

GRANULOMA, A CHARACTERISTIC "QUALITATIVE" CHANGE IN FOCAL ANAPHYLACTIC INFLAMMATION *

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I. INTRODUCTION

Nature of the Anaphylactic Reaction. The "Quantitative" Hypothesis

The effects of Arthus' "Repeated Injections of Horse Serum into the Rabbit,"¹ presented as a note to the Société de Biologie in June, 1903, became soon afterwards, under the name of "Arthus' phenomenon," the starting point of innumerable immunologic, physiologic, biochemical, and clinical researches on anaphylaxis, hypersensitiveness, and allergy. His second note, with Maurice Breton, on "Cutaneous Lesions Induced by Horse Serum Injections into Rabbits Anaphylactized by and for this Serum"² was presented to the same Society a few months later (November, 1903). This note, dealing with the histopathologic aspects of local shock, did not share the fate and importance of the first article, which dealt with its physiologic implication. The reason is to be found in the fact that the histopathologic picture of the focal anaphylactic response, as described therein, is not at variance with a "common" inflammatory reaction, except for some heightening of the process.

The assumption that focal anaphylaxis was pathologically similar to a common type of inflammation needed verification. Controlled researches were not performed, however, until 1914. Between 1914 and 1923, Rössle,^{11,12} and, under his instigation, Fröhlich,³ and Gerlach^{4,5} attacked the problem anew. The experimental method consisted either of a reproduction of the classical Arthus' phenomenon (on rabbits, and other animals; Gerlach) or of appropriate modifications. The latter comprised (1) watching the initial stages of the anaphylactic response and their development on the transparent mesentery of a living and sensitized frog, with substitution of the shock antigen for an inflammation-inducing agent (Fröhlich); (2) studying the local lesion induced by subcutaneous injection of avian erythrocytes into specifically sensitized guinea-pigs, the local shock being elicited when the serum of the guinea-pigs reached a high titer of anti-avian hemolysins (Rössle); (3) observing, through "abdominal windows," the gross peritoneal changes in specifically sensitized animals when the shock antigen is introduced into the peritoneal cavity (Rössle).

These controlled researches, which brought forth some additional

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data (presence of eosinophilia, for example) failed nevertheless to invalidate the initial findings of Arthus and Breton.² It is repeatedly maintained by the authors cited that the local anaphylactic reaction is not "qualitatively" different from any inflammatory response, the changes being simply and solely "quantitative." These quantitative changes concern the suddenness of the reaction; the immediately ensuing and more or less sustained blood stasis; the tremendous edema, which may or may not be hemorrhagic; the abundant eosinophilia, and the necrosis of the vessels walls. The process is heightened, accelerated, and shortened, and "stormy" in its unfolding. It fixes and limits the injurious shock antigen to its portal of entry, and therefore protects the organism as a whole, sometimes at the expense of local tissue death, through the interplay of capillary contraction, walling off by exudation, and the neutralizing and diluting effects of the edema.

Evidence Against the "Quantitative" Hypothesis

The accepted opinion, outlined above, that local anaphylaxis is only a "quantitative" variant of inflammation does not appear tenable, for the following reasons:

(a) Inflammation and anaphylaxis are two distinct phenomena. Inflammation follows a wide range of inciting agents and in final analysis is due to the injury or death of a cell or group of cells. Anaphylaxis, on the other hand, is highly specific, in that it depends upon an active or passive sensitization, a latency period, and, at least in part, on some form of antigen-antibody reaction. Anaphylaxis could be only a special case of inflammation, and as such its histologic picture should show more than "quantitative" changes.

(b) The anaphylactic response is the result of a primary antigen-antibody coupling, and occurs not in the blood stream or tissue fluids, but on or in cells which are able to remove or fix the circulating antibody. The anaphylactic state, moreover, is associated with the presence of a "fixed" antibody, and, conversely, with the absence of a freely circulating antibody. Immunologists have long since discarded the "humoral" or "anaphylatoxin" hypothesis, which supposes the formation of an antigen-antibody complex, and the adsorption by the latter of a stabilizing serum constituent with subsequent digestion of serum proteins or release of a toxic compound. Instead, immunologists now rally to the "cellular" hypothesis. It is beyond the aim of the present article to submit the experimental evidence which is so strongly in favor of the view that the anaphylactic response is cellular in nature and origin. However, we are still ignorant of whether the

phenomenon occurs at the surface or in the interior of cells, the types of cells concerned, and the histologic modalities of the phenomenon.

If, therefore, during the anaphylactic shock, the cells alone are operative, it seems fair to assume that a responsive cell may be found which will show by histologically analyzable signs its dominant rôle in the phenomenon. One may venture to say that its reactivity must be specific and "qualitatively" different from a common inflammatory response.

Rôle of the Histiocytes

Experimental evidence points to only two types of cells which seem to respond during the anaphylactic shock: the smooth muscle cell and the reticulo-endothelial cell. The reactivity of the smooth muscle cells explains, among other experimental and clinical data, the Schultz-Dale phenomenon and that of the "effector organ" (which varies from species to species, according to the richness of smooth muscle cells in these organs). However, in focal anaphylactic reactions of the Arthus type, the smooth muscle cells cannot be considered because of their scarcity in the subcutaneous tissue, where they are found only in the vessel walls. The reticulo-endothelial cells, on the other hand, are ubiquitous and can be found in the active mesenchyme of the subcutaneous tissue, not only as primitive mesenchymal cells, endothelial and perithelial cells, but also as histiocytes and monocytes.

The rôle of the histiocytes and their derivatives in anaphylaxis is still a debatable question (Jungeblut⁶), although experiments indicate a strict relationship between functional responsiveness of the reticulo-endothelial system and the anaphylactic shock. India-ink blockade attenuates and in cases inhibits shock when the specific antigen is injected. Similarly, the duplication of Arthus' initial experiment fails to induce an Arthus' phenomenon when the shock antigen is injected into a previously "blocked" area (experiment of the so-called local blockade, Klinge⁷).

Another argument in favor of the reactivity of the histiocytes is adduced by the behavior of the capillaries, which, with their potentially histiocytic endothelial and perithelial cells, are an integral part of the reticulo-endothelial system. Some of their reactions are only physiologic and not demonstrable microscopically, such as their functional insufficiency (the so-called irritability state of Doerr). In more advanced instances the damage becomes morphologically demonstrable as in the necrosis of the vessels, which is a prominent feature of the local anaphylactic reaction.

Finally, it has been shown by Rich,⁸⁻¹⁰ both clinicopathologically

and experimentally, that in serum sickness the lesion is essentially a monocytic adventitial and perivascular infiltration.

If, then, the histiocytes and derivative cells, and the cells making up the capillaries are physiologically reactive during the anaphylactic shock, an investigation of the histologic behavior of the histiocytes as a whole, and of the endothelial and perithelial cells in particular, seems indicated.

With the above-mentioned considerations as a starting point, experiments have been performed to investigate the reactivity of the histiocytes and capillaries in focal anaphylactic response. Other experiments, concerning the behavior of the histiocytes in local anaphylactic reactions in animals blocked with trypan blue, and the histopathology of sensitized areas after injection with histamine, are in progress.

II. EXPERIMENTAL DATA

All experiments were performed on healthy male and female guinea-pigs of 250 to 600 gm. average weight, kept on the usual diet of "kitchen greens" (cabbage, carrots, beets, etc.). Egg white was used as sensitizing and shock antigen. The sensitizing antigen was administered both intraperitoneally (for general sensitization) and subcutaneously (for focal sensitization). On occasion the sensitization of the focal area was reinforced by a subsequent subcutaneous injection given 24 hours after the first sensitizing injection. The site chosen for the focal injections was the mid-abdominal wall. Each subsequent injection, whether with the shock antigen or with the nonspecific antigen, was made in the same area as far as it was possible to do so, taking as a guide either the previous needle puncture mark or the grossly visible and palpable tumefaction.

In the identically sensitized control animals the shock antigen was replaced with nonspecific antigen (horse serum; in some experiments, human and rabbit serum). In one of the experimental groups, both the shock and nonspecific antigens were applied in dry form, as dressings, on a denuded area of the sensitized region.

The experiments are given in tabular form (Table I). The scope of each experimental group is summarized in the heading preceding its description in the text.

Most of the animals showed varying physiologic responses when the shock antigen was injected; they ranged from restlessness to polypnea, which was marked but of short duration. Only one guinea-pig showed the characteristic hair bristling over the head and neck ("lion's mane" sign), and recovered quickly.

The histologic technic was identical for all animals, and consisted

in Zenker's fixation, paraffin embedding, and staining with Mallory's phosphotungstic acid hematoxylin and with Mallory's phloxine and methylene blue. Occasionally Masson's trichrome stain and Foot's silver impregnation of reticulin fibers were used.

Group I. Inflammatory Changes Induced in Sensitized Animals by Subcutaneous Injection of Specific (or Shock) and Nonspecific (Control) Antigens

A. Shock Antigen. There were four findings of note in the animals receiving specific antigen.

(1) Presence of a cavity, heavily outlined by coarse and wavy fibrinous fibers. This cavity is believed to have contained the resorbed specific antigen.

(2) Presence of monocytes, histiocytes, and related cells in streamers or in loosely packed and ill-delimited islets. They were either scattered throughout or bordered the cavity which had been emptied of antigen.

(3) Presence of endothelial and perithelial hypertrophy and hyperplasia, of intraluminal blocking by monocytic forms, and evidence of endothelial and perithelial metamorphosis into monocytes, histiocytes, and related cells. The endothelial cells revealed their prospective potencies not only in the capillaries but also in precapillary arterioles.

(4) Presence of perivascular (Figs. 1 and 4), perineural (Fig. 3), and peri-adipose and intra-adipose granulomata (Fig. 2).

The remainder of the changes were consistent with a residual inflammation heightened by a recently superimposed acute inflammation. Edema, contiguous with the empty cavity, was tremendous and dissociated the stroma. The fragmented collagenous fibers showed swelling and occasional hyalinization, and were blurred or glassy. There was scattered erythrocytic diapedesis with no signs of a scavenger reaction. Neutrophilic polymorphonuclear leukocytes were scant, while eosinophils were numerous and lymphocytes rather rare. Venues occasionally were plugged with fibrin thrombi. Growth stimulation of fibroblasts was early.

B. Controls. In the animals which received nonspecific antigen the general picture was that of a residual inflammation with superimposed recent, mild, acute inflammatory changes and with no evidence of granulomata or of endothelial and perithelial reactivity. Coarsely granular coagula, presumably incompletely resorbed nonspecific antigen, were demonstrable. Edema was scant to moderate. Eosinophils were rare. In the scattered small areas of hemorrhages, groups of red cells were surrounded by macrophages.

TABLE I
Tabular Summary of Protocols of Experiments

Experiment	Group I		Group II	
	A	B (control)	A	B (control)
Number of guinea pigs	4	2	3	2
Day 0	1 ml. egg white intraperitoneally; 1 ml. egg white subcutaneously (sensitization)		3 ml. egg white intraperitoneally; 4 ml. egg white subcutaneously (sensitization)	
1	1 ml. egg white subcutaneously (reinforcement of local sensitization)			
10				
11			Linear incision, 1 cm. long on sensitized area. Wound dressed twice daily with:	
			Powdered egg albumin (shock antigen)	Powdered horse serum (nonspecific antigen)
12			Dressings as above	
13	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
14	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
15	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
16	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
17	*	*	*	*
20				
29				
31				
Granulomata	Present	Absent	Present	Absent

*. Animals sacrificed.

Group III				Group IV			
A	B	C	D (control)	A	B	C	D
x	x	x	x	3	3	3	3
3 ml. egg white intraperitoneally; 4 ml. egg white subcutaneously (sensitization)				2 ml. egg white intraperitoneally; 3 ml. egg white subcutaneously (sensitization)			
4 ml. egg white subcutaneously (reinforcement of local sensitization)				3 ml. egg white subcutaneously (reinforcement of local sensitization)			
				4 ml. egg white subcutaneously (shock antigen)	3 ml. horse serum subcutaneously (nonspecific antigen) (re-sensitizer)	3 ml. human serum subcutaneously (nonspecific antigen)	
4 ml. egg white (shock antigen) subcutaneously—diluted							
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
*	*	*	*				
				4 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen becomes shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	
				4 ml. egg white subcutaneously	3.5 ml. horse serum subcutaneously	3 ml. rabbit serum subcutaneously (nonspecific antigen)	3 ml. egg white subcutaneously (shock antigen)
				*	*	*	*
Present	Present	Present	Present	Present	Present	Absent	Present

* Animals sacrificed.

Group II. Wound Healing Type of Inflammation. Topical Application of Shock and Nonspecific Antigens, in Dried Form

A. Shock Antigen. In the animals given specific antigen the denuded area was covered by fibrin-entangled ghosts of polymorphonuclear leukocytes, which rested upon an irregularly patterned network of capillary sprouts. These sprouts had hyperchromic endothelial and perithelial nuclei and hypertrophic cytoplasmic bodies. The new capillaries centered or surrounded granulomata. Some granulomata showed early intratubercular or peritubercular fibrosis (Fig. 6). Beneath was a dense histiocytic as well as fibroblastic granulation tissue, which housed swollen and blurred collagenous bundles. It harbored a rich network of reactive, newly laid capillaries and also granulomata.

Granulomata were more numerous and larger in surrounding areas, outside, but near the wound, under the intact skin. Here evolutive and involutive changes were intermingled. In these granulomata the histiocytes might show fibroblastic metamorphosis or congregate into syncytia or giant cells, or be interspersed with plasmacytes. Encapsulation of solitary granulomata and conglomeration of granulomata, absent in the areas uncovered by epithelium, were well represented in the para-focal areas of the granulation tissue, which were protected by the skin.

B. Controls. In the animals given nonspecific antigen there was no evidence of granulomata, of focal accumulations of histiocytes or related cells, or of endothelial or perithelial cell reactivity. The network of capillary sprouts was radially patterned. The granulation tissue appeared somewhat looser and contained fewer histiocytes than that of the experimental animals. Connective tissue fibers showed no alterative changes.

Group III. Shock Antigen in Varying Dilutions

Granulomata were found in all of the experimental animals.

There seemed to be no necessary relationship between the strength of the shock antigen, on the one hand, and the number and size of the granulomata or of their evolutive and involutive changes and their tendency to conglomerate, on the other. Early granulomata might be found in the guinea-pigs which had received undiluted shock antigen, whereas intratubercular fibrosis with or without degeneration might be seen in the guinea-pigs which had received the more diluted doses. In some fields, the histologic pictures were similar, regardless of the dilution used.

This group of experiments was designed to show graded stages of development of the granuloma. From a histogenetic approach, how-

ever, the experiment is not more adequate than the above experiments or those still to be described.

Group IV

(A) *Specific Antigen, Repeatedly Administered, at Time Intervals of Latency Period Length, Induces Granuloma Formation.* Granulomata, either solitary or conglomerate, were found in varying stages of involution. In some granulomata, intertubercular or intratubercular connective tissue fibrils as well as mucoid degeneration of some of the connective tissue fibers might be seen. Many granulomata displayed giant cells. Lymphocytes and plasma cells were demonstrable, especially at the junction of two or more granulomata, when the latter were incompletely coalesced. There was also plasma cell and monocytic cuffing of capillaries and precapillary arterioles, as well as perineural monocytois. A recently superimposed mild acute inflammation with the usual marked edema and eosinophilia completed the histologic picture.

(B) *Nonspecific Antigen Repeatedly Administered, at Time Intervals of Latency Period Length, Re-sensitizes the Animal, Becomes Specific, and Induces Granuloma Formation.* Conglomerate granulomata were present, mostly in the stage of mucoid degeneration. There were also perivascular and perineural monocytois and histiocytosis, without definite clear-cut granuloma-like margins. The remainder of the changes were consistent with a residual inflammation with slight, recently superimposed, acute inflammation and marked eosinophilia. Thus, the changes were identical with those seen in animals A.

Animals A received four subcutaneous injections with specific antigen, counting the original sensitizing dose of egg white. Animals B received only three injections with horse serum. The first injection of horse serum, given 10 days after the initial dose of egg albumin, became a sensitizing injection, while the doses of horse serum given on the 20th and 29th days became shock antigen. Animals B had therefore only two doses of shock antigen (whereas animals A had three) and the action of this antigen upon the focal area was 10 days shorter; yet the changes were similar in both groups.

The experiment proves that "re-sensitization" to a new specific antigen is manifested in its focal response by granulomata, and that while it is possible to annihilate a first sensitization, it still influences the histologic picture by an "additive factor," as shown by almost identical findings in both experiments.

(C) *Various Nonspecific Antigens, Administered at Time Intervals of Latency Period Length, but Never Repeated, Do Not Induce Granu-*

loma Formation. There was no evidence of granulomata, of focal accumulations of histiocytes and monocytes, or of endothelial or perithelial reactivity. Residual inflammation with superimposition of slight and recent acute inflammatory stages was demonstrable. There were occasional perivascular eosinophilic infiltrates.

(D) *Nonspecific Antigens Intercalated between Sensitizing and Shock Antigens Do Not Prevent Granuloma Formation.* There were granulomata of varying size. In most of them the histiocytes were transforming into fibroblasts. Alterative changes were conspicuous and exhibited diverse modalities ranging from hyalinization to mucoid and myxomatoid degeneration. The granulation tissue, either in the areas where it harbored granulomata, or in the areas where granulomata were absent, showed fair numbers of monocytes and histiocytes. The microscopic picture appeared "too advanced" or "too speeded-up" for an interval of time of only 2 days between the injection of a single dose of shock antigen and the killing of the animals, as well as for animals which had received in all only one shock injection. This may be attributable to a sort of "additive effect" of the previously intercalated nonspecific antigens.

III. THE ANAPHYLACTIC GRANULOMA: MORPHOLOGY, HISTOGENESIS, EVOLUTION, AND INVOLUTION

The qualitative changes in a focal anaphylactic inflammatory response manifest themselves in two forms:

1. Histiocytic and monocytic accumulations, which may be irregularly shaped, ill-delimited, or streamer-like condensations in the granulation tissue. They are unrelated to vessels, nerves, or fat tissue. Such cell aggregates are rather rare and usually found outside the area housing the granulomata. (Diffuse histiocytosis.)

2. Round or ovoid cell accumulations, which vary in size and age, and may be solitary, or fused, encased into similar formations, or conglomerated. They are often well-delimited, and rarely faintly contoured. They may be encapsulated or not. They are related to nerves, adipose lobules, and mainly to capillaries as well as, in rarer instances, to precapillary arterioles. (Granulomata.)

- (a) The perineural granulomata (Fig. 3) are generally nonencapsulated and have peripherally fading histiocytes and monocytic streamers. In cross section they appear rounded. When the nerve is cut obliquely or lengthwise, their shape is irregular, vaguely triangular, with slightly curved, internally concave margins and smoothed angles. In contradistinction to the perivascular granulomata, they are more

readily evanescent and their histiocytes are more prone to undergo fibroblastic metamorphosis. Their histiocytes and monocytes usually do not pierce the perineurium, whereas the polymorphonuclear and, especially, eosinophilic leukocytes almost always invade the nerve.

(b) Granulomata developing in and around adipose lobules (Fig. 2) constitute a variant of the perivascular granulomata, structurally modified by environmental factors. They originate at the expense of the intralobular capillaries, by multiplication and unleashing of the prospective potencies of their endothelial and perithelial cells. The newly engendered cells impinge upon the neighboring fat cells and stem, by diffuse streamers of monocytes and related cells, into the argentaffine networks which house the adipose cells. Later the reactive cells penetrate into the fat-emptied adipose cells, and the lobule is ultimately entirely converted into a granuloma. Some of the peripheral cells may spread outside the lobule into the surrounding granulation tissue.

(c) The perivascular granulomata (Figs. 1 and 4) are by far the most numerous and the most conspicuous reactive cell aggregates. They may occasionally surround a precapillary arteriole (Fig. 2). As a rule, however, the perivascular granulomata are centered, at one or another of their evolutive stages, by a functional or vestigial capillary. This capillary may be stromal, but in most instances is a newly formed capillary of the granulation tissue. In some instances, a nerve and its flanking vessels may be embedded in a mass of granulomatous cells.

Shape. A pericapillary granuloma, when solitary, is more or less regularly circular or ovoid in its outline. Reactive cells from the surrounding granulation tissue may congregate towards a nonencapsulated or already encapsulating granuloma. If they surround concentrically the former layers moulded upon the central capillary, the granuloma will be spheroid. If the reactive cells become apposed to one or both of the spheroid's poles, the shape will assume that of a spheroid surmounted by two cones and later on that of an ovoid. When cells from the granulation tissue are apposed to an encapsulating granuloma, the inner rounded nodule is incarcerated within a larger one; such encased granulomata are not uncommon. Two or more granulomata may come into contact (Fig. 6), or they may fuse together without total loss of their individuality (Figs. 7, 8, and 9). They also may form a larger or coalesced unit, the polycyclic contours of which indicate their multicentric origin. Usually granulomata show a central vestigial capillary, except when the cut surface is a secant to the spheroid granuloma, or when the central capillary has undergone complete necrosis.

Size. Most of the solitary spheroid granulomata measure about

200 μ in diameter. Ovoid single granulomata measure up to about 450 by 250 μ . The largest single granuloma found so far measured 1.5 by 0.5 mm.

Cell Constituents. Histiocytes and Derivatives. Most of the cells are histiocytes, both in actual appearance and in origin. They may show slight variations in type. Some of the cells may lack one or several of the characteristics of histiocytes or monocytes, but they come nearest to such forms. Primitive mesenchymal cells may also be found, scattered among the histiocytes of the nodule. Entirely or predominantly monocytic granulomata are frequent. In occasional granulomata, reticular cells are demonstrable. They rest upon argentaffine fibrils and harbor in their meshes monocytes, giant cells, or syncytia. The enmeshed monocytes may congregate in sheet-like fused elements.

Syncytia, Epithelioid and Giant Cells. Reactive cells, regardless of type, may form small syncytia (Fig. 8) with crowded or piled up nuclei, or larger syncytia when the nuclei are less crowded or separated. The cells may also gather into an epithelioid pattern (Fig. 8) or form giant cells (Fig. 5). Giant cells are usually found at the periphery of the granulomata.

Alterative Changes; Metamorphoses. The cell types may change with the evolutive and involutive phases of the granuloma. It is not uncommon to see them in rows in which the histiocytes which undergo alterative changes are swollen by vacuoles of varying size which give them a foamy character. Before the granulomata undergo fibrosis or degeneration, some of the histiocytes and related cells show fibroblastic metamorphosis. The peripheral histiocytes are transformed into fibroblasts earlier than the central or midzonal elements. Sometimes the marginal fibroblasts, which either delimit or start to encapsulate a granuloma, are extratubercular in origin and stem from the interstitial granulation tissue.

Lymphocytes, Plasma Cells, Eosinophils. In some instances the histiocytes of a granuloma are intermingled with other cells, such as lymphocytes, and, more often, plasma cells. The latter are peripherally located, appear in groups, parallel rows, or, more frequently, in the granulation tissue which separates solitary granulomata (Fig. 13) or incompletely fused granulomata. Lymphocytes and plasma cells usually undergo degenerative changes earlier than the histiocytes. In other instances, when several coalescing granulomata show an almost total mucoid or myxomatous degeneration, the only viable or recognizable cells present are the peripheral or intertubercular plasmacytes. In very rare instances, small granulomata may be entirely plasmacytic.

Eosinophils wander among the granuloma cells. Granulomata with no admixture of eosinophils may also be found.

Vascularization. As a rule granulomata are nonvascular, except for the central capillary, the circulatory function of which is lost very early during the histogenesis of the granuloma. In some instances a newly formed capillary courses along an arc of the tubercular periphery and indicates the limit of the granuloma. In other cases a new capillary will branch into intratubercular sprouts which never fan out beyond the outer third of the tubercular area. Penetration of newly formed capillaries into a granuloma usually ushers in fibrosis, although sclerosis may occur also in absence of an intruding vessel, by direct fibroblastic metamorphosis of the histiocytes.

Histogenesis. The initial changes are either predominantly endothelial (Fig. 11) or more markedly (Fig. 10) or even exclusively perithelial (Fig. 1). Simultaneous endothelial and perithelial responses (Fig. 12) are not uncommon and proceed with more or less identical pace in both cell types.

The endothelial cells are swollen and increase up to three to four times their usual size (Fig. 10, and more characteristic in Fig. 12). The nucleus becomes more vesicular, with peripheral, dark, rod-like chromatin strands and a rather clear center; the cytoplasm is slightly darker and in cases basophilic; the cell outlines are precise. Soon the flattened endothelial shape is lost; the cell becomes vaguely fusiform, then more rounded (Fig. 11), and finally sessile or pedunculated. It may rest anchored to the reticular wall or become detached and fall into the lumen. Mitotic figures are frequent in the metamorphosing stage of the endothelial cells as well as in their derivatives. In certain cases most of the endothelial cells respond simultaneously, although the pace of their metamorphosis is not necessarily synchronous. In other cases only one endothelial cell will display reactivity, and the remainder will be quiescent. In a short time the lumen is crowded (Fig. 11) and, in cases, plugged with cells which resemble monocytes, although their cytoplasm may be larger than that of the typical monocyte and the nucleus less indented. Some of the monocytoid forms have cytoplasmic processes. The derivatives of the endothelial cells may be flanked by intraluminal eosinophils. They may phagocytize erythrocytic debris, although this is only rarely seen. Red cell or hemoglobin engulfment, when it occurs, is imperfect or clumsy. No fibrinous thrombi are seen at this or any other stage in capillaries, but may be seen in postcapillary venules. The clump of intraluminal cells does not elicit at any stage a parietal fibroblastic reaction, nor does it

undergo any abortive, involutive, or degenerative process prior to granuloma formation.

The perithelial cells likewise show mitotic figures (Fig. 10) and more frequently than the endothelial cells. Clumps of cells appear at points where usually only one pericyte is expected. The newly formed cells are piled up, sometimes in an epithelial-sheet fashion. The forms are not clear-cut at the beginning; and their evolution, step by step, is more difficult to follow than in the case of the endothelial derivatives. Although they may not be typical histiocytes, they resemble most closely this type, as they display round to flattened cytoplasmic bodies with vaguely ragged outlines and bean-shaped nuclei with inconspicuous nucleoli. Likewise some cell derivatives come nearest to monocytic or original mesenchymal forms, but cannot in all instances be indisputably termed as such. Histiocytic and monocytic patterns predominate, however.

Concomitantly with the endothelial and perithelial metamorphoses, the capillary wall undergoes changes of its own. The various stages range from irregular, blurred staining, fragmented straight or wavy fibril-like structures to a dot-like disintegration. In some cases, when the capillary walls have disappeared, the cells of endothelial ancestry can still be differentiated from those of perithelial origin, the former, mostly monocytic in type, being encased by the latter, which are mainly histiocytic. In subsequent stages, in most instances, this differentiation is no longer possible.

When cell aggregates surround fibrillar remnants of a capillary wall the impression may be gained that the anaphylactic granuloma may have an origin similar to that of an Aschoff body. This impression is false, however. Indeed, (1) all stages ranging from centering capillaries that are intact to capillary wall débris are demonstrable; (2) the dot-like disintegrated walls of the central capillary are reticular and not collagenous; (3) these remnants do not swell, but disintegrate; (4) they do not undergo a fibrinoid degeneration; (5) the reactive cells of a granuloma, even at their earliest evolutive stages, do not show bar-like central nucleoli, conspicuous nuclear vesiculation, or markedly ragged cell outlines; (6) the granuloma at any stage shows more compactness of texture and cell richness than an Aschoff body; (7) the intracapillary and pericapillary origin of a granuloma is in most cases clearly evident in younger granulomata, and traceable in retrospect in older ones.

At this stage of its formation the granuloma is made up of crowded cells, arranged either concentrically or in no definite patterns. Although no marginal fibroblasts are as yet present, the granuloma has a

clear-cut outline, except when it sends out cell streamers. But even in such cases, the bulk of the cell aggregate constitutes an anatomic unit.

At various stages of its development, the granuloma acts as a center of attraction for neighboring cells when these are endowed with identical morphologic and functional characters. Histiocytes and monocytes from the surrounding granulation tissue appear to have pivoted and turned one of their poles towards the periphery of the nodule, to have come nearer to it, and finally to have reinforced the cells of the granuloma.

Encapsulation, Fibrosis, Involution. Involution changes may be seen at any time, even when the granuloma shows signs of growth. One or several histiocytes, whether peripheral or midzonal, may undergo a fibroblastic metamorphosis. This is heralded by a change in their axis of orientation, which from radial becomes tangential, or by their coursing in a curvilinear direction, with their concavity facing the center of the granuloma. They may lie in single files or in concentric layers, be localized on only one of the sectors of the circumference, or be scattered all around the periphery of the granuloma (Fig. 6). They may or they may not be accompanied by connective tissue fibrils. Encapsulation may therefore be only polar or on an arc of the circumference before becoming circular. In many cases, in which the wrapping collagenous fibers are missing, the encapsulation is only apparent and rather in the nature of a fibroblastic delimitation. Fibroblastic metamorphosis of midzonal histiocytes brings about a delineation of a small circular arc which finally becomes a spheroid encased into the granuloma. Sometimes encapsulation is begun, but new histiocytes which come from the granulation tissue are apposed to the fibroblastic barrier. In the course of time, the granuloma will be either partially or entirely fibrosed.

Fibrosis may or may not be followed by degeneration. This degeneration may be mucoid (Fig. 8) or myxomatoid (Fig. 9). In the mucoid type the fibrils, and later on the cell processes and the fibroblastic bodies as well, take the basic components of the Mallory stain and assume afterwards a greenish hue (pale orange with phosphotungstic acid hematoxylin). Subsequently parts of the granuloma are replaced by greenish patches where vestigial cells are seen, either shriveled or swollen. In the myxomatoid type the cells become separated but are still in contact with their polar or stellate processes; the intervening matrix is ridged, or coarsely granular, or homogeneously swollen and of the same greenish hue (when stained with Mallory's phloxine and methylene blue). The picture suggests Wharton's jelly.

Degenerating granulomata may show a diversified structure. In the

mottled greenish mass of mucoid matrix, the cells may or may not be identifiable. In other cases the degeneration is preceded by the appearance of giant cells or syncytia. Fibrosed or degenerated, the granulomata are always recognizable by their shape, by the concentric or vaguely whorled arrangement of their cell remnants, and by the richness of the circumscribing granulation tissue in histiocytes and plasma cells.

No experiments have been performed to ascertain the length of time necessary for a granuloma to disappear.

SUMMARY

Subcutaneous injections of shock antigen into specifically sensitized guinea-pigs induce a local inflammatory reaction which is at variance with nonanaphylactic ("normergic") inflammation, both quantitatively and qualitatively.

1. The quantitative changes are known from the work of Arthus and Breton, Rössle, Gerlach, and others, and consist of a stormy and intense unfolding of the process with initial ischemia, overwhelming edema, marked polynucleosis, eosinophilia, and early fibrosis as main characteristics.

2. The qualitative changes are characterized by:

A. Complete local resorption of the specific antigen. Whether this resorption is in the nature of a local fixation by either edema or reactive histiocytes is, at this stage of the investigation, only conjectural.

B. A histiocytosis, which may manifest itself:

I. Rarely in diffuse aggregates of histiocytes and related cells.

II. Most frequently by granulomata, which are:

(a) Perineural, the least reactive cell aggregate;

(b) Perivascular, and especially pericapillary, the best represented and the most reactive cell aggregate;

(c) Intra-adipose and peri-adipose, an environmentally conditioned variant of the pericapillary granuloma.

3. The morphology, cell constituents, histogenesis, evolution, and involution of the anaphylactic granuloma have been described.

4. The origin of the granulomata is twofold:

A. Endothelial and/or perithelial, through prospective potencies of these cells.

B. In a minor degree, through apposition of histiocytes from the surrounding reactive granulation tissue.

5. The qualitative reaction occurs constantly. Dilution of the shock antigen is immaterial. There are, however, variations with regard to

the intensity of the reaction (number, size of granulomata, their tendency to conglomerate) and to its modalities (presence or absence of plasma cells; epithelioid patterns; giant cells). This variability in intensity and modality of the focal anaphylactic response is at present not attributable to any factor. Such variations may be seen from experiment to experiment, or in cases, from animal to animal within the same experimental group, and even from tissue block to block.

6. The part played by intermingled cells (lymphocytes, plasma cells) is not clear. However, plasma cells are more frequently seen: (a) at the periphery of the granulomata, especially when the nodules fuse or congregate; (b) at the periphery of fibrosing and, especially, degenerating granulomata; (c) in granulomata induced by shock antigen repeated several times at long intervals; (d) in pericapillary mantlings; (e) occasionally as small plasmacytic granulomata.

7. Local injections of nonspecific antigen do not induce granulomata, except when the nonspecific antigen, by repeated injections, at time intervals of latency period length, re-sensitizes the animals and becomes specific (or shock) antigen.

8. Nonspecific antigens, when preceding specific antigens, or when intercalated between the sensitizing and shock antigens, may speed up the histogenesis, evolution, and involution of granulomata, and act as "additive factors" which elicit "additive effects."

9. The anaphylactic granuloma is not patterned histogenetically and histologically after the Aschoff body of rheumatic fever.

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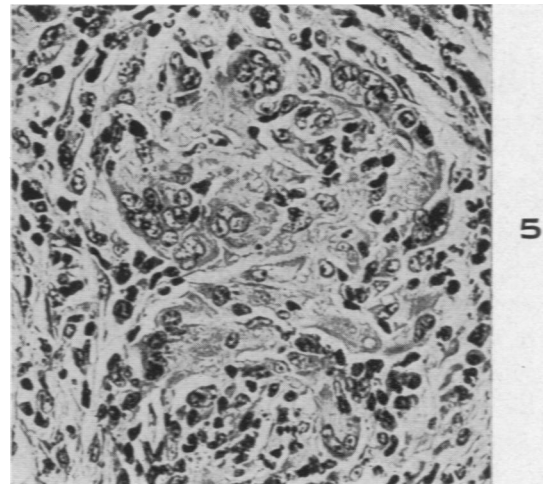
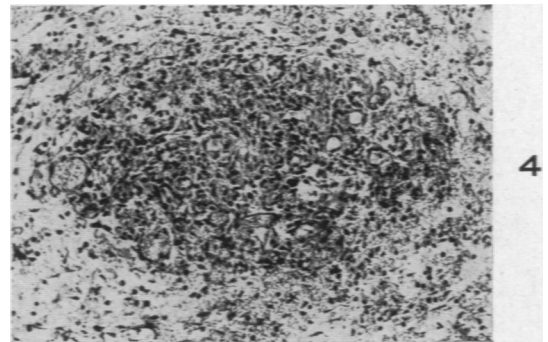
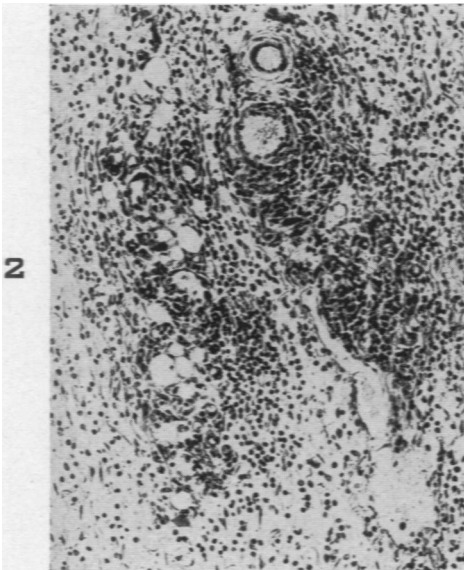
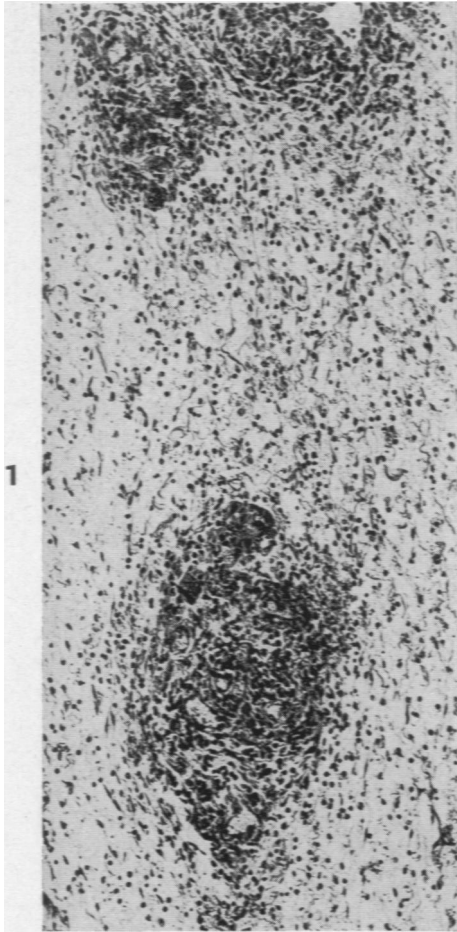
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DESCRIPTION OF PLATES

PLATE 140

- FIG. 1. Group I, A. Three granulomata, with intervening area of edema with monocytic infiltration. The upper left granuloma is centered about an arteriole. The lower, although appearing as an anatomic unit, is multicentric. Of note are the widely dilated capillaries and the absence of endothelial reactivities; this is an example of a granuloma of exclusively perithelial origin. Phloxine and methylene blue stain. $\times 140$.
- FIG. 2. Group I, A. Peri-adipose and intra-adipose granuloma, early. The lobule is not yet entirely converted into a granuloma. Next to it (separated by lymphatics) two coalescing peri-arteriolar granulomata can be seen. Phloxine and methylene blue stain. $\times 140$.
- FIG. 3. Group I, A. Granuloma embedding a nerve and its vascular satellites. Of note is the appositional growth. Phloxine and methylene blue stain. $\times 140$.
- FIG. 4. Group I, A. Well outlined, but nonencapsulated granuloma. Phloxine and methylene blue stain. $\times 140$.
- FIG. 5. Group IV, A. Small granulomata converted into groups of giant cells. Phosphotungstic acid hematoxylin stain. $\times 460$.

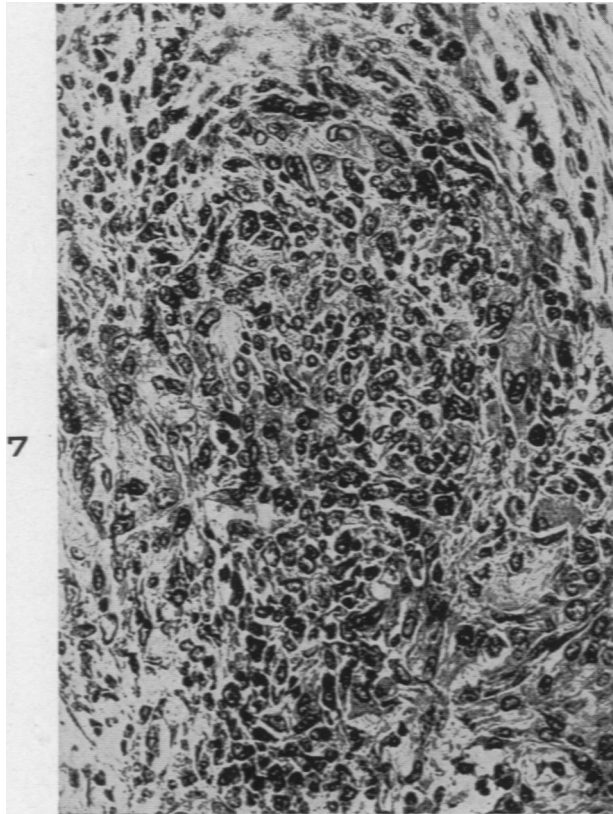
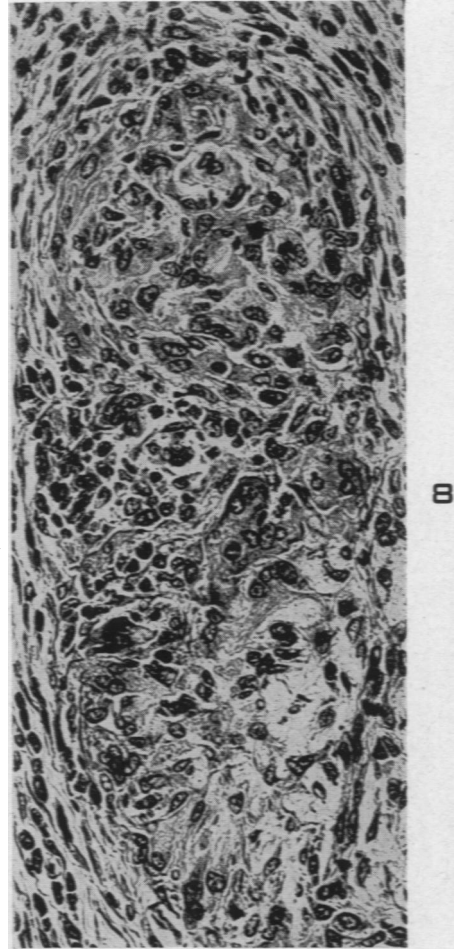
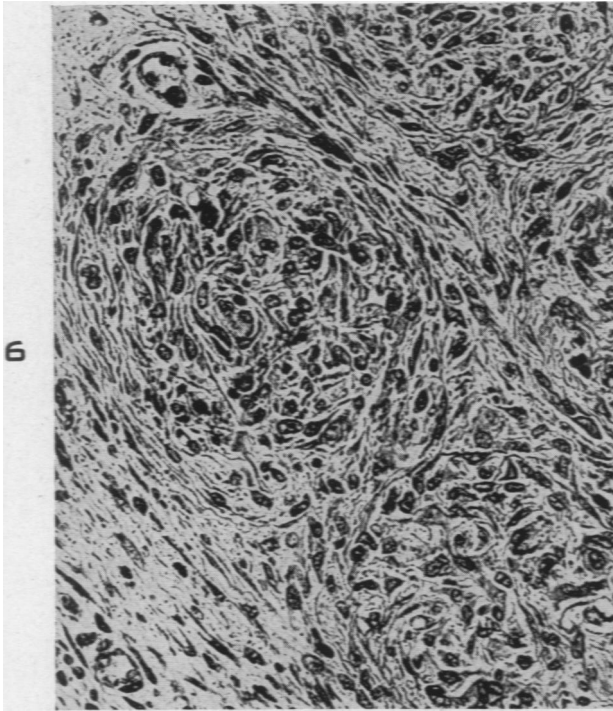


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Granuloma in Focal Anaphylactic Inflammation

PLATE 141

- FIG. 6. Group II, A. Group of granulomata with fibrosing internodular granulation tissue. The upper (and leftwards directed) granuloma shows the vestigial capillary with obliterated lumen (group of whorling cells). In all granulomata there is evidence of actual or impending fibroblastic metamorphosis. Masson's trichrome stain. $\times 555$.
- FIG. 7. Group IV, A. Histiocytic granulomata, with compact texture. Phosphotungstic acid hematoxylin stain. $\times 595$.
- FIG. 8. Group IV, A. Granulomata and internodular granulation tissue. Upper granuloma made up mostly of histiocytes (fibrillar structure of cytoplasm brought out by Mallory's phosphotungstic acid hematoxylin stain). Vestigial capillary is slightly eccentric. In lower granuloma, where an early, central mucoid degeneration is demonstrable, the marginal histiocytes show an epithelioid pattern and formation of syncytia. Of note is the perinodular fibrosis. Phosphotungstic acid hematoxylin stain. $\times 595$.
- FIG. 9. Group II, A. Group of degenerating granulomata. Myxomatoid degeneration (best seen in the granuloma on the left). Phloxine and methylene blue stain. $\times 280$.



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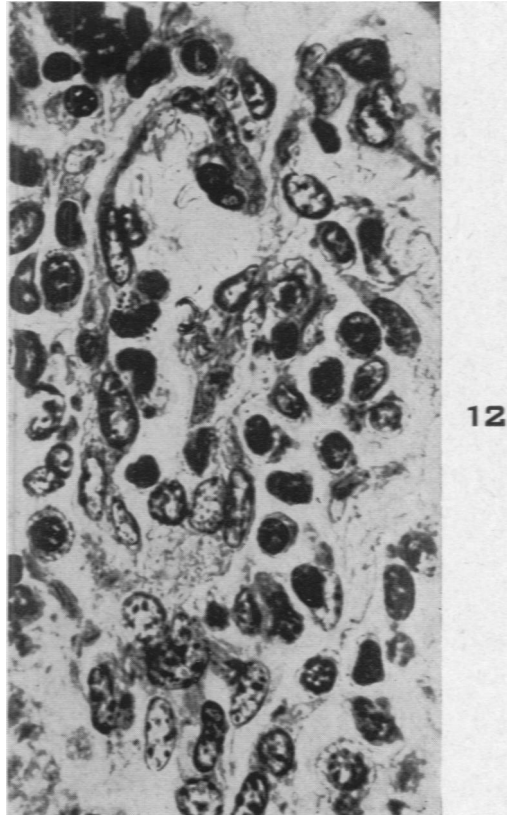
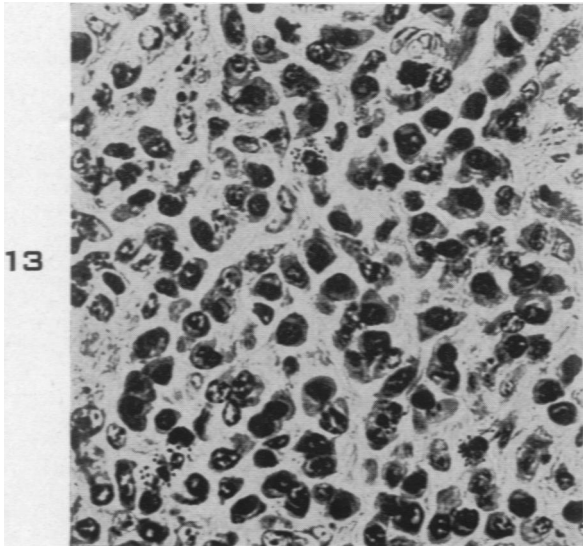
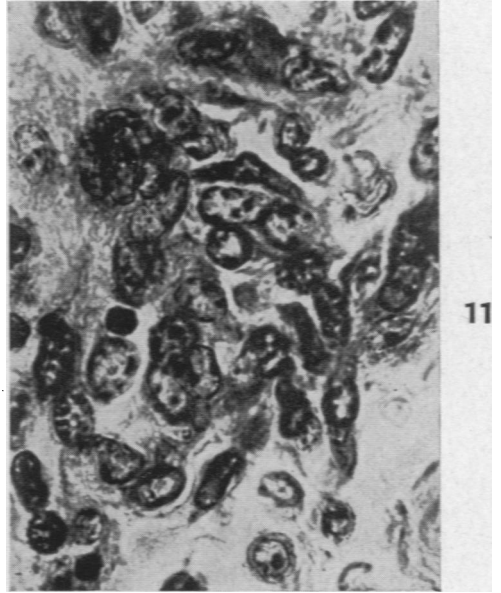
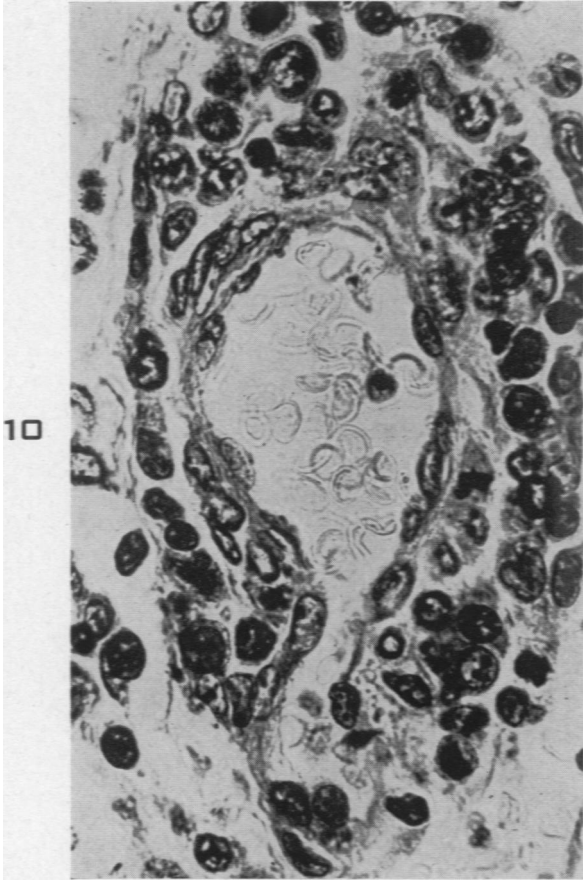
PLATE 142

FIG. 10. Group I, A. Histogenesis of a pericapillary granuloma. Marked perithelial proliferation (several mitotic figures) and metamorphosis (histiocytes, monocytes). Earliest endothelial hyperplasia. Phloxine and methylene blue stain. $\times 1110$.

FIG. 11. Group I, A. Histogenesis. Section through an S-shaped capillary, showing narrowing of the lumen and endothelial hyperplasia, hypertrophy, and metamorphosis. There is also slight perithelial reactivity. Phloxine and methylene blue stain. $\times 1110$.

FIG. 12. Group I, A. Two neighboring capillaries. The capillary towards the left of the figure shows slight to moderate endothelial and perithelial reactivity, but no luminal blockage. The perithelial cells (left margin) are still metamorphosing. The capillary on the right no longer shows its reticulum wall; only a few endothelial cells can be identified; the lumen is crowded with metamorphosing cells. In the lower sector of the figure a vaguely triangular to trapezoidal patch shows the derived cells at almost the end-stage of their metamorphosis. Phloxine and methylene blue stain. $\times 1110$.

FIG. 13. Group IV, A. Plasma cell infiltrate in intertubercular granulation tissue. In places the circular arrangement may give the false impression of plasma-cytic granulomata. Phloxine and methylene blue stain. $\times 742$.



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