THE DEVELOPMENT OF THE ACUTE INFLAMMATORY RESPONSE TO EXPERIMENTAL CUTANEOUS MUCORMYCOSIS IN NORMAL AND DIABETIC RABBITS*

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(Received for publication, July 21, 1959)

Previous experiments have shown that the behavior of experimental mucormycotic infection shows striking differences in the normal as compared with the metabolically abnormal host. In rabbits with acute alloxan diabetes and acidosis the inoculation of the fungus produced an acute spreading and fatal infection while the fungus lesions in the metabolically normal host remained confined to the site of inoculation and healed spontaneously (1). These observations referred primarily to fully developed acute lesions. Therefore, the present experiments were designed to compare the development of the inflammatory response to mucormycosis in normal and metabolically abnormal rabbits at various time intervals during the first 24 hours after inoculation.

Methods

White male rabbits ranging from 1800 to 2300 gm. in weight were used. In 38 animals acute alloxan diabetes with acidosis was produced and the presence of hyperglycemia, glycosuria, and ketonuria were determined as previously described (1). The presence of fully developed acute diabetes was established by blood sugar levels above 400 mg. per cent and 4 plus ketonuria. Forty-one rabbits received no alloxan and served as controls. All 79 animals were inoculated intradermally, without anesthesia, in four sites on the shaved back with 0.3 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).[1]

Both diabetic and control rabbits were sacrificed at 5, 10, 20, 30, 45, 60 minutes and at 2, 4, 6, 12, and 24 hours after inoculation by intravenous injection of air preceded by 1 cc. of undiluted nembutal administered rapidly. The skin lesions were excised, fixed in Helly's fluid, and each lesion was examined in its entire extent, with multiple sections stained with Giemsa. Complete autopsies were performed and histologic preparations of tissues other than

* Aided by Grant A-954 (C3) from the United States Public Health Service, Department of Health, Education and Welfare.

[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. Dr. Ajello also ascertained that the addition to it of India ink in the concentration used had no effect on the fungus.

TABLE I

Acute Inflammatory Reaction in Rabbits

Time	Normal						Diabetic					
	Rabbit No.	Conges.	Leukocytes			Fungus prolif.	Rabbit No.	Conges.	Leukocytes			Fungus prolif.
			Margin.	Exud.	Phagoc.				Margin.	Exud.	Phagoc.	
5 min.	15-05	++	1	0	0	0	15-04	+	tr.	0	0	0
	15-10	++	1	0	0	0	15-89	+	tr.	0	0	0
	15-12	++	1	0	0	0	15-93	0	0	0	0	0
	15-13	++	1	1	0	0	16-01	+	0	0	0	0
							16-04	0	0	0	0	0
10 min.	14-63	++	1	0	0	0	15-85	+	0	0	0	0
	14-64	++	1	1	0	0	16-02	0	0	0	0	0
	14-65	++	1	1	0	0	16-05	+	0	0	0	0
20 min.	13-98	++	2	2	0	0	15-02	+	tr.	tr.	0	0
	14-66	++	2	2	0	0	15-03	0	tr.	0	0	0
	14-67	++	2	2	0	0	15-99	0	0	0	0	0
	15-11	++	3	2	1	0						
30 min.	14-19	+++	3	2	1	0	14-33	++	0	0	0	1
	14-41	+++	3	2	1	0	14-34	++	0	0	0	2
	14-42	+++	2	3	1	0	14-35	++	1	tr.	0	0
	14-43	+++	2	2	1	0	14-55	++	0	0	0	0
	14-47	+++	1	2	0	0						
	14-60	+++	2	2	0	0						
45 min.	14-61	++	2	2	1	0	14-56	+	0	0	0	1
	14-62	+++	2	3	2	0	14-57	++	0	0	0	0
	15-14	++	2	2	2	0	15-98	++	0	0	0	1
1 hr.	14-18	++	2	2	2	0	14-36	++	1	0	0	1
	14-44	+++	2	3	2	0	14-37	++	0	0	0	2
	14-45	+++	2	2	2	0	14-38	++	1	tr.	0	2
	14-46	+++	2	3	3	0	15-92	+	1	0	0	0
	14-49	+++	2	2	3	0						
2 hrs.	14-13	++	2	3	3	0	14-31	++	0	0	0	1
	14-39	+++	2	3	3	2	14-32	++	1	tr.	2	2
	14-40	+++	2	3	3	0	15-96	+	1	tr.	0	1
	15-97	+++	2	2	2	0						
4 hrs.	15-21	+++	2	4	4	2	14-85	+	2	1	0	3
	15-86	+++	2	4	4	0	14-86	++	1	1	1	3
							16-00	+	0	0	0	2

TABLE I (*Continued*)

Time	Normal						Diabetics					
	Rabbit No.	Conges.	Leukocytes			Fungus prolif.	Rabbit No.	Conges.	Leukocytes			Fungus prolif.
			Margin.	Exud.	Phagoc.				Margin.	Exud.	Phagoc.	
6 hrs.	14-89	++	2	4	4	2	14-81	++	2	2	2	3
	14-90	+++	2	4	4	2	14-83	+++	2	2	2	2
	15-19	+++	2	4	4	2	15-90	+++	2	3	3	3
	15-20	+++	2	4	4	2	15-91	+	1	2	2	3
12 hrs.	15-87	+	2	4	4	1	15-94	+	1	2	2	3
	15-88	+	0	4	4	2	15-95	++	1	2	3	3
							16-03	+++	1	3	2	4
24 hrs.	15-17	+++	3	4	4	2	15-15	+++	2	3	2	4
	15-18	+++	3	4	4	2	15-16	++++	3	4	3	4

skin were stained with hematoxylin and phloxine. Cultures of lung tissue on Sabouraud-dextrose agar were done only in animals with lesions of 12 and 24 hours' duration.

The histologic findings were arbitrarily graded from 1 to 4 plus for the following features: congestion, cellular response, and fungus proliferation. The evaluation of cellular response included margination and diapedesis of polymorphonuclear leukocytes, their infiltration of tissues, and their clustering around the fungus and carbon particles which was regarded as evidence of phagocytosis. Response by large mononuclear cells, and proliferation of fibroblasts, were also noted but were not suitable for grading.

Morphologic Findings

The morphologic findings in the skin lesions are summarized in Table I.

Normal Rabbits.—The core of all lesions formed was at the site of inoculation and around it the subsequent changes developed. At first the lesion consisted of disrupted and separated connective tissue fibers, with fungus spores and carbon particles. Later this appearance was modified by the inflammatory response and the proliferation of the fungus.

In all animals sacrificed 5 minutes after inoculation, early leukocytic margination was present in the capillaries and venules of the corium (Fig. 1). In one animal rare leukocytes were present in the tissues outside the blood vessels. At 10 minutes, slight leukocytic infiltration of the tissues was present in 2 of the 3 animals. At 20 minutes, both leukocytic margination and tissue infiltration were more pronounced in all rabbits and in one instance the leukocytes were beginning to cluster around some of the fungus spores and carbon particles (Fig. 2). At 30 minutes, slight leukocytic clustering around the inoculum was observed more frequently while margination and infiltration continued. At 45 minutes, many more spores were surrounded by larger aggregates of

leukocytes while the area showing leukocytic margination and infiltration had increased in size. At 60 minutes, all aspects of the leukocytic response had become more widespread and clustering was more marked (Fig. 3). At 2 hours, tissue infiltration was intensified and extended further. Virtually all fungus and carbon particles were now surrounded by masses of leukocytes. At 4 hours, these findings were even more marked. At 6 hours, the lesions appeared to have reached their maximum size. In the center, the polymorphonuclear leukocytes began to degenerate but leukocytic margination and tissue infiltration continued unabated at the periphery where, around small blood vessels, a few large mononuclear cells appeared (Fig. 4). Simultaneously the fibroblasts at the margins of the lesions showed the first signs of proliferative activity. At 12 hours leukocytic margination was decreased while infiltration and clustering remained unchanged. The large mononuclear cells had become more numerous and now were part of the cellular reaction around the fungus and carbon particles. There was definite proliferation of fibroblasts towards the center of the lesion. At 24 hours the major change consisted of a marked increase in macrophages and proliferating fibroblasts with beginning organization of the inoculation site and demarcation of the lesions (Fig. 5).

The first sign of fungus proliferation occurred at 2 hours after inoculation and was manifested by the presence of a single early mycelia in one of four animals. Budding spores and early mycelia formation were observed at all subsequent time intervals but were confined to the center of the lesion and occurred only in small numbers (Fig. 6). Fungus invasion of the surrounding tissues and blood vessels or systemic dissemination were never encountered.

Congestion of blood vessels was already pronounced in the earliest lesions and maximal engorgement of individual vessels was noted at 30 minutes. This persisted throughout the entire experiment with increasing numbers of vessels being involved.

Diabetic Rabbits.—As in the normal animals, the site of inoculation showed disrupted and separated connective tissue fibers and the inoculum of fungus spores and carbon particles. At 5, 10, 20, 30, and 45 minutes after inoculation an occasional granulocyte was seen attached to the endothelium of one or two capillaries at the periphery of the inoculation site. This change was found only in a few of many sections and occurred in 5 of 19 rabbits representing the various time intervals listed above. Definite margination of polymorphonuclear leukocytes was first noted at 1 and 2 hours after inoculation (Fig. 7), did not increase appreciably in extent and degree until 6 hours, was diminished at 12 hours and was most marked at 24 hours. Until 4 hours after inoculation leukocytic infiltration consisted of a rare granulocyte in the tissues around the inoculation site and was encountered in only 5 of 25 rabbits (Fig. 8). At 6 and 12 hours tissue infiltration had become more marked and widespread and was most pronounced at 24 hours. An appreciable degree of clustering of polymorphonuclear leukocytes around fungus spores and carbon particles did not occur until 6 hours following inoculation (Fig. 9). At 12 and 24 hours, the process had become more intense and extensive (Fig. 10).

At all times the granulocytes of the exudate displayed regressive nuclear changes consisting of pyknosis and karyorrhexis. Large mononuclear cells were first observed around capillaries and venules at the periphery of the lesions at 6 hours after inoculation (Fig. 11). At 12 and 24 hours, the monocytic response was increased and more

widespread and some of the macrophages formed part of the cell aggregates around the fungus and carbon particles. There was no evidence of fibroblastic proliferation at any time.

Occasional active spores and rare early mycelia were first encountered in the center of the lesions at 30 minutes in 2 of 4 rabbits and in 2 of 3 animals at 45 minutes. Fungus proliferation was more frequent at 1 and 2 hours and at 4 hours had extended beyond the site of inoculation. At 6 and 12 hours mycelia formation was extensive and there was early invasion of blood vessels at the periphery of the lesions. At 24 hours after inoculation the growth of the fungus was massive with invasion of deep tissues and large blood vessels (Fig. 12). The lesions showed no signs of demarcation but systemic dissemination of the fungus did not occur.

Some congestion of blood vessels was noted at 5 minutes after inoculation. This became more marked after 30 minutes and subsequently increased, progressively involving more vessels over a wider area.

In both normal and diabetic rabbits the mononuclear cell response appeared to consist chiefly of macrophages as shown by the frequent occurrence of phagocytosis of carbon particles and cellular debris. Lymphocytes and plasma cells were searched for but not recognized among the cellular response.

DISCUSSION

Although the basic pattern of the inflammatory response was the same in both normal and diabetic rabbits, striking differences between the metabolically normal and abnormal animals existed in the onset, intensity and extent of the morphologic changes associated with acute inflammation. Despite the inaccuracies inherent in the quantitative evaluation of a dynamic process by histologic techniques, the differences between the two groups were so marked and consistent that a rough system of grading could be applied to the study of some aspects of the lesions.

The results of these experiments indicate that the early inflammatory reaction in rabbits with acute alloxan diabetes and acidosis is strikingly altered when compared with that of normal controls. The granulocytic response, which in the normal host begins within a few minutes after inoculation and increases rapidly in degree and extent, is delayed and of diminished intensity. Fibroblastic proliferation, which in the normal animals is present within some hours and contributes to the early demarcation of the lesions, is lacking in the diabetic rabbits. In addition to these impairments of the exudative and proliferative processes of inflammation, fungus growth begins early, progresses rapidly, and soon spreads widely beyond the site of inoculation. This behavior of the agent never occurs in the normal host and fungus growth begins later, is much less marked, remains confined to the site of inoculation, and, as shown in previous experiments, is soon altogether suppressed (2).

In contrast to the marked differences of the reaction in the diabetic as compared with the normal rabbits the large mononuclear cell response was remark-

ably similar in the two groups in time of appearance, relative numbers, distribution, and evidence of phagocytic activity. Vascular congestion seemed slightly less marked in the diabetic animals but otherwise appeared to behave quite similarly in the two groups.

We have found in the literature only a few experimental studies in which the morphologic changes of early inflammation were compared in metabolically normal and abnormal animals. Striking differences in the acute inflammatory reaction in the skin of normal as compared with abnormal guinea pigs have been described by Miles and Niven (3). These authors used a variety of pathogenic bacteria to produce lesions and altered the internal environment of the host before or after inoculation by shock induced by bacterial exo- and endotoxins and other agents. They found that shock with severe hypotension interfered with the emigration of polymorphonuclear leukocytes into the lesions and concluded "that the decisive reactions of both the bacterium and the host tissues are displayed in the first 3 hours of infection, and determine, within narrow limits, the subsequent course and outcome of the disease."

Cruickshank observed in staphylococcal skin lesions of rabbits with acute alloxan diabetes and ketosis an almost complete failure of the inflammatory reaction while control animals responded in a normal manner (4). The times after inoculation at which the lesions were studied are not specified in the text but the legends of the illustrations mention intervals of 17, 18, and 32 hours. Cruickshank attributes the unrestrained multiplication of the bacteria to the absence of leukocytes and the latter phenomenon to peripheral vascular collapse.

The experiments of Miles and Niven and of Cruickshank differ in several respects from those reported here. These investigators employed bacteria which are capable of producing disease in normal animals whereas *Rhizopus oryzae*, used in our experiments, is innocuous to the metabolically normal host. In our experiments, the development of acute inflammation was studied in the lesions procured at multiple time intervals from 5 minutes until 24 hours after inoculation. The lesions from each rabbit were used to study only a single time interval while in the study of Miles and Niven multiple lesions representing time intervals of 0, ½, 1, 2, 3, and 5 hours' duration were obtained from the same animal. The histologic observations of Cruickshank refer to lesions which appear to be of more than 15 hours' duration and do not include the early phase of acute inflammation.

Miles and Niven as well as Cruickshank attribute the failure of the inflammatory response to an absence of leukocytes resulting from peripheral vascular collapse. While shock with peripheral vascular collapse might explain the failure of the inflammatory response in diabetic rabbits during the first 4 to 6 hours after inoculation it is difficult to reconcile this concept with the development of acute inflammation at 12 and 24 hours, which was observed in our experiment. By that time, most of the rabbits with acute alloxan diabetes were obviously much worse. They were more dehydrated and hypothermic than at the

time of fungus inoculation and the presence of severe peripheral vascular collapse was shown by the fact that in many instances blood samples could be obtained only by cardiac puncture.

At present there is no adequate explanation for the increased susceptibility to infection, which is often clearly related to severe alterations in host metabolism. Some facts, however, concerning the relationship of specific metabolic abnormalities to certain defects of the body defences are beginning to accumulate (5). Morphologic and functional changes of the polymorphonuclear leukocytes are known to occur in association with metabolic alterations in man and experimental animals and they have been reviewed recently (6). There is evidence that acidosis may impair host resistance (7).

The present experiments have shown that the increased susceptibility of the diabetic and acidotic host to experimental mucormycosis is in part related to a deficiency of the body defenses which manifests itself most strikingly at the very onset of the acute inflammatory response and is less readily appreciated a few hours later. A comparison of the morphology of the early lesions in normal and diabetic rabbits reveals that in the metabolically abnormal host the response by polymorphonuclear leukocytes is markedly delayed, less intense and apparently less effective and that fibroblastic proliferation is lacking. These impairments of host defences correspond with a period of early and unrestrained growth of the fungus which later leads to a rapid spread of the infection. These findings indicate that during the critical period immediately following exposure of the host tissues to the infectious agent the metabolically abnormal animal lacks its most effective cellular defence mechanism.

SUMMARY

The histologic changes associated with the development of the acute inflammatory response to experimental cutaneous mucormycosis were studied at various times from 5 minutes to 24 hours after inoculation into normal rabbits and in rabbits with acute alloxan diabetes and acidosis. In normal animals the response by polymorphonuclear leukocytes began within a few minutes after inoculation, increased rapidly in extent and intensity and reached its peak within 6 to 12 hours. By that time there was also early proliferation of fibroblasts and of large mononuclear cells and these cellular reactions, together with the accumulated granulocytes, began to produce a demarcation of the lesions. Beginning 4 to 6 hours after inoculation the fungus showed some growth but this remained confined to the necrotic center of the lesions. In the diabetic rabbits the onset of the response by polymorphonuclear leukocytes was delayed by several hours, reduced in intensity and was apparently less effective. There was no proliferation of fibroblasts and the lesions were spreading rather than circumscribed. Fungus growth in the tissues began shortly after inoculation, was marked, progressed rapidly, and soon extended beyond the site of inocula-

tion. The large mononuclear cells, however, appeared at about the same time and in equal number in the lesions of both diabetic and non-diabetic animals and showed no morphologic changes.

It is concluded that a significant delay and impaired effectiveness of the response by polymorphonuclear leukocytes, a lack of fibroblastic proliferation and an enhanced growth of the fungus lower host resistance to infection with it and are directly consequent on severe alterations in host metabolism.[2]

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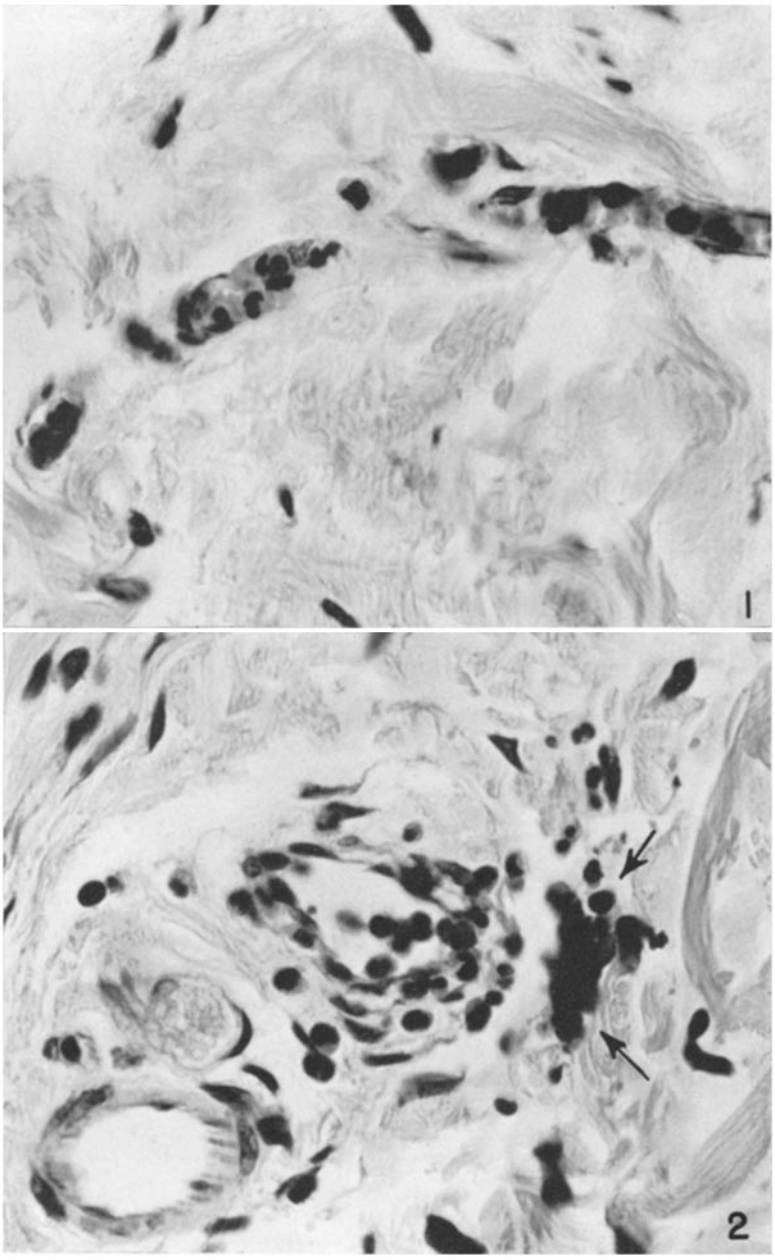
[2] The technical assistance of Miss Hillma Gheesling and Mrs. Elaine S. Kester is gratefully acknowledged.

EXPLANATION OF PLATES

PLATE 83

FIG. 1. Control rabbit, 5 minutes after inoculation. A congested capillary shows margination by polymorphonuclear leukocytes: Giemsa. × 695.

FIG. 2. Control, 20 minutes after inoculation. Capillary with leukocytic margination and early infiltration of wall. A few granulocytes are approaching a small mass of inoculum (arrows). Giemsa. × 695.

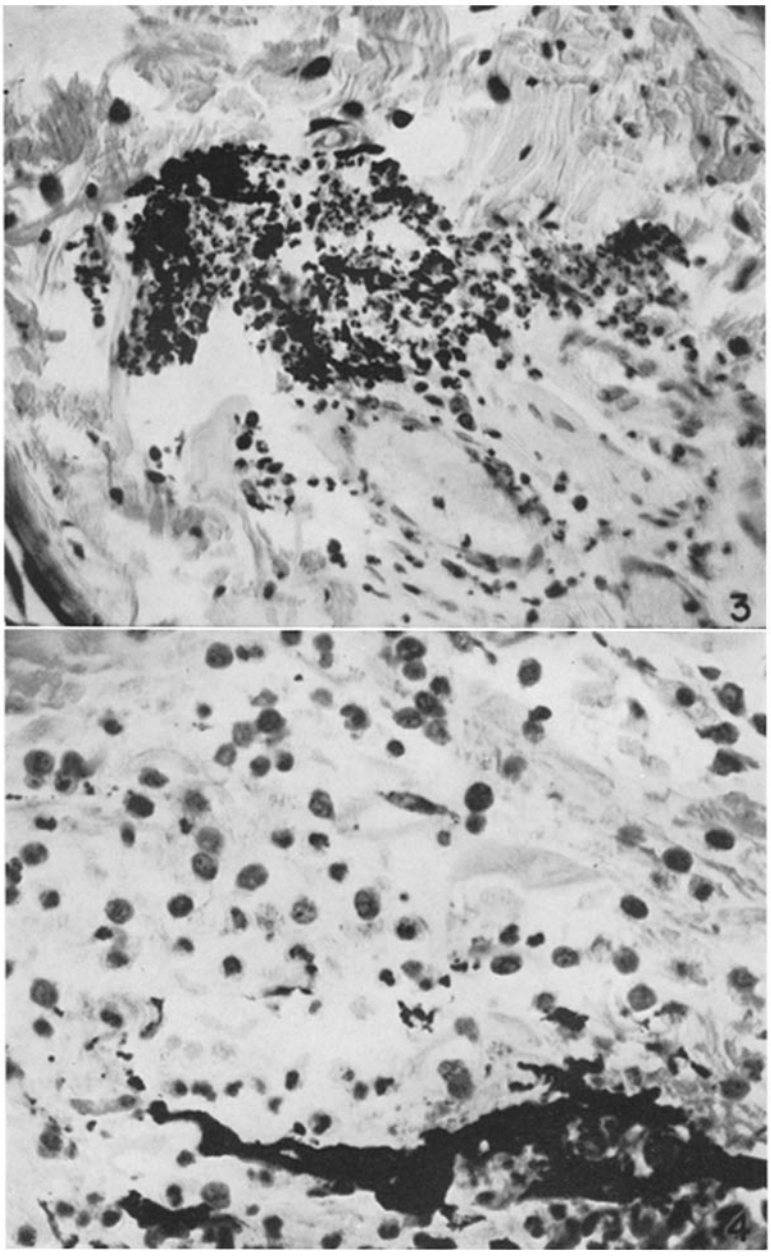


(Sheldon and Bauer: Inflammatory response to cutaneous mucormycosis)

PLATE 84

FIG. 3. Control, 1 hour after inoculation. The inoculum is surrounded by polymorphonuclear leukocytes. Giemsa. × 325.

FIG. 4. Control, 6 hours after inoculation. Large mononuclear cells and some polymorphonuclear leukocytes are present at the periphery of the inoculum (black mass at bottom). Giemsa. × 530.

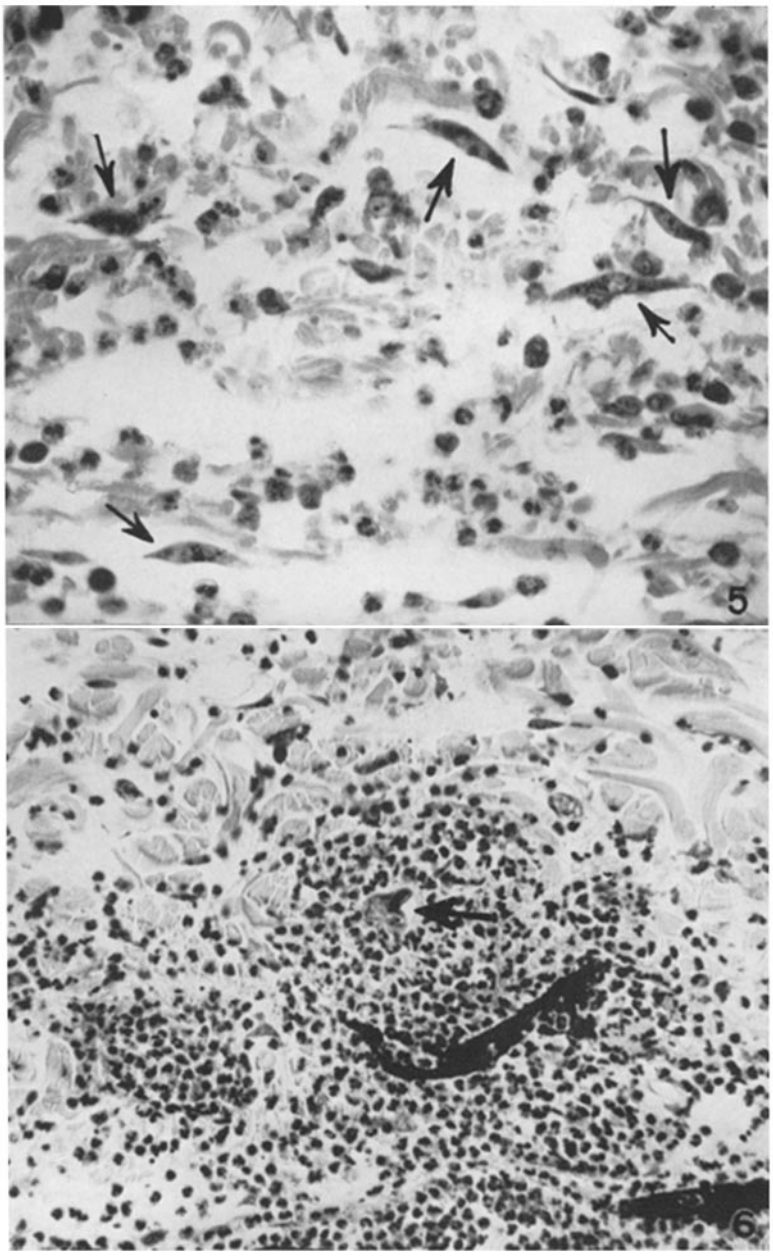


(Sheldon and Bauer: Inflammatory response to cutaneous mucormycosis)

PLATE 85

FIG. 5. Control, 24 hours after inoculation. Proliferating fibroblasts are present (arrows) among inflammatory cells at the periphery of the lesion. Giemsa. × 500.

FIG. 6. Control, 12 hours after inoculation. Single budding spore (arrow) surrounded by polymorphonuclear leukocytes. Giemsa. × 320.

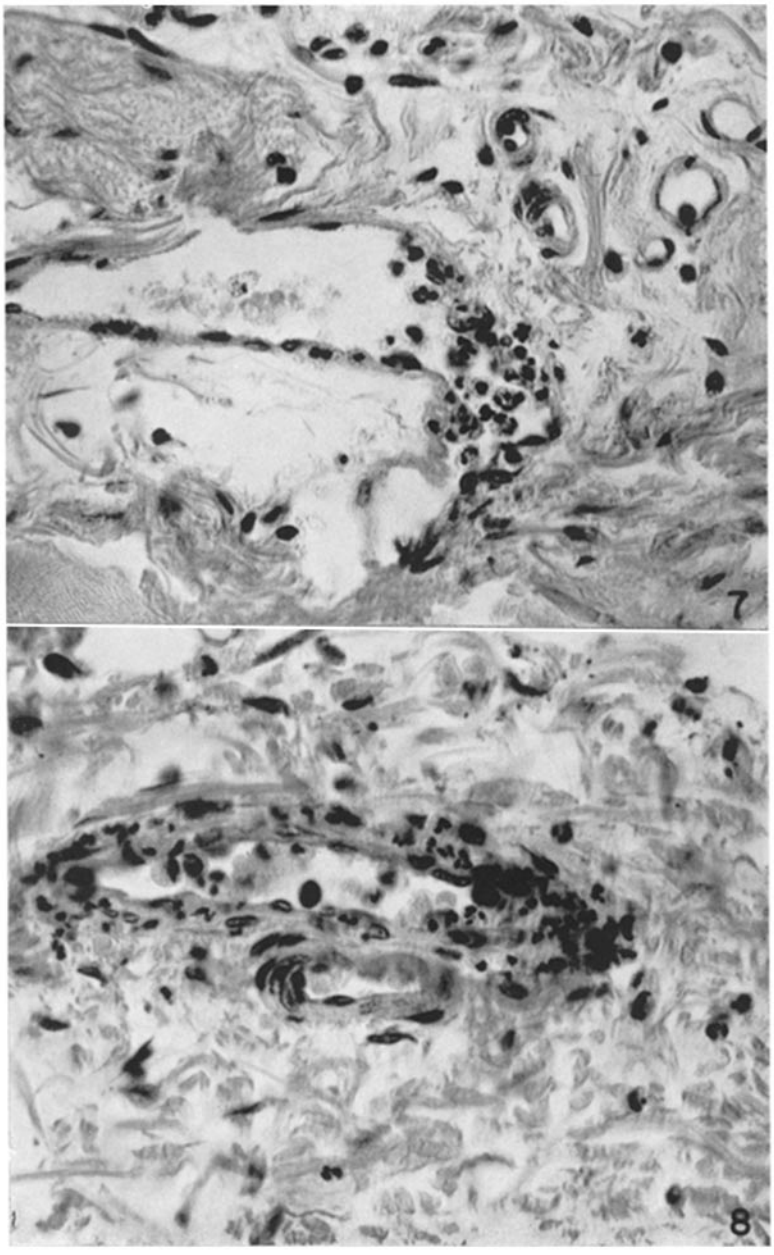


(Sheldon and Bauer: Inflammatory response to cutaneous mucormycosis)

PLATE 86

FIG. 7. Diabetic, 2 hours after inoculation. Small vein with a few polymorphonuclear leukocytes, showing some margination and early infiltration of wall. Giemsa. × 520.

FIG. 8. Diabetic, 4 hours after inoculation. Polymorphonuclear leukocytes are slightly more numerous in the wall of a small vessel and begin to infiltrate surrounding tissues. Giemsa. × 570.

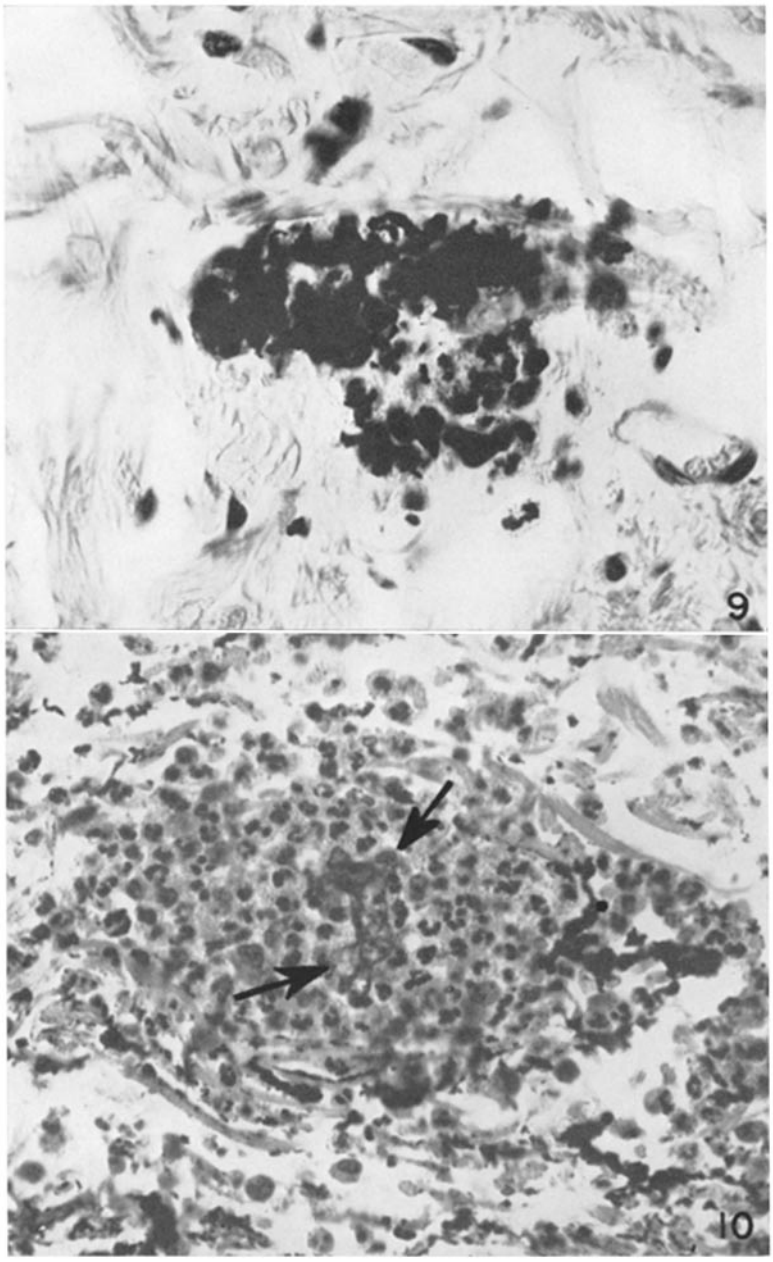


(Sheldon and Bauer: Inflammatory response to cutaneous mucormycosis)

PLATE 87

FIG. 9. Diabetic, 2 hours after inoculation. Mass of inoculum without any significant cellular response about it. Giemsa. × 680.

FIG. 10. Diabetic, 12 hours after inoculation. Mycelia (arrows) surrounded by a mass of polymorphonuclear leukocytes. Giemsa. × 570.

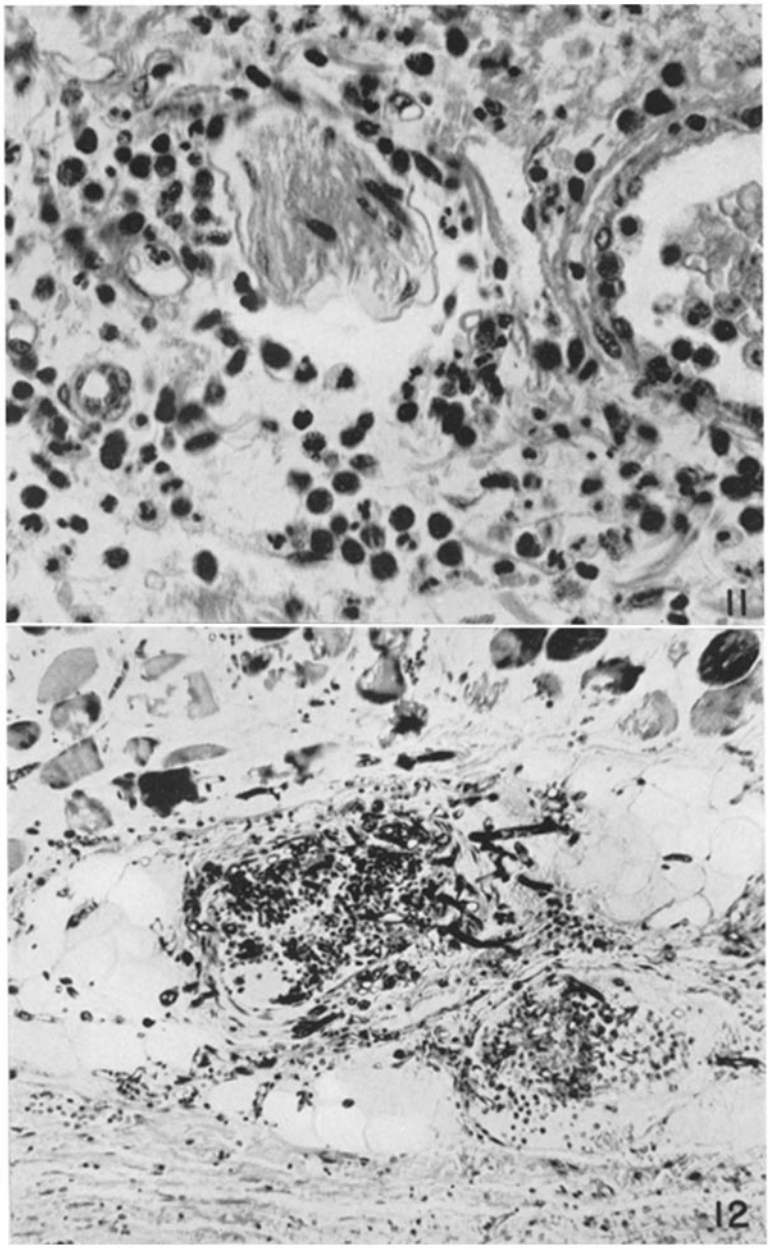


(Sheldon and Bauer: Inflammatory response to cutaneous mucormycosis)

PLATE 88

FIG. 11. Diabetic, 6 hours after inoculation. Large mononuclear cells appear among the inflammatory cells at the periphery of lesion. Giemsa. × 570.

FIG. 12. Diabetic, 24 hours after inoculation. Acute spreading mucormycosis with masses of mycelia invading a subcutaneous artery and vein. Giemsa. × 155.



(Sheldon and Bauer: Inflammatory response to cutaneous mucormycosis)