to thrombosis subsequent to vascular damage by the organisms or a hypothetic toxin.

Redaelli[1] found that intravenous injection of *C. albicans* resulted in rapid diffusion in guinea-pigs, rats, and rabbits, and, that the kidney was the most altered organ although its medulla was rarely involved. He also recorded involvement of the liver, brain, heart, spleen, adrenal glands, and mesenteric lymph nodes although no description of the pathologic alterations was recorded. Redaelli believed that the lesions were of embolic character, the organisms comprising the emboli.

Stovall and Pessin[2,3] disagreed with this concept and contended that embolism was not the pathogenetic mechanism of systemic moniliasis. They indicated that there were species of Candida much larger in diameter than *C. albicans* which were not pathogenic to laboratory animals. They further demonstrated that septicemia in animals infected with *C. albicans* was always present. This they failed to demonstrate with other species of Candida. It was concluded that *C. albicans* was a very virulent species capable of long survival in animal tissues and, therefore, capable of producing lesions.

Fuentes, Schwarz, and Aboulafia[4] have shown that the rat, rabbit, guinea-pig, and mouse were susceptible to *C. albicans* in the order indicated. They noted in their histologic studies that the lumina of capillaries contained blastospores and pseudohyphae. These were associated with desquamation of endothelium and diapedesis of red blood cells. They suggested the possibility of local toxic damage to endothelium as a mechanism for pathogenicity.

Salvin, Cory, and Berg[5] showed that the use of mucin as a vehicle for the injection of *C. albicans* permitted the employment of relatively smaller numbers of organisms which produced higher mortality in a

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shorter period of time. More recently, Salvin[6] proved that cells of *C. albicans* contain a soluble endotoxin. He demonstrated that filtrates from suspensions of the organisms produced the same lesions as live organisms.

Scherr,[7,8] in his study of the use of yeast cells to enhance the virulence of *C. albicans* for mice and in his subsequent work on the effect of cortisone on moniliasis in mice, used gross pathologic changes in his observations. Many other workers have described isolated organic lesions in case reports.[9-13]

In this paper, we will endeavor to show the distribution and nature of lesions in experimental moniliasis in mice.

Materials

Four groups of mice were studied in addition to a control series. The mice were all white males of the same strain and weighed approximately 20 gm. They were obtained from the same dealer and were reared under identical conditions. The animals were obtained in three batches at different times. The first batch was used in group I and the controls, the second in groups II and IV, and the third in group III. Studies with groups II and IV were carried out 1 year after study of group I was begun. The group III study was carried out a few months after groups II and IV were started. Groups I, II, and III were inoculated intravenously with an isotonic saline suspension of our *C. albicans* "Fuentes"* grown overnight on Saboraud's dextrose agar. The fourth (group IV) group was inoculated with formalin-killed *C. albicans*. The control group was not given any inoculation.

The difficulty of an exact count presented a problem. Count in a hemocytometer included dead organisms while count in a pour plate reduced the true number of organisms by the presence of clumps which grew in a single colony. Our suspension was made by comparison with tube 3 of McFarland's nephelometer.†

Methods and Results

The *control group* consisted of 10 mice which did not receive an inoculum of *C. albicans*. They were kept separate from the inoculated group for 2 weeks and sacrificed at the termination of that period. All mice were necropsied and organs preserved in 10 per cent formalin. Tissue sections were prepared and stained with hematoxylin and eosin. Organs from the 10 mice of the control group showed no recognizable lesions.

* *C. albicans* "Fuentes" was isolated from a case of vaginal moniliasis.

† Tube 3 of McFarland's nephelometer corresponds to 50,000,000 monilia per cc.

Group I

Group I consisted of 80 mice. All were inoculated intravenously under light chloroform anesthesia with 0.1 cc. of a suspension, this amount containing 100,000 organisms. In the process of injection, the suspension of organisms was agitated constantly to prevent sedimentation. Three mice died instantly after injection, probably as a result of faulty technique. The remaining 77 mice were inspected three times a day during the period of study. Dead animals were removed promptly, necropsied, and all organs preserved in 10 per cent formalin. Tissue sections were stained with hematoxylin and eosin and the periodic acid-Schiff stain.

Table I indicates the survival period following inoculation. All animals died within 13 days. Two died after a period of 18 hours. The average survival was 81 hours.

TABLE I

Survival Results, Group I

Number of mice	Survival
	hrs.
2	18
5	30
12	40
5	50
16	65
6	75
14	90
5	110
3	120
5	144
2	168½
2	310
Total 77	

Of 77 animals examined, brain, heart, lungs, and kidneys were not available in 2; brain, heart, and lungs were not available in 3; and brain alone was not available in 5. These organs had been eaten by other animals in the cage before the dead bodies were removed.

The heart was the seat of myocarditis and abscess formation (Fig. 1). The myocarditis was characterized by focal and/or diffuse interstitial inflammation with lymphocytes, and, to a lesser degree, histiocytes and neutrophils. Focal areas of inflammation usually were fairly well circumscribed. Microabscesses were found singly or in coalescent form in cases with more severe myocardial involvement. Yeast cells and pseudohyphae were present in all lesions and their demonstration was facilitated by examination of sections stained with the periodic acid-Schiff stain (Fig. 2). They were in large numbers, particularly in animals which died from 18 to 40 hours after inoculation. These lesions were found in increased number and extent in inverse proportion to survival period. In the 9 animals which survived longer than 144 hours, only 2 had minimal focal myocarditis.

Granulomatous lesions in the heart were seen on two occasions in animals which survived up to 65 hours. These were well circumscribed,

non-caseating nodules made up of histiocytes and bordered by a zone of lymphocytes. No organisms were found in the granulomatous lesions.

The kidneys contained many isolated and coalescent abscesses, predominantly among animals which succumbed between 50 to 120 hours after inoculation. Organisms were present in large numbers in these lesions. In several instances, cortical abscesses had broken through the renal capsule to produce perinephric abscesses (Fig. 3). The abscesses were found regularly in the renal cortical areas of animals surviving less than 120 hours and were much less common in those living longer than 140 hours. When present in the latter group, they were located invariably in the medulla. Three of these, indeed, did not have abscesses at all. In addition to renal abscess formation, all 77 cases exhibited a monotonous pattern of focal or mild diffuse interstitial infiltration of lymphocytes and histiocytes. As with the abscesses, the extent of the reaction was greater in animals which succumbed early and less in animals which survived longer. In the latter group, the interstitial lesions appeared in medullary areas and frequently were associated with pyelitis. In the pelvis of the kidneys in which inflammation was present, numerous organisms were found also in yeast form and as pseudohyphae in calyces, mucosa, and tips of renal pyramids.

Granulomatous lesions of the kidneys were met with regularity among animals surviving 50 to 120 hours (Fig. 4). Fifty-six of the 73 animals examined showed tubercles of recent development. They were generally non-caseating microtubercles consisting of histiocytes concentrically arranged and surrounded by a zone of lymphocytes. They were located both in cortical and medullary regions. Organisms were not demonstrated in the granulomatous lesions.

Lesions of the brain appeared early but were most extensive between 50 and 120 hours. They consisted of foci exhibiting glial cell degeneration and localized infiltrations with lymphocytes and histiocytes (Fig. 5). Organisms were present in many of these lesions.

The spleen in 50 per cent of the animals was the seat of marked histiocytic proliferation in sinusoidal areas and exhibited progression to frank granulomatous formation with central necrosis (Fig. 6). Organisms, ordinarily in the form of yeasts, were noted in these lesions. Animals surviving 50 to 120 hours showed these lesions in greater numbers.

The stomach of the majority of animals surviving less than 65 hours had a superficial mucosal inflammatory process upon which were

superimposed dense plaques or organisms, mostly in the form of pseudohyphae. The organisms overlaid the mucosa and rarely were observed to penetrate the muscular layers. The small intestine, in two instances, exhibited lesions of the same type.

Voluntary muscle from the paravertebral region at the level of the kidneys demonstrated diffuse interstitial histiocytic and lymphocytic infiltration with occasional abscess formation and numerous organisms.

In one instance for each, pancreas, lacrimal gland, prostate, and seminal vesicle were the seats of inflammatory reactions similar to those occurring elsewhere. Organisms were found in these lesions.

Lungs in all animals showed acute congestion, focal hemorrhages, and focal edema. In a few there was non-specific lobular pneumonia. Organisms were never found in the lungs.

The eye, salivary gland, liver, adrenal gland, and urinary bladder failed to show pathologic alterations.

Group II

Group II consisted of 11 mice. These were inoculated with 0.1 cc. of a suspension containing 1,000,000 organisms in the same manner as animals in group I. Two animals were sacrificed 1 hour after inoculation, 2 at 4 hours, 2 at 10 hours, and 1 at 20 hours. The remaining 4 mice died spontaneously between the 10th and 20th hours after inoculation. The mouse sacrificed at the end of the 20th hour was moribund. Heart, lungs, liver, spleen, and brain were fixed in 10 per cent formalin and tissue sections prepared in the same manner as for group I.

Four mice sacrificed 4 hours after inoculation showed no detectable alterations. A few yeasts and pseudohyphae were present in a section of heart from an animal sacrificed after 1 hour. The earliest lesions appeared at 10 hours in the form of interstitial myocarditis, dense hyaline casts in the kidneys, and focal histiocytic proliferation in the liver. In this mouse, also, organisms were seen in the heart. The mice which died between 10 and 20 hours and the last animal sacrificed at the end of 20 hours showed essentially the same pathologic features as described for animals in group I. The lesions were unusually severe.

An unexpected finding in the liver was the presence of focal but widespread interstitial histiocytic proliferation in portal areas or around blood vessels. There were organisms in these lesions. They were found in 7 of 11 animals and were present extensively in the 4 animals which died spontaneously and in the last one sacrificed. These lesions were generally circumscribed and showed no necrosis.

There was lobular pneumonia in 4 mice which died spontaneously. Organisms were present in 2 of the 4 lungs exhibiting pneumonia.

Group III

Group III consisted of 54 mice. These animals were inoculated with 0.1 cc. of a suspension containing 1,000 organisms in the same manner as in groups I and II. Two mice died promptly after injection and were discarded. The remaining 52 mice were sacrificed as follows: 4 mice at 6 hours after inoculation, 4 at 12 hours, 4 at 19 hours, and 3 animals daily until the 15th day.

None of the 52 animals in group III died spontaneously. At necropsy, heart, lungs, liver, kidneys, and brain were preserved in 10 per cent formalin, and tissue sections stained with hematoxylin and eosin and by the periodic acid-Schiff method were prepared.

Thirty-one of 52 animals (59.6 per cent) showed myocarditis of focal or diffuse character, similar to that previously described for groups I and II. Focal myocarditis was recognized in one mouse sacrificed 6 hours after inoculation. This lesion appeared with maximum intensity among animals sacrificed on the fourth day. It was found in all animals up to the ninth day, after which only a single mouse (sacrificed on the twelfth day) had mild focal myocarditis. Only 4 subjects exhibited micro-abscesses in the myocardium and these were encountered among mice sacrificed as early as the first day and as late as the sixth day after inoculation. Two myocardial granulomas were seen, one in an animal sacrificed on the second day and another in one sacrificed on the fourth day. Only two hearts contained organisms. The nature of the lesions was similar to that described in groups I and II but they were less severe.

As in the heart, renal lesions consisting of focal and diffuse interstitial infiltrates with lymphocytes and histiocytes were found as early as 6 hours after inoculation. They appeared regularly among animals sacrificed up to the 13th day, a total of 36 (69 per cent) exhibiting this process. Twelve (23 per cent) of those sacrificed after the fourth day showed abscesses, but organisms appeared in only 2 of the mice sacrificed before the seventh day. From the eighth to the twelfth day, the interstitial inflammation was associated with marked pyelitis, many cellular casts in the tubules, abscesses, and innumerable organisms in the medullary and pelvic regions. Granulomas were found in only 2 animals.

Lesions in the brain were similar to those observed in group I. They were present in 48 per cent and were most common among animals sacrificed from the fourth to the eleventh day. There were abscesses

in 8 cases and organisms in 5 of these. A granuloma was seen only once.

In the liver, the peculiar periportal and perivascular histiocytic infiltrations were observed in all but 6 cases. The 6 animals without this lesion were sacrificed latest in the series (14th and 15th days). There were abscesses in 5 and a granuloma with central necrosis in one. Organisms were encountered in one abscess. The hepatic lesions were similar to those encountered in group II (Figs. 7 and 8).

Group IV

Group IV consisted of 12 mice inoculated intravenously with 0.1 cc. of a suspension containing 1,000,000 dead organisms. The organisms were killed in formalin and washed five times in isotonic saline solution. Pairs of mice were sacrificed at 1 hour, 4 hours, 10 hours, 20 hours, 2 days, and 5 days after inoculation. None of these animals died spontaneously. As in groups II and III, sections were prepared from heart, lungs, spleen, kidneys, and brain.

There were no recognizable pathologic alterations in the heart. Six animals had focal lymphocytic infiltrations in the kidneys and 4 had similar lesions in the brain. The liver in all cases had histiocytic proliferation similar to that found in groups II and III. However, no organisms were demonstrated.

Discussion

It was apparent from group I that mice succumbed at an average of 3 to 4 days after intravenous inoculation with 100,000 organisms *(C. albicans)*. Renal lesions in the form of interstitial inflammation, abscesses, and granulomas were present in all cases. The intensity of the renal process suggested that all animals might have succumbed without associated lesions in other organs. However, in view of the coexistent myocarditis and encephalitis, most prominent in animals which died early, it seemed likely that early death was brought about by a combination of all the lesions.

There is no ready explanation for the predominance of lesions in the heart, kidneys, and brain, nor for the absence of hepatic changes in group I. Special organ predilection has been observed with other microorganisms. In point, *Histoplasma capsulatum* often localizes in the liver and rarely in the kidney. It was interesting to note that in the presence of considerable kidney damage, the urinary bladder remained free of lesions. *Candida albicans* grows best in a slightly acid medium (pH 5.0). Urine of rodents has a very alkaline reaction and this may have inhibited the growth of organisms in the bladder.

Inflammation and abscesses in voluntary muscles adjacent to the kidneys were probably the result of local extension from cortical abscesses of the kidney which had broken through the capsule. The hematogenous route of localization cannot, of course, be excluded.

The greater number of granulomas encountered among animals which succumbed later in group I would indicate that such a lesion is a manifestation of chronicity or indolence. This, however, did not prove to be the case in group III, in which granulomas occurred with much less frequency. We believe that in the latter study the number of organisms introduced was insufficient to incite granulomatous lesions.

Group II demonstrated that the intravenous inoculation of a heavy dose of *C. albicans* in mice resulted in rapid fatality (within 24 hours). Judging from the nature and extent of the lesions observed in the 4 mice which succumbed between 10 and 20 hours, it seems reasonable to conclude that the other animals in the series would have expired within 24 hours had they been permitted to live. In fact, the mouse sacrificed at 20 hours was moribund.

This method might present a speedy means of determining the pathogenicity of certain species of Candida. The intravenous introduction of a suspension of an unknown Candida would be an easy and inexpensive procedure.

The presence of a considerable number of circumscribed collections of histiocytes accompanied by demonstrable organisms in the liver was an unexpected occurrence since this had not been encountered in any animals in group I. This phenomenon prompted us to proceed with studies in group III and IV. We believed originally that hepatic lesions were the result of massive inoculation. However, since 46 of 52 animals (88.5 per cent) in group III showed the same lesions, although without demonstrable organisms, this was obviously not the case. Indeed, 5 animals showed hepatic abscesses and 3 showed granulomas. Moreover, focal histiocytic proliferation also appeared in group IV. At present there is no ready explanation for the variation of response in the liver.

A small inoculum of *C. albicans* has proved to be incapable of producing fatalities in mice. Lesions of mild nature have been produced, however.

The mechanism of death in mice with intravenous inoculation of *C. albicans* would appear to be attributable to toxemia. This is in keeping with Salvin's[6] demonstration of an endotoxin in *C. albicans*. It is not possible, however, to rule out myocardial damage as an im-

mediate cause of death since the cardiac lesions were extensive and severe. This was especially true with a heavy inoculum. It does not seem reasonable to consider vascular occlusion from embolization of fungi an explanation for the pathogenesis. In all sections examined, only one artery contained a mycotic occluding embolus.

Summary

Four studies were made to determine the pathogenicity of *Candida albicans* "Fuentes." Group I consisted of 80 mice inoculated intravenously with 0.1 cc. of a suspension of *C. albicans* (containing 100,000 organisms). All animals succumbed within 13 days. Two died as early as 18 hours. The average survival period was 3½ days.

Group II consisted of 11 mice inoculated with 0.1 cc. of a suspension of *C. albicans* (containing 1,000,000 organisms). This proved fatal to 4 animals within 20 hours. From the nature of the lesions found in the remaining 7 animals, we assumed that all would have died spontaneously within a day if the animals were not sacrificed earlier.

Group III consisted of 54 mice inoculated intravenously with 0.1 cc. of a suspension of *C. albicans* (containing 1,000 organisms). No fatalities resulted.

Group IV consisted of 12 mice inoculated intravenously with 0.1 cc. of a suspension of killed and washed *C. albicans* (containing 1,000,000 organisms per ml.). No fatalities resulted after 5 days and no lesions comparable with those produced in groups I, II, and III were encountered.

Experimental moniliasis in mice was manifested by a disseminated disease characterized by interstitial inflammation, abscesses, and formation of granulomas. These lesions were most prominent in the heart, kidney, brain, and spleen. Hepatic changes were variable. Eyes, salivary glands, adrenal glands, urinary bladder, and lungs failed to show pathologic alterations directly attributable to the infectious agent.

Myocarditis appeared to be the earliest manifestation of the disease and probably was the immediate cause of death in the majority of animals in groups I and II.

Interstitial nephritis was of constant occurrence. Medullary localization was prominent among animals which survived for the longer periods.

Encephalitis was a late manifestation of the disease.

Granulomas, judging from observations in group I, indicated chronicity or indolence.

Survival time and tissue reaction depended largely on the number of inoculated organisms.

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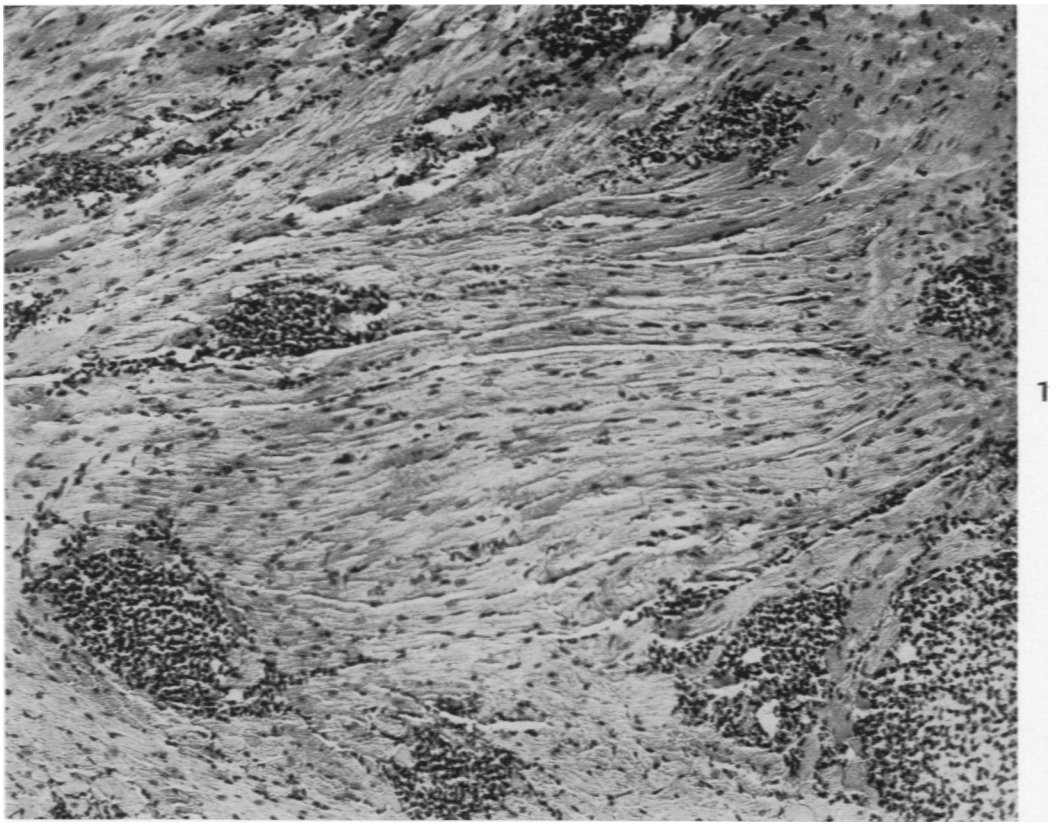
LEGENDS FOR FIGURES

FIG. 1. Heart, from mouse of group II. Interstitial infiltrate of lymphocytes, histiocytes, and neutrophils with abscess formation. × 160.

FIG. 2. Numerous yeast cells and pseudohyphae in a myocardial abscess. Animal from group I which died between 30 to 40 hours after inoculation. × 900.



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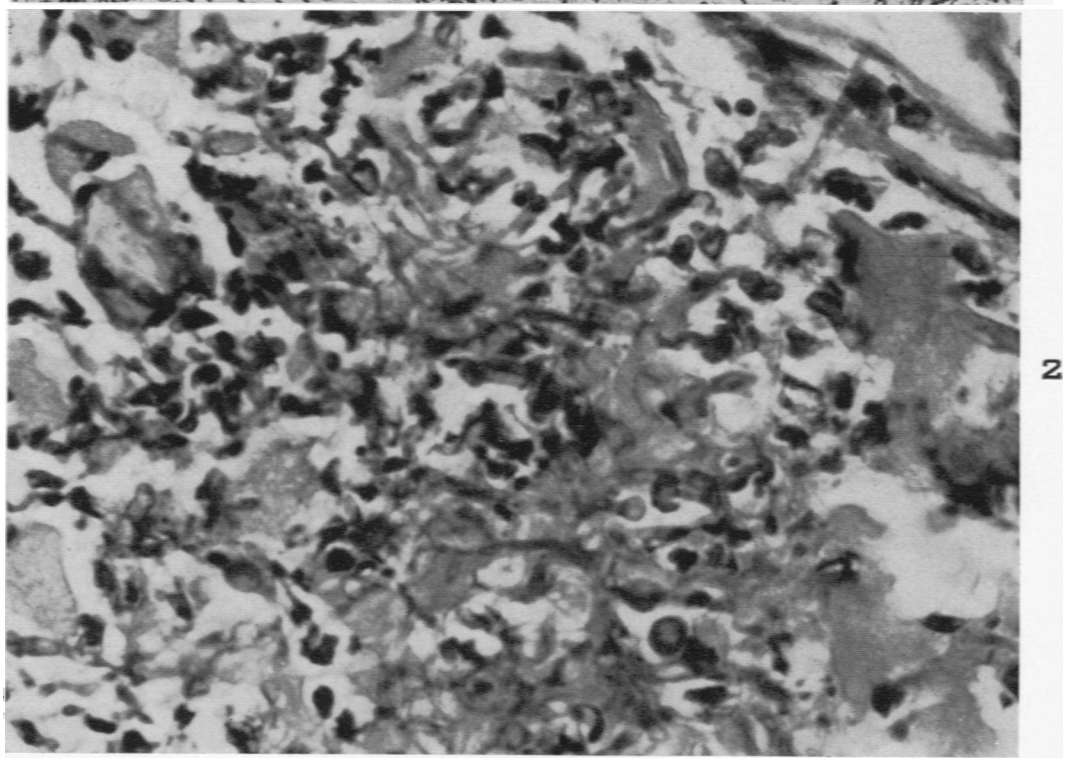

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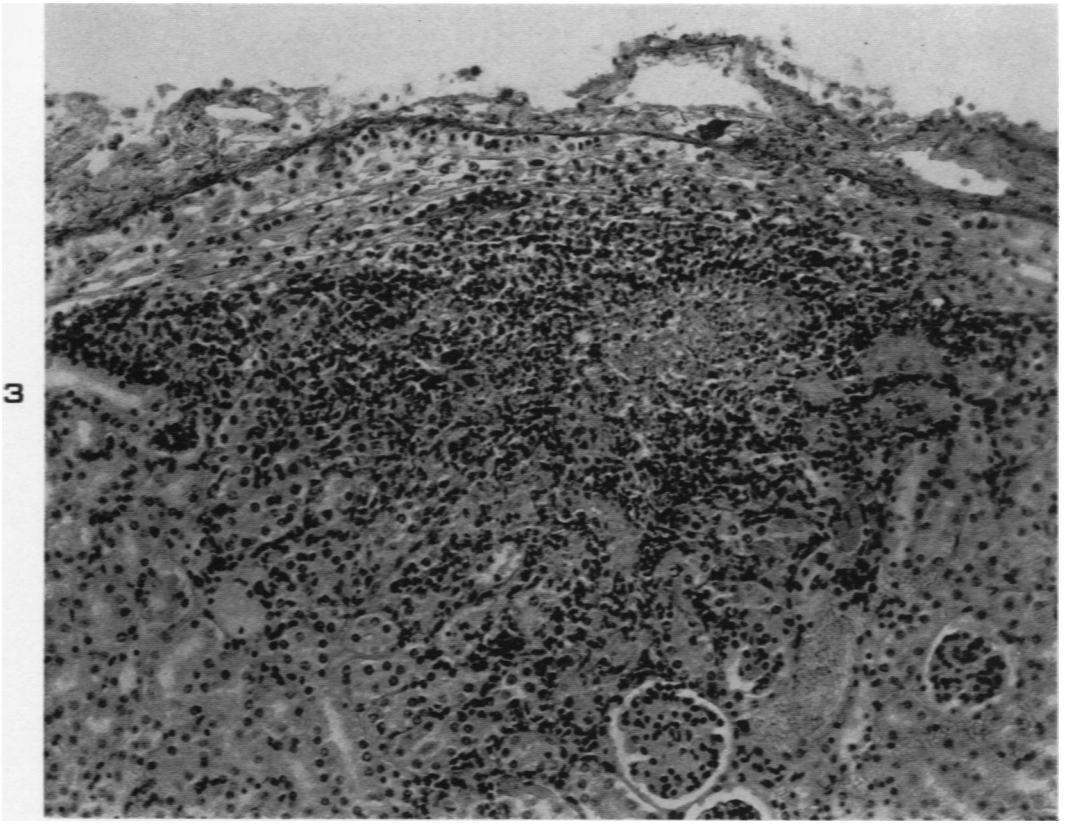
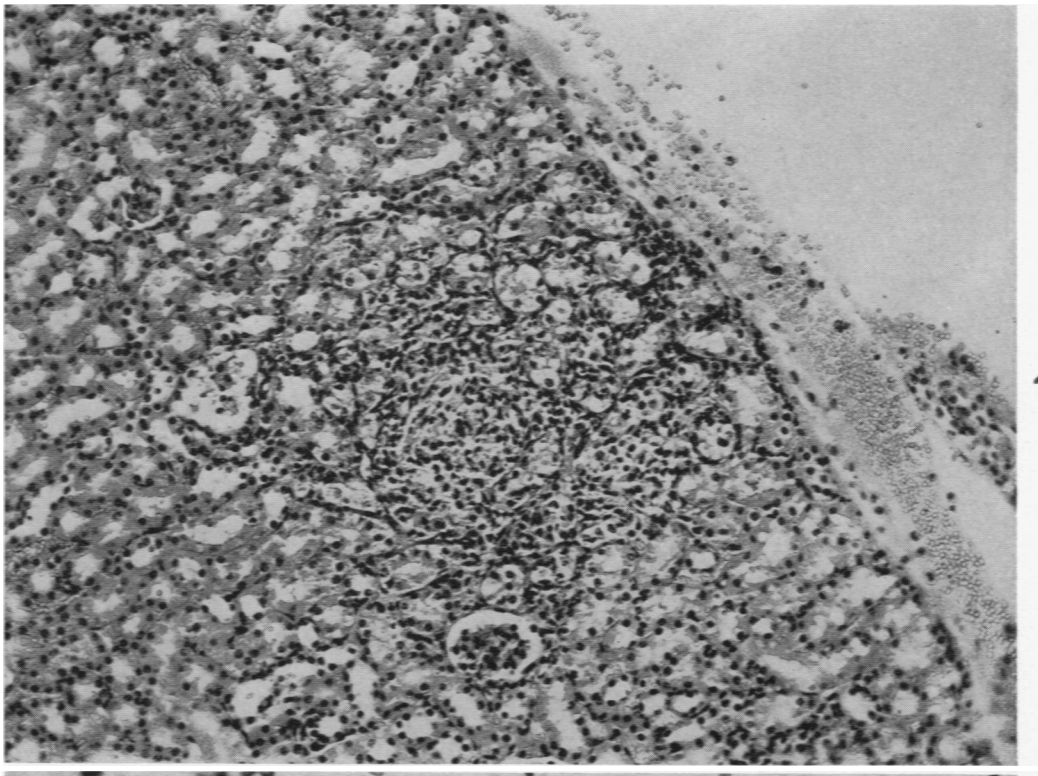


FIG. 3. Kidney. group I. A cortical abscess in an animal which died between 120 and 144 hours. The lesion has broken through the surface. with perinephric abscess formation. × 200.

FIG. 4. A granuloma of recent development in the kidney of a mouse from group III. The lesion consists primarily of histiocytes and lymphocytes. × 170.

FIG. 5. Focal encephalitis (mouse from group I). characterized by degeneration and necrosis of neuronal elements. Infiltrate of histiocytes and lymphocytes. × 650.



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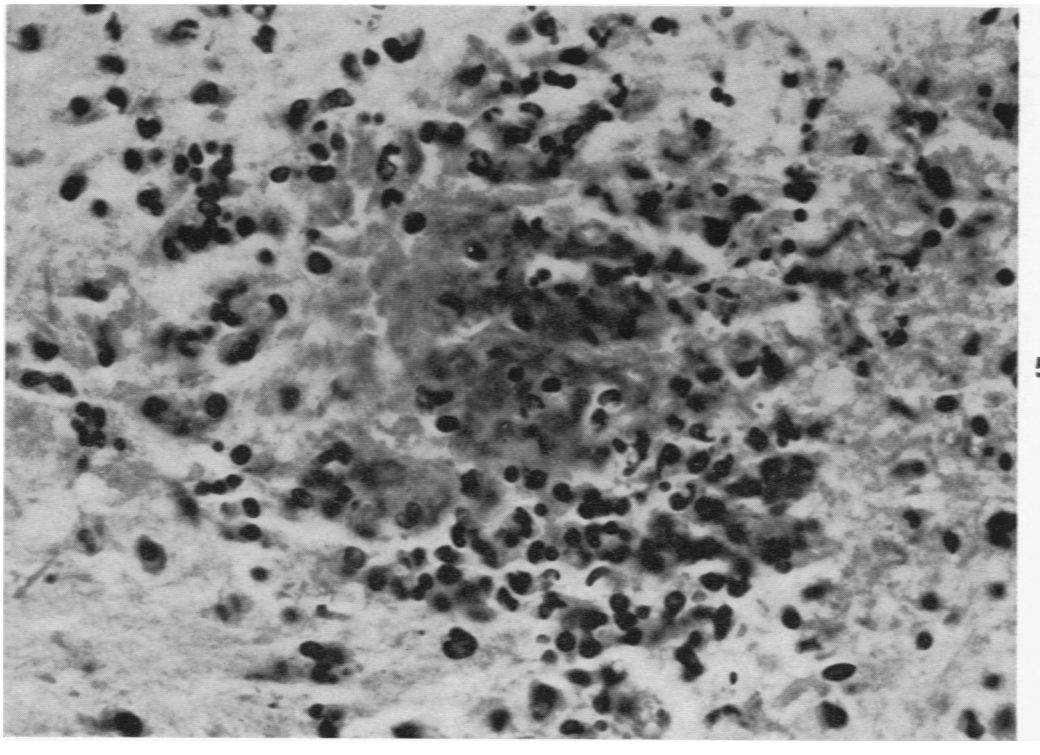

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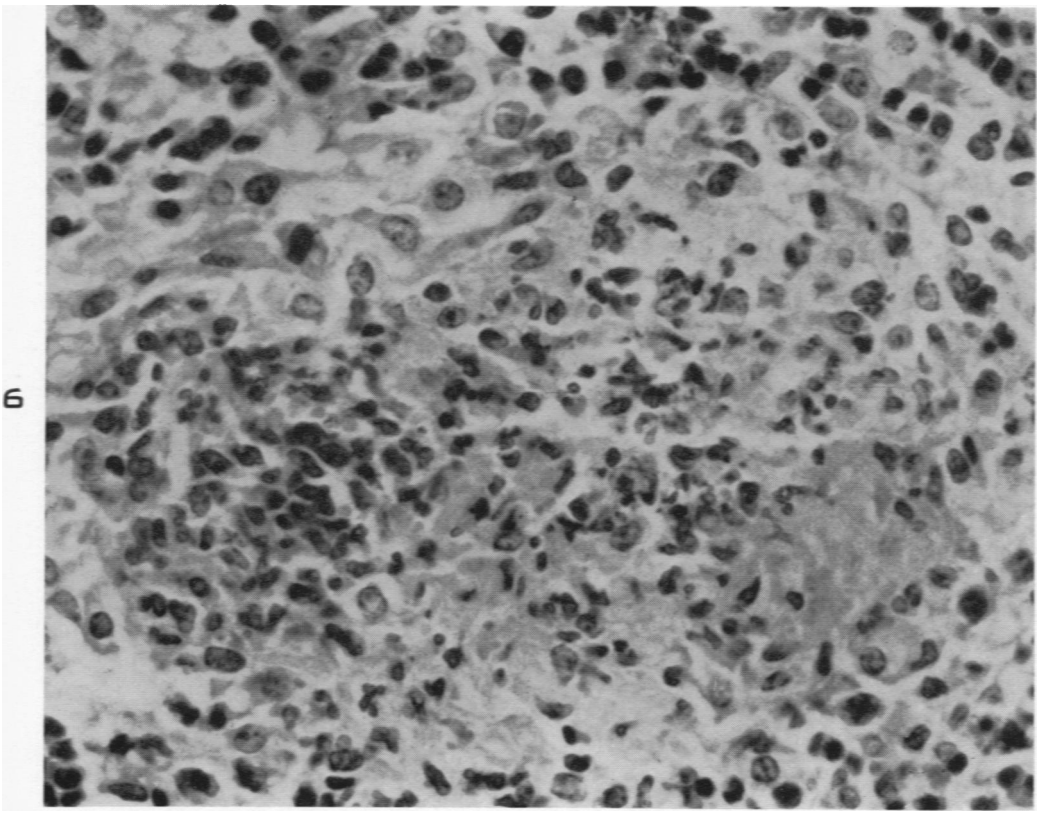
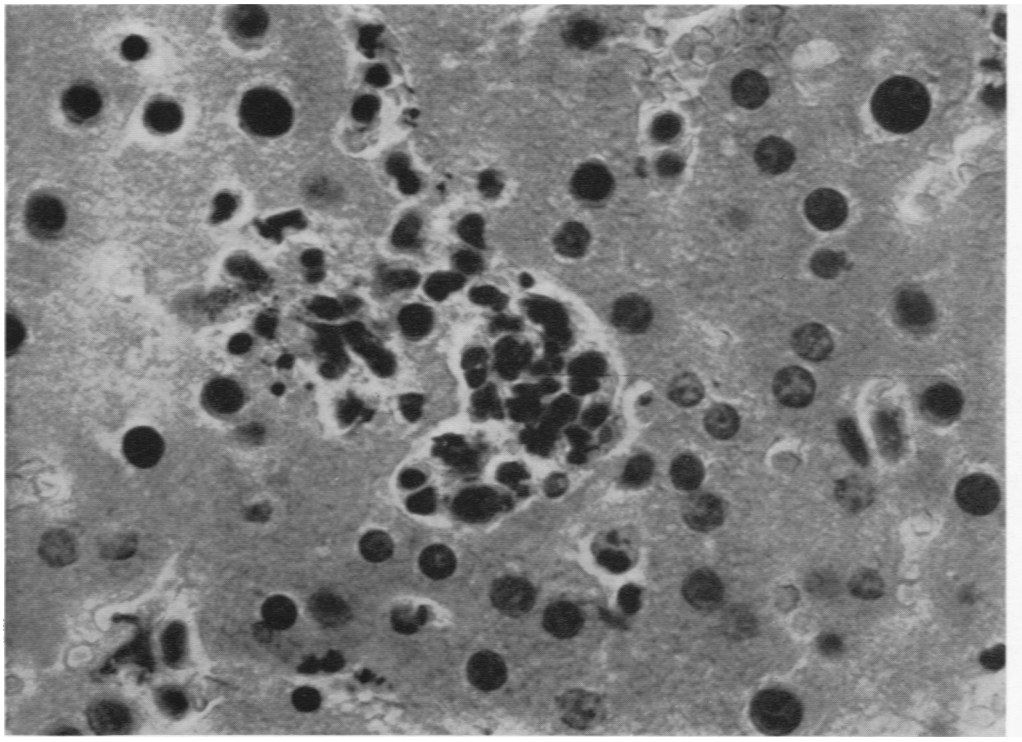


FIG. 6. Two coalescent granulomas of recent development in the spleen (mouse from group I). The reaction is primarily histiocytic, although the lesion on the left shows early necrosis. × 650.

FIG. 7. Liver, group II. Area of histiocytic cell proliferation in sinusoidal space. × 900.

FIG. 8. Liver, group III. Perivascular granuloma with early central necrosis. × 170.



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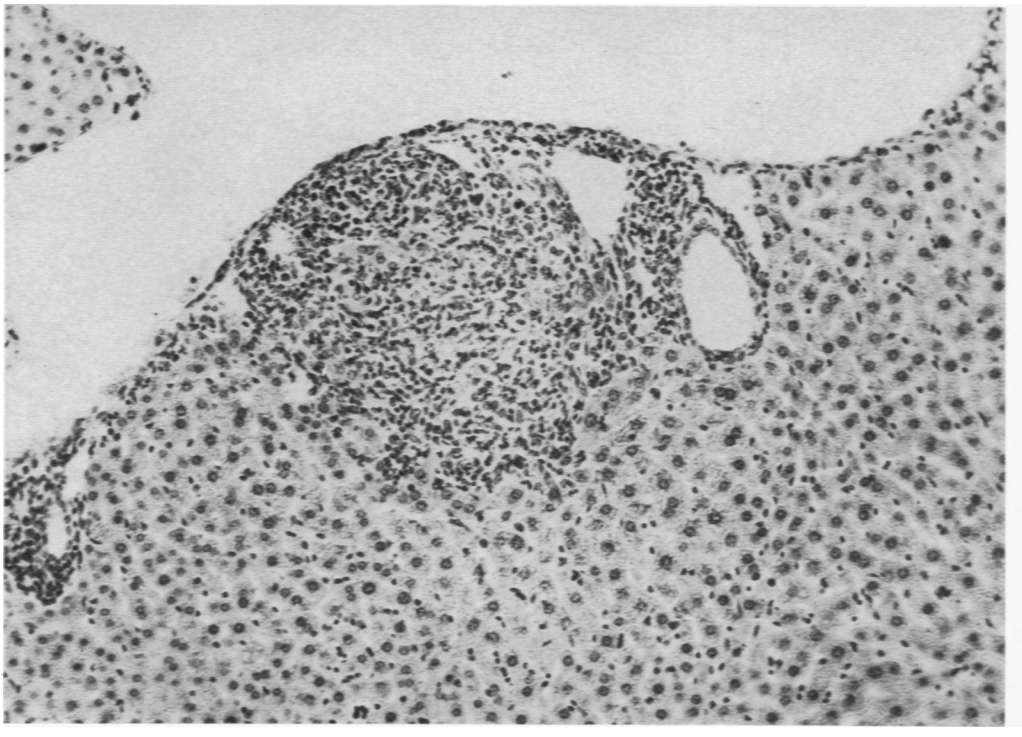

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