TISSUE MAST CELLS AND ACUTE INFLAMMATION IN EXPERIMENTAL CUTANEOUS MUCORMYCOSIS OF NORMAL, 48/80-TREATED, AND DIABETIC RATS*

By WALTER H. SHELDON,‡ M.D., and HEINZ BAUER, M.D.

(From the Department of Pathology, Emory University School of Medicine, Atlanta)

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The tissue mast cells in the rat have been shown to play a part in the development of hyperemia, vascular permeability, edema formation, and wound healing (1–3). It appears that these processes, which are essential components of the normal inflammatory and reparative reaction, are elicited by the release of substances located in or on the cytoplasmic granules of the tissue mast cells. In the rat these substances have been identified as histamine, heparin, and serotonin (5-hydroxytryptamine) (4, 5). It has also been shown that the tissue content of histamine closely parallels the number and granularity of the tissue mast cells and that histamine, heparin, and serotonin are released into the tissues when the tissue mast cells are depleted of their granules by various experimental means (1, 4).

The following experiments were devised to study in three different biologic settings the role of the tissue mast cells in the development of acute inflammation. The first experiment was designed to obtain information in normal rats regarding the behavior of these cells within the general framework of acute inflammation in response to a localized infection. In the second experiment the relation between the inflammatory reaction and infection was investigated in animals pretreated with a compound which temporarily depletes the tissue mast cells of their granules and chemical constituents but is not otherwise toxic for the rat. In the third experiment the influence of a severe, general alteration of host metabolism on the susceptibility to this infection in general and on the tissue mast cells in particular was studied and for this purpose acute alloxan diabetes with acidosis was produced. The same infection was employed in all animals and consisted of the subcutaneous injection of the fungus *Rhizopus oryzae* since previous studies had shown that in the normal host mucormycotic infection is self-limiting and remains confined to the site of inoculation while in animals with acute alloxan diabetes and acidosis the infection progresses rapidly and spreads widely (6, 7). The results of the experiments were evaluated by histologic examination of the mucormycotic skin lesions.

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‡ Present address: Department of Pathology, Johns Hopkins Hospital, Baltimore.

Methods

White, female, Sprague-Dawley rats, ranging in weight from 170 to 200 gm., were used. All animals were inoculated subcutaneously under light ether anesthesia in two sites of the shaved back with 0.2 ml. of a standardized spore suspension of *Rhizopus oryzae* to which 1 per cent of sterilized India ink had been added to facilitate the identification of the inoculum in the tissues[1] (6). The inoculum was placed into the layer of subcutaneous tissue immediately beneath the corium.

The first group consisted of 30 normal rats which were inoculated as described. Three rats each were sacrificed with ether anesthesia at 10, 20, and 30 minutes and at 1, 2, 4, 6, 12, 24, and 48 hours after inoculation. Terminal blood sugar levels were done on blood obtained by cardiac puncture and repeated determinations of urine sugar and acetone were performed on one or more animals for each time interval (6).

The second group of 40 rats was given intraperitoneal injections of the histamine-liberator compound 48/80, a condensation of *p*-methoxyphenethylamine with formaldehyde, kindly supplied by Burroughs, Wellcome, and Company, Inc., Tuckahoe, New York. The compound was given intraperitoneally, as described by Riley, twice daily for 5 days beginning with an injection of 100 micrograms and increasing the dose by 100 micrograms each day reaching a final dose of 500 micrograms on the 5th day (4). The animals were inoculated with fungus as described above 18 hours after the last injection of the compound and were sacrificed in the same manner, and at the same time intervals after inoculation as the previous group. Blood and urine determinations for sugar and acetone were performed as described above.

The third group consisted of 40 rats in which acute alloxan diabetes with acidosis was produced by the injection into the tail vein of 150 to 200 mg. per kg. body weight of alloxan dissolved in normal sterile saline (0.9 per cent NaCl). Hyperglycemia, glycosuria, and acetonuria were determined as previously described (6). All animals were inoculated when acetonuria was 3 plus or greater. Four rats each were sacrificed in the same manner and at the same time intervals as in the other groups.

No chemical determinations for histamine, heparin, or serotonin were done on these animals. However, in a preliminary study, the virtually complete degranulation of the tissue mast cells following treatment with 48/80 was demonstrated in two groups of 3 rats each injected with the compound as described above and sacrificed 18 and 66 hours after the last administration of the compounds.

Five rats not included in the third group were rendered diabetic and acidotic by the injection of alloxan but received no fungus inoculation. In these animals the tissue mast cells were studied histologically at various times ranging from about 2½ to 26 hours after the onset of marked acetonuria. The tissue mast cells showed no loss of cytoplasmic granules or other morphologic changes.

The skin lesions with the underlying soft tissues were excised and one lesion from each rat was fixed in Helly's fluid and the other was fixed either in buffered 10 per cent formalin or in absolute alcohol. Complete autopsies excluding the central nervous system were performed and representative slices of heart, lung, spleen, pancreas, liver, small intestine with attached mesentery, adrenal, with surrounding retroperitoneal tissues, and kidney were fixed in the same manner as described. Each skin lesion, including a wide margin of uninvolved tissues, was examined with multiple blocks and sections. The histologic preparations were stained with Giemsa.

In preliminary studies on normal rats it had been shown that histologic preparations of

[1] We are indebted to Dr. L. Ajello, Chief, Mycology Section, Communicable Disease Center, Department of Health, Education and Welfare, Chamblee, Georgia, for the preparation of the fungus spore suspension.

tissues fixed in Helly's fluid and stained with Giemsa were as satisfactory for the study of the tissue mast cells as preparations fixed in buffered 10 per cent formalin or absolute alcohol and stained with an 0.1 per cent aqueous solution of toluidine blue. It was also ascertained that sacrifice by ether anesthesia as compared to decapitation without anesthesia did not affect the distribution, number, or appearance of the tissue mast cells.

The histologic findings were arbitrarily graded from 0 to 4 plus for the following features: congestion and edema, cellular response, decrease in number and granularity of the demonstrable tissue mast cells as compared with those of normal uninjured subcutaneous tissue and the degree of fungus proliferation. The evaluation of the cellular response included margination and diapedesis of granulocytes, infiltration of tissues by neutrophiles and eosinophiles, their clustering around the fungus and carbon particles which was regarded as evidence of phagocytosis, and proliferation of large mononuclear cells and fibroblasts.

Morphologic Observations

Normal Rats.—No abnormal gross findings were noted at autopsy. Blood sugar as well as urine sugar and acetone determinations were not remarkable. The data regarding the histologic findings in the skin lesions are summarized in Table I.

At 10 minutes after inoculation, the site of injection consisted of a deposit of spores and carbon particles displacing the connective tissue elements some of which were disrupted. All animals showed early margination and diapedesis of neutrophiles in capillaries adjacent to the inoculum. At 20 minutes, neutrophilic margination and exudation into the tissues had noticeably increased in extent and degree, had become quite marked, and an occasional eosinophile was seen in the capillaries and tissues (Fig. 1). At 1 hour the neutrophile response had reached its maximum extent and increasing numbers of neutrophiles and some eosinophiles appeared in the tissues around the inoculation site. At the same time, the first traces of clustering of neutrophiles occurred around spores and carbon particles at the periphery of the inoculum. At 2 hours, tissue infiltration and clustering around the inoculum had become more marked while granulocytic margination had decreased. At 4 hours, the acute inflammatory response had become quite intense and reached its maximum at 6 hours when the entire inoculum was completely surrounded and infiltrated by masses of neutrophiles and some eosinophiles. The latter participated in the clusters around the inoculum. Thus, the inoculation site was transformed into an abscess and at the periphery of the inflamed area early but distinct proliferation of mononuclear cells and fibroblasts was seen. At 12 hours, polymorphonuclear leukocytic margination had virtually subsided and granulocytes were less numerous in the tissues around the abscess in the center of which these cells began to show regressive changes At this time, a beginning demarcation of the abscess by large mononuclear cells and some fibroblasts was found. At 24 hours, leukocytic margination had stopped. In the abscesses the neutrophiles and eosinophiles were degenerating and at the periphery they were being supplanted by an increasing number of fibroblasts and large mononuclear cells which began to form the outermost wall

of the abscess. Some plasma cells were now present at the periphery of the inflamed area. At 48 hours, large mononuclear cells and fibroblasts had proliferated further and now formed a distinct wall around the purulent core (Fig. 2).

TABLE I

Normal Rats

Time	Rat No.	Congestion*	Edema*	Granulocytes				Tissue Mast Cells‡		Fibroblasts	Mononuclears	Fungus
				Margin.	Exud.	Phagoc.	Eosinoph.	Decreased				
								No.	Granularity			
10 min.	16-65	++	++	+	+	0	0	+	++	0	0	0
	16-68	++	++	+	+	0	0	+	++	0	0	0
	16-93	++	++	+	+	0	0	++	++	0	0	0
20 "	16-70	+++	+++	++	++	0	+	+++	+++	0	0	0
	16-92	+++	+++	++	++	0	+	++	++	0	0	0
	16-94	+++	+++	+++	++	0	++	++	++	0	0	+
30 "	16-66	+++	+++	+++	+++	0	++	+++	+++	0	0	0
	16-84	+++	+++	+++	+++	0	++	+++	+++	0	0	0
	16-95	+++	+++	+++	+++	0	++	+++	+++	0	0	0
1 hr.	16-64	++++	++++	+++	+++	+	+++	+++	+++	0	0	0
	16-67	+++	++++	++	++++	+	+++	+++	+++	0	0	0
	16-83	++	+++	+++	++++	+	+++	+++	+++	0	0	0
2 hrs.	16-69	++	+++	++	++++	++	+++	++	++	+	0	0
	16-85	++	++	++	++++	++	+++	++	++	+	+	0
	16-91	++	++	++	++++	++	+++	++	++	+	+	+
4 "	16-53	++	++	+	++++	+++	+++	+	+	+	+	++
	16-54	++	++	+	++++	+++	+++	+	+	++	++	++
	16-55	++	++	+	++++	+++	++++	+	+	++	++	++
6 "	16-61	+	++	+	++++	++++	++++	+	+	++	++	++
	16-62	+	+	++	++++	++++	++++	+	+	++	++	++
	16-63	+	+	+	++++	++++	++++	+	+	++	++	++
12 "	16-50	+	+	+	+++	++++	+++	0	0	++	++++	++
	16-51	+	+	+	+++	++++	++++	0	0	++	++++	++
	16-52	+	+	+	+++	++++	++++	0	0	++	++++	++
24 "	16-33	+	+	0	++	++++	++++	0	0	+++	++++	++
	16-48	+	+	0	++	++++	++++	0	0	+++	++++	++
	16-49	+	+	0	++	++++	++++	0	0	+++	++++	++
48 "	16-45	+	+	0	+	++++	0	0	0	+++	++++	++
	16-46	+	++	0	+	++++	+	0	0	+++	++++	++
	16-47	+	+	0	+	++++	+	0	0	+++	++++	++

* Denotes extent and degree.

‡ Compared with normal uninjured subcutaneous tissue.

At 2 hours some active spores and at all later times some early mycelia formation was found. The fungus always remained confined to the site of inoculation and never invaded adjacent tissues or blood vessels.

At 10 minutes the tissue mast cells in and around the inoculation site, when compared with those of other parts of the same section, appeared less numerous and showed a beginning loss of their cytoplasmic granules (Fig. 3). The latter

was manifested by the presence of basophilic granules apparently free within the tissue around the mast cells which showed correspondingly less granules within the cytoplasm. At 20 minutes this change had spread centrifugally for a short distance around the inoculation site and appeared to have reached its maximal extent and degree at 30 minutes. By that time, virtually no recognizable tissue mast cells were found in the center of the lesions while, more peripherally, only degranulated mast cells were seen which displayed a small dense nucleus and violescent cytoplasm. At the periphery of the lesion a belt containing both normal appearing and partly degranulated tissue mast cells was seen. No further changes in the mast cells were noted until 2 hours after inoculation except for the clustering of a few neutrophiles and rare eosinophiles around an occasional degranulated mast cell. At 2 hours the tissue mast cells at the periphery of the lesion seemed to increase slightly in number and granule content and no basophilic granules were present outside their cytoplasm. At 4 and 6 hours these cells at the periphery of the lesion had resumed a virtually normal appearance and number but none could be found either within or in the immediate vicinity of the abscesses. At 12 hours, however, granulated tissue mast cells were encountered in close proximity to the abscess wall. At 24 and 48 hours no appreciable differences in the tissue mast cells of the lesions were noted in comparison with the uninvolved areas of the section except for the continued absence of these cells among the contents of the abscesses (Fig. 2). All the above described changes in the tissue mast cells occurred only at the inoculation site and its immediate vicinity.

Appreciable edema and congestion were seen at the inoculation site at 10 minutes, increased at 20 minutes, and had reached their peak at 30 minutes and 1 hour. Thereafter, they decreased and after 6 hours only slight congestion and edema remained in the lesions.

Rats Treated with 48/80.—The gross autopsy findings as well as blood and urine determinations for sugar and acetone were not remarkable. The histologic findings in the skin lesions are summarized in Table II.

At 10 minutes after inoculation, the inoculum in the tissues presented a similar appearance to that seen in the previous group. At its periphery an occasional capillary showed a few marginating neutrophiles and at 20 minutes the first evidence of minimal exudation of these cells was observed. At 30 minutes and 1 hour neutrophilic margination and exudation had increased slightly in degree and extent (Fig. 4) and had become somewhat more widespread 2 and 4 hours after inoculation but the intensity of the neutrophile response did not increase until 6 hours. After 12 hours margination had ceased while tissue infiltration remained unchanged. Clustering of neutrophiles around spores and carbon particles first occurred at 2 hours after inoculation, increased at 4 and 6 hours (Fig. 5) and had reached its peak at 12 hours when all the inoculum was enveloped by

TABLE II

Rats Treated with 48/80

Time	Rat No.	Congestion*	Edema*	Granulocytes				Tissue Mast Cells‡		Fibroblasts	Mononuclears	Fungus
				Margin.	Exud.	Phagoc.	Eosinoph.	Decreased No.	Decreased Granularity			
10 min.	17-36	0	0	0	0	0	0	++++	++++	0	0	0
	17-46	0	0	0	0	0	0	++++	++++	0	0	0
	17-47	0	0	0	0	0	0	++++	++++	0	0	0
	17-65	0	0	0	0	0	0	++++	++++	0	0	0
20 "	17-29	+	+	+	+	0	0	++++	++++	0	0	0
	17-40	+	+	+	+	0	0	++++	++++	0	0	0
	17-54	+	+	+	+	0	+	++++	++++	0	0	0
	17-64	+	+	+	+	0	0	++++	++++	0	0	0
30 "	16-31	+	+	++	+	0	+	++++	++++	0	0	0
	17-34	+	+	++	+	0	+	++++	++++	0	0	0
	17-45	+	+	+	+	0	+	++++	++++	0	0	0
	17-60	+	+	++	+	0	+	++++	++++	0	0	0
1 hr.	16-30	+	+	++	++	0	+	++++	++++	0	0	0
	17-52	+	+	++	+	0	+	++++	++++	0	0	0
	17-55	+	+	++	+	0	+	++++	++++	0	0	0
	17-63	+	+	++	+	0	+	++++	++++	0	0	0
2 hrs.	17-28	++	++	++	++	+	+	++++	++++	0	+	+
	17-33	++	++	++	++	+	+	++++	++++	0	+	+
	17-41	++	++	++	+	+	+	++++	++++	0	+	+
	17-61	++	++	++	+	+	+	++++	++++	0	+	+
4 "	17-27	+	+	++	++	++	+	++++	++++	0	++	++
	17-31	+	+	+	++	++	+	++++	++++	0	++	++
	17-39	+	+	++	++	+	+	++++	++++	0	++	++
	17-44	+	+	++	++	++	+	++++	++++	0	++	++
6 "	17-26	++	++	++	+++	+++	+++	++++	+++	0	+++	++
	17-30	++	++	++	+++	+++	++	++++	+++	0	+++	++
	17-37	++	++	++	+++	+++	+++	++++	+++	0	+++	++
	17-42	++	++	++	+++	+++	++	++++	+++	0	+++	++
12 "	17-50	+	+	+	+++	++++	++	++++	+++	0	+++	++
	17-53	+	+	+	+++	++++	++	++++	+++	0	+++	++
	17-56	+	+	+	+++	++++	++	++++	+++	0	+++	++
	17-58	+	+	+	+++	++++	++	++++	+++	0	+++	++
24 "	17-32	+	+	0	+++	++++	+	++++	++	0	+++	++
	17-35	+	+	0	+++	++++	+	++++	++	0	+++	++
	17-59	+	+	0	+++	++++	+	++++	++	0	+++	++
	17-62	+	+	0	+++	++++	+	++++	++	0	+++	++
48 "	17-38	+	0	0	+++	++++	0	++++	++	++	++++	++
	17-43	+	0	0	+++	++++	0	++++	++	++	++++	++
	17-51	+	0	0	0	++++	0	++++	++	++	++++	++
	17-57	+	0	0	0	++++	0	++++	++	++	++++	++

* Denotes extent and degree.

‡ Compared with normal uninjured subcutaneous tissue.

the inflammatory cells. Thus the lesions became circumscribed and at 24 and 48 hours appeared as well defined abscesses (Fig. 6).

The demarcation of the lesions was enhanced by the presence of mononuclear cells which first appeared in the periphery at 2 hours and increased at 6 hours

after inoculation. By 12 hours these cells constituted the major portion of the peripheral inflammatory response and at 24 and even more at 48 hours formed a distinct belt surrounding the entire lesion. Some plasma cells and lymphocytes were also present.

Fibroblasts did not appear to participate in the cellular response around the lesions during the first 24 hours after inoculation. Some degree of fibroblastic proliferation was seen only at 48 hours when it contributed to the demarcation of the lesions.

The first indications of fungus growth, consisting of active and budding spores in the center of the inoculum, were noted at 2 hours after inoculation. Thereafter, mycelial growth was present in all lesions but always remained confined to the necrotic center. Spread to adjacent tissues or distant sites was never observed. At 24 and 48 hours many spores appear to be degenerating.

Normal appearing tissue mast cells were not seen in the histologic preparations of the tissues at any of the time intervals, but cells resembling degranulated tissue mast cells containing rarely a few basophilic granules were seen in the subcutaneous tissue in their usual locations. However, at 6 and 12 hours after inoculation which was 24 and 30 hours after the last injection of compound 48/80, a slightly larger number of cells with the appearance and staining qualities of tissue mast cells and containing somewhat larger numbers of basophilic cytoplasmic granules were encountered in the uninvolved subcutaneous tissue. A similar appearance of the tissue mast cells in other sites was not observed until 24 hours following inoculation. The number and granularity of these cells had increased slightly at 24 and 48 hours after inoculation but a restitution of the normal incidence and morphology of these cells was never attained within the period of time of this experiment. Extracellular basophilic granules bearing any relationship to the tissue mast cells were never observed.

The peritoneal surfaces and underlying tissues in all animals revealed a slight reaction consisting of mesothelial and mononuclear cell proliferation. Scattered eosinophiles were also present some of which were degenerating and had been phagocytized by large mononuclear cells. The peritoneal reaction had become less marked at 6 hours after inoculation (24 hours after the last injection of 48/80) and progressively diminished thereafter.

In the skin lesions rare eosinophilic granulocytes were first seen in the capillaries at 20 minutes and in the tissues at 30 minutes after inoculation. These cells did not increase until 6 hours after inoculation at which time they formed part of the inflammatory infiltrate. Their number had decreased by 24 hours and at 48 hours they were no longer seen. Phagocytosis of eosinophiles by mononuclear cells was not observed in the skin lesions.

Congestion and edema at the inoculation site were not seen until 20 minutes after inoculation and were of minimal extent and degree until 2 hours. At 4 and 6 hours congestion and edema had increased somewhat in extent but decreased again thereafter.

TABLE III

Diabetic Rats

Time	Rat No.	Congestion*	Edema*	Granulocytes				Tissue Mast Cells‡		Fibroblasts	Mononuclears	Fungus
				Margin.	Exud.	Phagoc.	Eosinoph.	Decreased				
								No.	Granularity			
10 min.	16-98	+	+	0	0	0	0	0	0	0	0	0
	17-01	+	+	0	0	0	0	0	0	0	0	0
	17-18	+	+	0	0	0	0	0	0	0	0	0
	17-24	+	+	0	0	0	0	0	0	0	0	0
20 "	16-99	+	+	0	0	0	0	0	0	0	0	0
	17-17	+	+	0	0	0	0	0	0	0	0	0
	17-22	+	+	0	0	0	0	0	0	0	0	0
	17-25	+	+	0	0	0	0	0	0	0	0	0
30 "	17-07	+	+	0	0	0	0	0	0	0	0	0
	17-10	+	+	+	0	0	0	0	0	0	0	0
	17-20	+	+	+	+	0	0	0	0	0	0	0
	17-23	++	++	+	+	0	0	0	0	0	0	0
1 hr.	17-12	++	++	+	+	0	0	0	0	0	0	0
	17-15	++	++	+	+	0	0	0	0	0	0	0
	17-16	++	++	+	+	0	0	0	0	0	0	+
	17-21	++	++	+	+	0	0	0	0	0	0	0
2 hrs.	17-05	++	++	++	++	0	0	0	0	0	0	0
	17-09	+	+	+	+	0	0	0	0	0	0	0
	17-13	++	++	++	++	0	0	0	0	0	0	+
	17-19	++	++	++	++	0	0	0	0	0	0	+
4 "	16-96	++	++	++	++	0	+	0	0	+	+	++
	17-04	++	++	++	++	0	+	0	0	+	+	++
	17-06	++	++	++	++	0	+	0	0	+	+	++
	17-14	++	++	++	++	++	+	0	0	+	+	++
6 "	17-00	++	++	++	+++	++	+	0	0	+	+	++
	17-03	++	++	+++	+++	++	+	0	0	+	+	++
	17-08	++	++	++	+++	++	+	0	0	+	+	+++
	17-11	++	++	++	+	++	+	0	0	+	+	+++
12 "	16-32	+	+++	+	++++	++++	+	+§	+§	+	++	++++
	16-34	+	+++	+	++++	++++	+	+	+	++	++	++++
	16-39	+	+++	+	++++	++++	+	+	+	++	++	++++
	16-42	+	+++	+	++++	++++	+	+	+	++	++	++++
24 "	16-19	+	+++	+	++++	++++	+	+	+	+++	+++	++++
	16-21	+	+++	+	++++	++++	+	+	+	+++	+++	++++
	16-23	+	+++	+	++++	++++	+	+	+	+++	+++	++++
	16-24	+	+++	+	++++	++++	+	+	+	+++	+++	++++
48 "	16-22	+	+++	+	++++	++++	0	+	+	+++	+++	++++
	16-40	+	+++	+	++++	++++	0	+	+	+++	+++	++++
	16-97	+	+++	+	++++	++++	0	+	+	+++	+++	++++
	17-02	+	+++	+	++++	++++	0	+	+	+++	+++	++++

* Denotes extent and degree.

‡ Compared with normal uninjured subcutaneous tissue.

§ Refers to disruption in necrotic areas of lesions.

Diabetic Rats.—All animals in this group had terminal blood sugar levels above 400 mg. per cent as well as 4 plus glycosuria and acetonuria. The histologic findings in the lesions are summarized in Table III.

At 10 and 20 minutes no discernible reaction was found at and around the

inoculation site which showed only spores and carbon particles displacing the connective tissue elements (Fig. 7). At 30 minutes slight neutrophilic margination and exudation into the tissues were seen in an occasional capillary at the periphery of the inoculum. At 1 hour this process involved more vessels but had not increased in intensity. At 2 and 4 hours both the extent and degree of the neutrophile response had become somewhat more marked but without appreciable infiltration of the inoculum. A further increase of neutrophile exudation was present at 6 hours when a moderate degree of clustering of the inflammatory cells around the spores and carbon particles was first observed. At the periphery of the lesions beginning proliferation of a few fibroblasts and large mononuclear cells was seen, but the lesions failed to show demarcation. Fungus growth, which was first noted at 2 hours and had increased at 4 hours, was now becoming more active with beginning invasion of adjacent tissues. At 12 hours the neutrophile reaction was marked and had reached its maximal intensity and the response by fibroblasts and mononuclear cells had also increased somewhat. The lesions, how ever, continued to spread with massive mycelial growth and invasion of deep tissues including large blood vessels (Fig. 8). The center of the lesions showed tangles of hyphae and some carbon particles against a background of necrotic tissue without any inflammatory cells. More peripherally, a dense belt of neutrophiles was seen mixed with many hyphae and carbon particles. Most of the neutrophiles showed some regressive changes consisting of nuclear pyknosis and some karyorrhexis. At 24 and 48 hours no change in the neutrophile response was noted but there was a further increase in the proliferation of fibroblasts and mononuclear cells without demonstrable effect on the continuing spread of the infection. A few eosinophiles were noted among the granulocytic infiltrate at 4 hours. These cells were never present in large numbers and were no longer found in the lesions of 48 hours duration. Occasional plasma cells appeared among the mononuclear cells at 48 hours.

The tissue mast cells at the inoculation site and throughout the uninvolved portions of the sections showed no changes at any time during the course of the experiment in their number, distribution, and appearance (Fig. 7) except for those along the needle tract leading to the inoculum. Here an occasional tissue mast cell was found surrounded by a few basophilic granules but the cells from which the granules appeared to have originated showed no appreciable changes. Until 1 and 2 hours after inoculation, no other changes were seen in these cells anywhere (Fig. 9). At that time, the tissue mast cells at the site of the inoculum were less numerous and a few appeared pale blue with diminished staining intensity of their cytoplasmic granules. No granules were found free in the tissues around these cells. At 4 and 6 hours, while the center of the lesions enlarged with the spread of the infection, correspondingly more tissue mast cells showed the above described changes, but the majority of the tissue mast cells at the inoculation site and elsewhere appeared morphologically normal. At 12, 24, and 48 hours fully granulated and normally staining tissue mast cells were seen in vari-

ous stages of disintegration in the necrotic core of the lesions (Fig. 10). In the immediately adjacent belt of inflammatory cell infiltration and fungus proliferation as well as in the advancing edge of the lesions the tissue mast cells were normal in number, distribution, and appearance.

Appreciable edema and congestion were not found until 1 hour after inoculation. Congestion was somewhat increased at 2, 4, and 6 hours after inoculation but was never marked and was diminished at 12, 24, and 48 hours. Edema increased somewhat at 2, 4, and 6 hours and was fairly marked at 12, 24, and 48 hours after inoculation.

DISCUSSION

The results of the first experiments indicate that in normal rats the tissue mast cells play a part in the development of the acute inflammatory response which rapidly contains and suppresses mucormycotic infection. Within minutes after the fungus injection the site of inoculation and its immediate periphery show degranulation of the tissue mast cells as well as the rapid development of edema, congestion, and of granulocytic margination. While the degranulation of tissue mast cells progresses and spreads centrifugally for a short distance, reaching its peak at 30 minutes, the vascular changes and the margination of granulocytes also continue to increase. After the discharge of granules from the tissue mast cells has ceased, congestion, edema, and margination persist for a short period of time and decrease thereafter. Granulocytic exudation, infiltration of tissues and, somewhat later, clustering around the inoculum increase progressively until 6 hours at which time the lesions have become circumscribed and begin to assume the appearance of abscesses. Eosinophilic leukocytes appear early among the exudate, are present in large numbers from 6 to 24 hours after inoculation, and virtually disappear thereafter. The demarcation of the lesions is further enhanced by the proliferation of large mononuclear cells and fibroblasts which begins at 4 hours after inoculation, forms a distinct peripheral zone at 12 hours and sharply circumscribes the lesions at 48 hours. After initial degranulation cytoplasmic granules begin to reappear in the tissue mast cells at the periphery of the lesions at 2 hours. These cells continue to increase in number and granularity of the cytoplasm and by 12 hours their incidence and appearance, except in the necrotic center of the lesions are similar to those of the adjacent uninvolved tissue. Fungus proliferation consists only of budding spores and occasional mycelia formation which always remained confined to the core of the lesions.

In the second experiment the absence of tissue mast cell granules results in a delayed onset and diminished intensity of the acute inflammatory response and a slight, transient enhancement of the infection. Virtually no granulated tissue mast cells are present anywhere at the time of fungus inoculation in rats pretreated with 48/80. In these animals minimal congestion, edema, and granulo-

cytic margination and exudation first appear at 20 minutes and do not increase appreciably until 2 hours after inoculation at which time the earliest evidence of granulocytic clustering around the inoculum is seen. Exudation and envelopment of the inoculum by granulocytes do not reach a significant degree until 6 hours and attain their maximum at 12 hours after inoculation. The eosinophile response in the lesions is also slightly delayed and distinctly decreased in intensity. By 48 hours the lesions are localized and circumscribed abscesses. Budding spores appear among the inoculum somewhat earlier and in larger numbers than in the normal rats, but the fungus growth fails to progress and in the later stages only small numbers of budding spores and occasional early mycelia are found confined to the center of the lesions.

In contrast to the delay and diminution of the granulocytic response in this group, the large mononuclear cells appear at the periphery of the inoculation site at the same time as in the normal rats and contribute increasingly to the demarcation of the lesions. However, there is a striking absence of demonstrable fibroblastic proliferation until 48 hours after inoculation. Rare, partly granulated tissue mast cells begin to reappear in the subcutaneous tissues 6 hours after inoculation and slightly increase thereafter without ever approaching the number and granularity of normal tissue mast cells or showing evidence of granule discharge. In sites other than the subcutaneous tissue, the reappearance of cytoplasmic granules in these cells does not begin until 24 hours after inoculation.

The third experiment shows that a severe alteration of host metabolism impairs the function of the tissue mast cells, interferes with the inflammatory response and increases the susceptibility to infection. In the diabetic rats with acidosis in which the tissue mast cells at and around the inoculation site and elsewhere show no detectable discharge of granules, the onset and intensity of the acute inflammatory response are markedly delayed and diminished. Congestion and edema reach a significant degree only at 1 hour after inoculation, but considerable edema related to the rapid spread of the lesions is present at 12, 24, and 48 hours. Clustering of granulocytes around the inoculum is markedly delayed and obviously ineffective as shown by the spreading character of the fungus lesions. Eosinophilic granulocytes appear late and are always scarce. At 6 hours after inoculation many budding spores and mycelia are present throughout the entire extent of the lesions and fungus proliferation rapidly progresses thereafter. Large mononuclear cells begin to participate in the reaction of the diabetic rats at about the same time, but in somewhat lesser numbers than in the two other groups. The appearance of fibroblastic proliferation at the periphery of the lesions in the diabetic animals occurs somewhat later than in normal rats, but much earlier than in those pretreated with compound 48/80. Despite the proliferation of large mononuclear cells and fibroblasts, the lesions of the diabetic animals continue to spread with massive fungus invasion of adjacent

tissues and blood vessels and never assume the self-limiting character of those in the other 2 groups of animals. In striking contrast to the rapid granule discharge following injury to the subcutaneous tissues of normal rats this response of the tissue mast cells completely fails in and around the spreading lesions of the diabetic rats. These cells remain as numerous and as heavily granulated as those in the adjacent uninjured tissues and normal appearing tissue mast cells continue to be present throughout the entire experiment even in the necrotic areas of the lesions where eventually they show necrosis with disruption of the cytoplasm but no discharge of granules.

Our findings suggest that in the normal rat the rapidity with which acute inflammation follows injury of the subcutaneous tissues, is related to the presence and ready discharge of the cytoplasmic granules from the tissue mast cells. The granule discharge appears to be the morphologic manifestation of the release of histamine and 5-hydroxytryptamine which have been shown to initiate hyperemia and increased vascular permeability (1, 4). These processes precede or coincide with granulocytic margination and exudation; in short, the acute inflammatory response. In the normal rat this reaction rapidly increases in extent and intensity and quickly localizes and soon suppresses cutaneous mucormycotic infection.

The benign course of mucormycotic infection in the rats pretreated with compound 48/80 indicates that the pharmacologically induced degranulation of the tissue mast cells does not constitute a major disturbance of host metabolism since spreading mucormycosis occurs only in severe metabolic disorders (7). However, the inflammatory response in animals pretreated with 48/80 is somewhat delayed and of decreased intensity and the early lesions show an increase in fungus proliferation. It appears that the absence of available tissue mast cell granules affects only the earliest phase of acute inflammation and that, when these cells are unable to function, another mechanism initiates the inflammatory response (2, 8). This altered response is adequate to suppress mucormycotic infection, but the outcome under otherwise similar conditions might be less favorable in infections with organisms of greater pathogenicity.

Mucormycotic infection in the diabetic and acidotic rats produces rapidly spreading lesions which are characterized by a markedly delayed onset and decreased intensity of the acute inflammatory response and by massive fungus growth with invasion of adjacent tissues and blood vessels. The increased susceptibility of the diabetic and acidotic host seems in part related to the retardation of the acute inflammatory response which in the rat appears to result from an impairment of the tissue mast cells. These cells completely fail to discharge their cytoplasmic granules which probably initiate inflammation in the metabolically normal rat. The behavior of the infection in the diabetic animals resembles that previously observed in normal and diabetic rabbits and represents a striking example of the influence of host metabolism on resistance to infection (6).

These findings indicate one effect of altered host metabolism on the complex of reactions by which the host responds to infection, but the nature of the underlying biochemical defect remains unknown. It is clear from many studies that lowered host resistance to infection resulting from altered host metabolism cannot be attributed to a single factor acting upon one particular mechanism but probably represents the summation of multiple factors interfering with many aspects of the body defenses (6, 9, 10).

Although our observations do not confirm the finding of others that eosinophilic granulocytes phagocytize discharged tissue mast cell granules, a relationship of eosinophiles to the activity of the tissue mast cells appears to exist and may be related to the histamine content of the extracellular mast cell granules (11). In normal rats in which the tissue mast cells discharge their granules and presumably their histamine, many eosinophiles are present in the granulocytic response. In the diabetic animals in which the tissue mast cells retain their granules, few eosinophiles are encountered in the cellular exudate. In rats in which the tissue mast cells have been depleted of their granules, the eosinophile response in the lesions is less marked than in the normal rats, but more pronounced than in the diabetic host. An eosinophile response in the absence of tissue mast cell granules may be attributed to a residual blood eosinophilia which had occurred in response to the histamine liberated by the repeated injections of 48/80 (12).

An interesting observation, incidental to the design of these experiments, is the marked delay of fibroblastic proliferation in the lesions of the rats pretreated with 48/80. The retardation of the fibroblastic reaction suggests that one of the functions of the tissue mast cells affects the fibroblastic response. Delayed healing of experimentally produced wounds has been shown to occur in rats in which the tissue mast cells had been depleted of their cytoplasmic granules by the administration of compound 48/80 (3).

SUMMARY

The role of the tissue mast cells in relation to the acute inflammatory reaction to experimental cutaneous mucormycosis was studied histologically in normal rats, in animals whose tissue mast cells had been depleted of their cytoplasmic granules prior to infection by the administration of compound 48/80 and in others in whom acute alloxan diabetes with acidosis had been produced before injection of the fungus.

The discharge of the tissue mast cell granules in normal rats occurred within minutes at the site of infection and appeared to initiate the rapid onset of acute inflammation. The degranulation of the tissue mast cells subsided in a short time and the cells reassumed a normal histologic appearance while inflammation progressed with the formation of circumscribed lesions. In animals pretreated with compound 48/80 in which the tissue mast cells contained no granules, the

onset of inflammation was briefly delayed, the intensity of the process was somewhat decreased, fibroblastic proliferation was retarded, and the fungus growth in the early lesions was increased. However, the infection did not spread and the lesions were well localized. The tissue mast cells in the diabetic and acidotic rats completely failed to discharge their cytoplasmic granules, the onset and intensity of the acute inflammatory response were markedly delayed and decreased and the infection progressed rapidly with massive fungus growth invading adjacent tissues. A relationship between the discharged tissue mast cell granules and eosinophilic granulocytes was noted since the latter were numerous among the inflammatory cell exudate in normal rats and scarce in the lesions of the diabetic animals.

It is concluded that a function of the tissue mast cells in the normal rat is the rapid initiation of acute inflammation at the site of injury and that degranulation of these cells prior to infection somewhat delays the inflammatory response and therefore slightly diminishes host resistance. Furthermore, a severe metabolic disorder such as acute alloxan diabetes with acidosis, inhibits the normal function of the tissue mast cells, delays and decreases inflammation, and in this manner contributes to the greatly increased susceptibility of the host to infection.[2]

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[2] The technical assistance of Miss Hillma Gheesling and Mrs. Elaine S. Kester is gratefully acknowledged.

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EXPLANATION OF PLATES

PLATE 87

FIG. 1. Normal rat, 30 minutes after inoculation. Congested venule with granulocytic diapedesis and early tissue infiltration. Discharge of cytoplasmic granules from three tissue mast cells (arrows). Giemsa. × 630.

FIG. 2. Normal, 48 hours after inoculation. Segment of abscess wall with outer layer of large mononuclear cells and fibroblasts. Fully regranulated tissue mast cells (arrows) at and near wall of lesion. Giemsa. × 300.

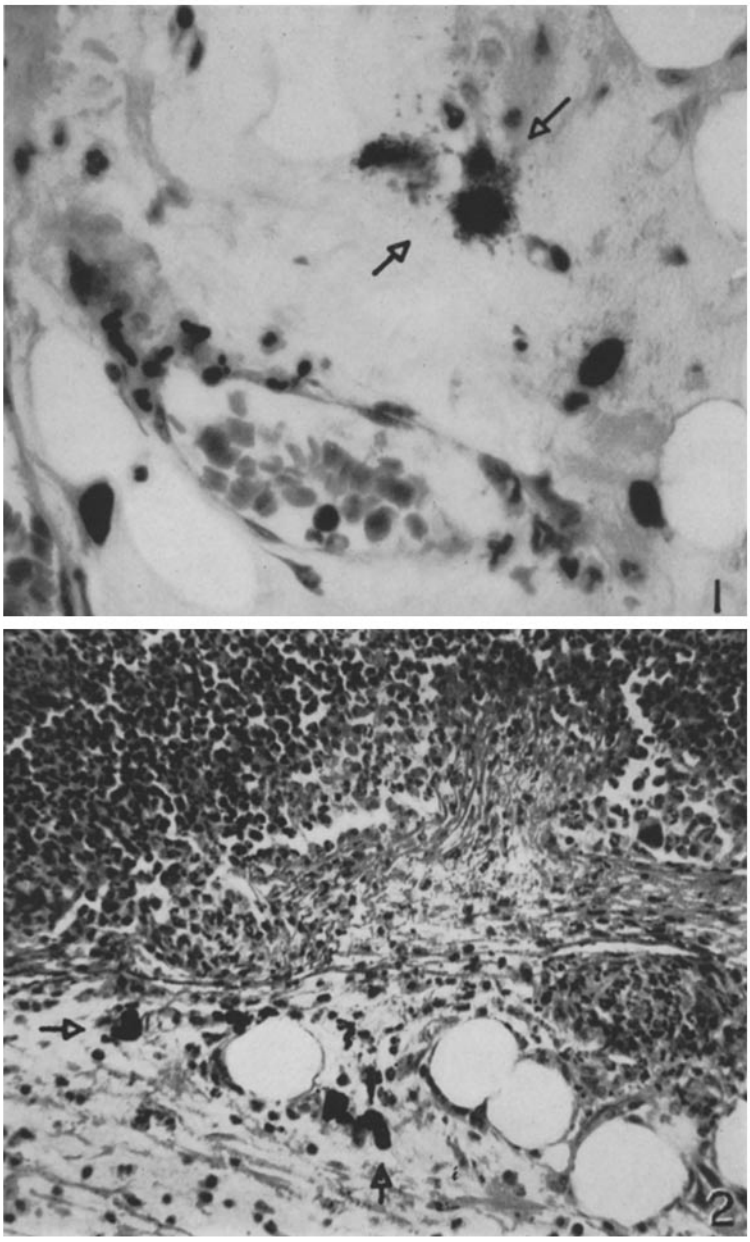

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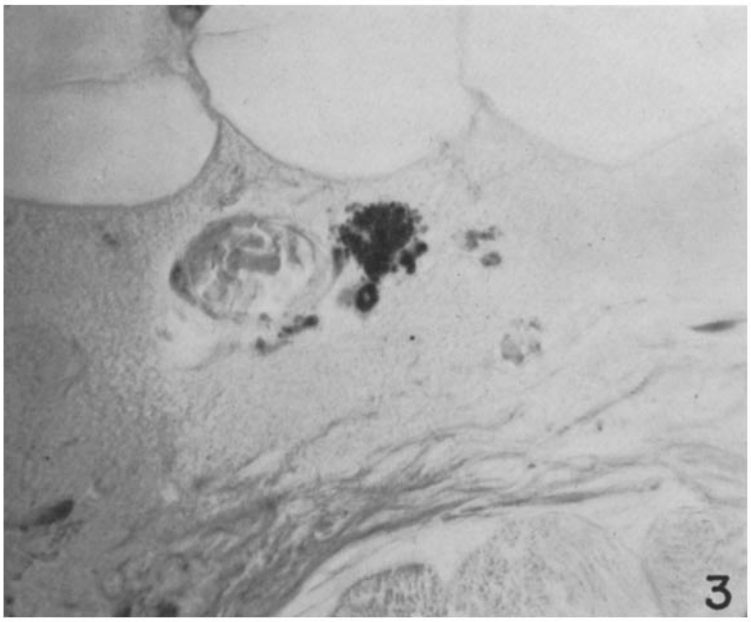
PLATE 88

FIG. 3. Normal, 10 minutes after inoculation. Inoculation site with congested capillary and single tissue mast cell showing beginning discharge of cytoplasmic granules. Giemsa. × 740.

FIG. 4. Rat pretreated with 48/80, 1 hour after inoculation. Note comparatively slight degree of congestion, granulocytic exudation, and complete absence of granulated tissue mast cells. Giemsa. × 330.



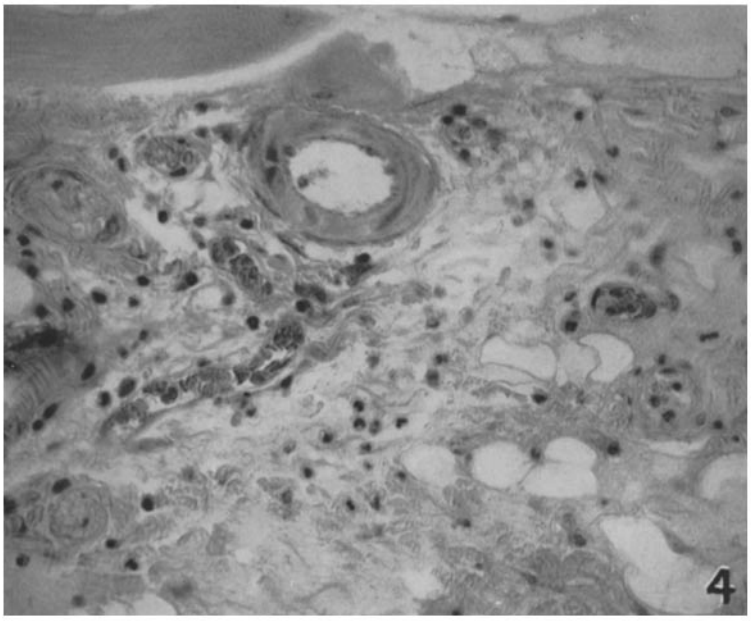


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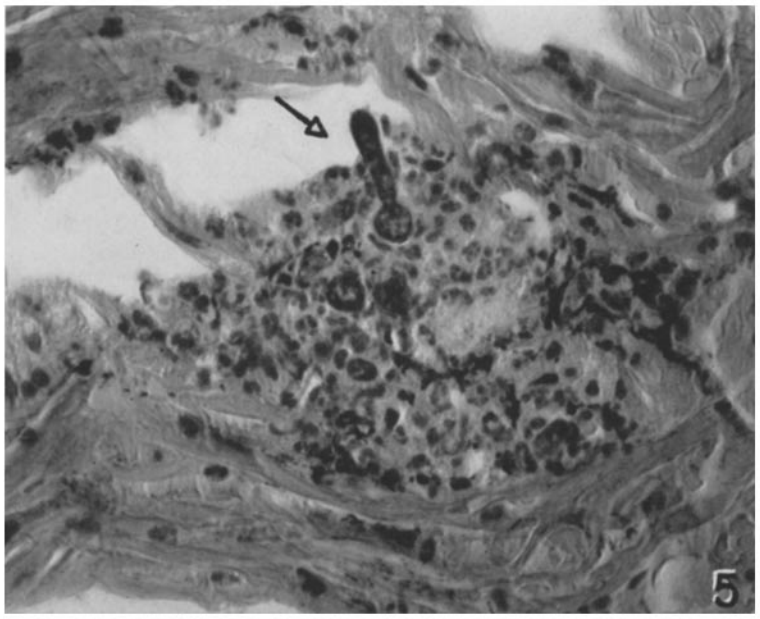
PLATE 89

FIG. 5. Pretreated with 48/80, 4 hours after inoculation. Granulocytic clustering around one active spore and single early mycelia (arrow). Giemsa. × 550.

FIG. 6. Pretreated with 48/80, 48 hours after inoculation. Segment of well defined abscess wall with many large mononuclear cells but virtually no fibroblasts. Note absence of granulated tissue mast cells. Giemsa. × 275.



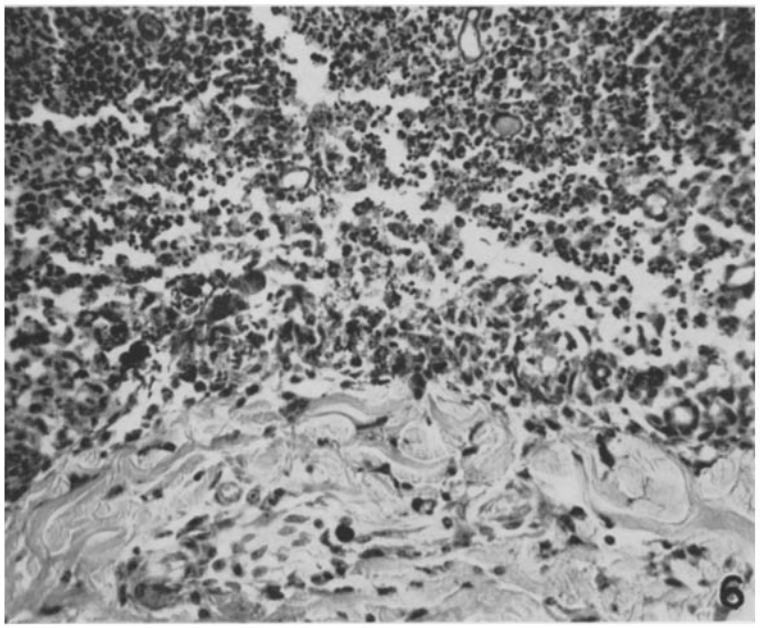


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PLATE 90

FIG. 7. Diabetic rat, 10 minutes after inoculation. Periphery of inoculation site (bottom left and center) with spores and carbon particles (black granular material). Note cluster of fully granulated tissue mast cells (arrows) and absence of congestion and cellular response. Giemsa. × 330.

FIG. 8. Diabetic, 12 hours after inoculation. Edge (top) of spreading fungus lesion with many mycelia. Many granulated tissue mast cells (arrows) persist. Giemsa. × 320

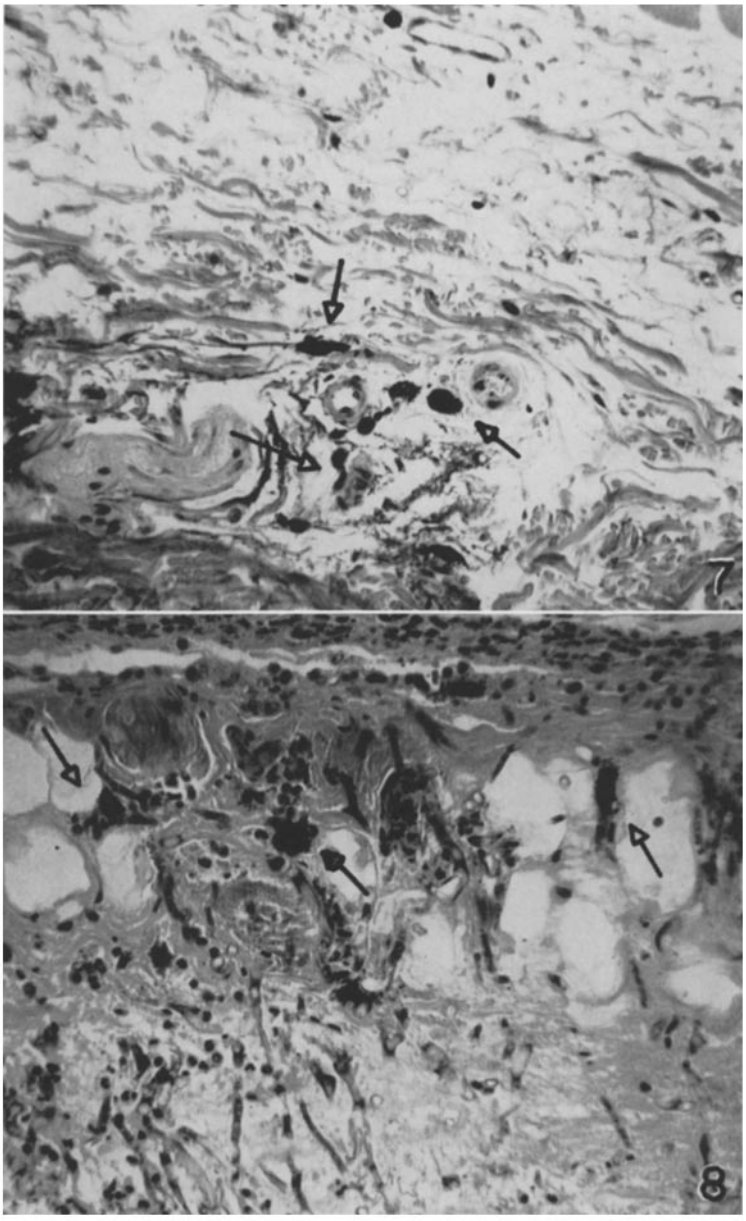

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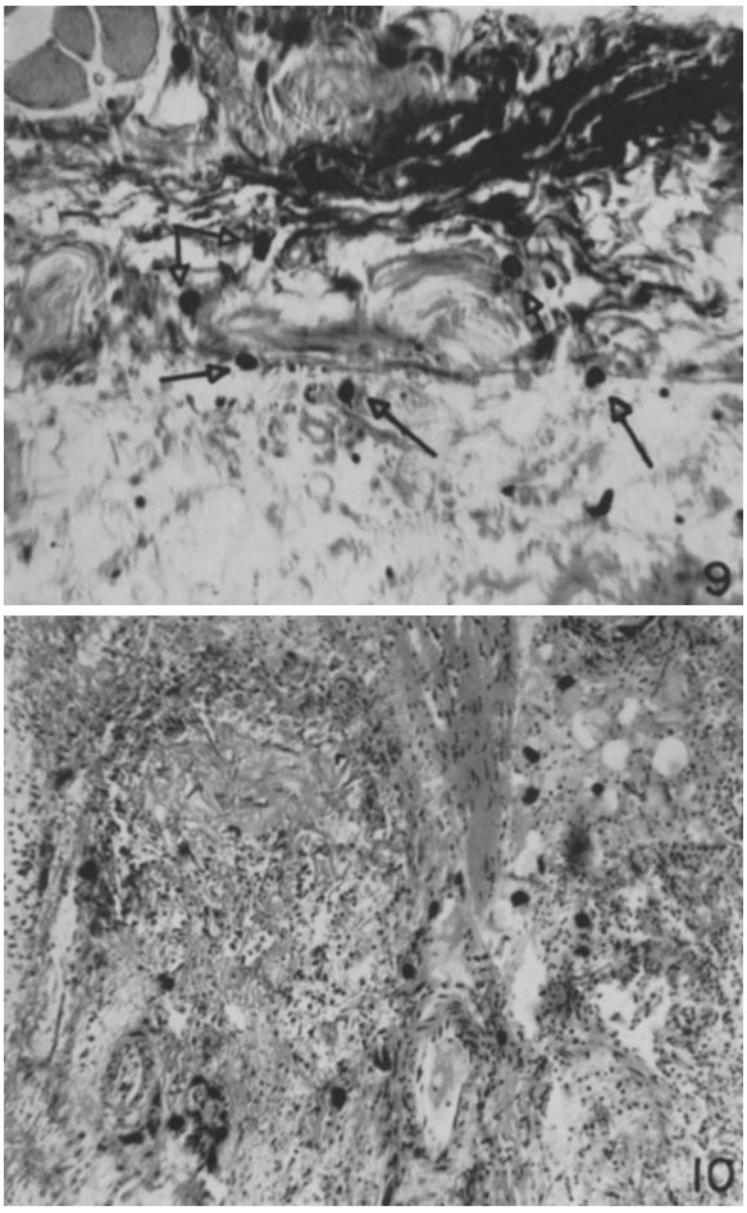
PLATE 91

FIG. 9. Diabetic, 1 hour after inoculation. Edge of inoculum (black mass at right top and upper center) with many granulated tissue mast cells (arrows). Giemsa. × 320.

FIG. 10. Diabetic, 48 hours after inoculation. Spreading fungus lesion with many granulated tissue mast cells (small black masses) in necrotic tissue. Giemsa. × 140.



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