A STUDY OF THE GENERALIZATION OF VACCINE VIRUS FROM ENHANCED SKIN LESIONS

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Plates 16 to 18

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One of us has shown in a previous paper (1) that extracts from normal testicles are endowed with the power of enhancing to an extraordinary degree the lesions produced by vaccine virus. Extracts from kidney, liver, skin, brain and placenta share this property only to a certain extent. Extracts from suprarenal, retina, muscle and ovary do not modify the vaccinal infection, whereas blood, spleen and bone marrow extracts frankly interfere with and even suppress the activity of the virus.

Rabbits injected intradermally with mixtures of neurovirus and testicle extract become ill, develop a very high temperature, lose considerable weight, and approximately 25 per cent of the animals die from the disease. The present study is chiefly concerned with the histological characteristics of the wide-spread, typical vaccina, together with the non-specific lesions found at autopsy, and with the bearing of these findings on the much discussed question of the tissue affinities of the vaccine virus.

Calmette and Guerin (2) found that when vaccine virus was injected intravenously, it localized selectively in the shaved areas of the skin, whereas no localization was observed in mesodermic organs. This phenomenon was also observed by Camus (3), who found moreover that the virus localized in the buccal mucous membrane, genital organs and in cutaneous *naevi*. It was likewise noted by Levaditi and Nicolau, Rivers and Tillett, Noguchi and others (4, 5). Borrel and Burnet (6, 7) introduced the term *épithélioses* for a group of diseases produced by filter-passing viruses; viz., vaccinia, variola, fowl-pox, sheep-pox and molluscum contagiosum, all of which were said to attack epithelium almost exclusively. Lipschütz (8) promulgated the theory that the tissue localization of a virus is

conditioned by so-called specific affinities. A similar hypothesis was sustained by Menze (9). The doctrine of the *ectodermoses neurotropes*, however, has been defended mainly by Levaditi and his coworkers. Vaccine virus is regarded as a typical member of this group, showing a marked affinity for the internal segment of the ectoderm (neuraxis) and a more or less pronounced affinity for the external segment of the same ectoderm. The mesoderm and blood, therefore, would be considered wholly non-susceptible, and only certain entodermic organs would be involved.

In 12 experiments performed by Levaditi and Nicolau (10) vaccine virus was injected into rabbits, usually by the intravenous route. These rabbits were killed in from 6 to 10 days. The virus did not localize, or else was poorly localized, in mesodermic organs, whereas it did localize in the mammary glands, adrenals, tongue and buccal mucosa, and likewise in the skin and brain, but only after irritation. It localized abundantly in the lungs and moderately in the liver and the submaxillary salivary glands. Histological lesions, infiltrative or necrosing, were found in the mammary glands, and on two occasions in the lungs and liver. They were always absent in mesodermic organs. Of all the structures studied by Levaditi and Nicolau, the testes and ovaries were regarded as the most sensitive to vaccine virus because the virus could readily be recovered from them and because, at histological examination, necrotic lesions, interstitial in distribution, were consistently found.

The susceptibility of the testicle had already been shown by the work of Noguchi (11), Ohtawara (12), Rivers and Tillett (loc. cit.) and others, who found that the testis was the most suitable organ in which to demonstrate very small amounts of virus. Moreover, Parker (13), Merwin and Schemerling (14), and Eagles and McClean (15) reported data showing that testicular tissue is an excellent substrate for the *in vitro* cultivation of vaccine virus.

The concept of *ectodermoses neurotropes* was enunciated in 1921 (16). Since then there has been a reaction against it, since later experimental work has failed to bring supporting evidence. Thus, Blanc and Caminipetros (17), Chaumier (18), Walthart (19) and Ledingham and McClean (20) succeeded in implanting and passing vaccine virus in pure mesodermic tissues, such as muscle, kidney, lymphatic tissue and derma (corium) without any loss of activity. Moreover Ohtawara (loc. cit.) and Gildemeister and Heuer (21), after intratesticular inoculation, withdrew the virus from the blood as late as the 15th day after the injection. Recently Wilson and Smith (22) by the use of a special technique of fractionation, found the virus adherent to the white blood cells up to 8 days after injection. Vaccine virus has been successfully cultured *in vitro* in a kidney medium by H. B. and M. C. Maitland (23).

Watanabe (24), in a small series of animals injected intravenously, found that the virus localized especially in the dermis without involvement of the epithelial cells; it was likewise found in the liver and spleen.

A very extensive study of the subject has been made by Douglas, Smith and Price (25), who, after intravenous injections in a particularly sensitive strain of

rabbits, noted that the virus produced macroscopic specific lesions (pocks) in the following organs, arranged in order of their frequency of involvement: first, lung, skin and mucosa, spleen, and liver; secondly, genital organs and adrenals, both with the same percentage incidence of lesions. After intradermal and cerebral inoculation some generalization was detected, especially in the lungs, liver, skin and spleen. The tissues were tested by sub-inoculation for infectivity and it was found that during the first two weeks the virus was abundant in the lungs, spleen, bone marrow and genital organs; after this time, however, the skin, tongue, adrenals, ovaries and testicles seemed the most prone to harbor the virus. These authors' conclusion was that vaccine virus "possesses varying degrees of affinity for different tissues and general statements of tissue susceptibility to vaccinia virus should however be received with caution."

Ledingham (25) believes that the reticulo-endothelial system is in the main affected and responsible for the vaccinal infection, the epithelial lesions being secondary to the attack on the mesodermal derivatives. This conclusion was reached both by the histological study of the experimental lesion and by the fact that a blockade of the system in the skin with India ink frankly interfered with the infection. Several authors have already regarded the variolous pocks in the skin as being purely inflammatory. MacCallum and Moody (27) state that in alastrim the corium is involved before the epithelium.

The literature concerning the pathological anatomy in variola and allied diseases is most extensive. A complete account of the work previous to 1904 will be found in the memoirs of Councilman, Magrath and Brinkerhoff and their associates (28), but most of these papers are concerned with the parasitic nature of the virus and deal chiefly with skin and corneal lesions where Guarnieri bodies are easily found. Nevertheless the above-mentioned authors give a very complete description of the generalized lesions in variola, noting in the testes specific "anemic focal necrosis," and furthermore degeneration apparently not truly anemic in origin, but "due to the action of toxins," in the blood-forming cells of the bone marrow which constitute for them lesions almost pathognomic of variola but nevertheless devoid of "parasites." Chiari described these bone marrow lesions, applying the term "osteomyelitis variolosa." Councilman and his associates likewise noted the constant occurrence of more or less acute diffuse degenerative changes in liver, kidney, suprarenals and testes.

The most common lesion found in the lung and one which was very rarely absent was bronchitis, usually combined with more or less extensive bronchopneumonia even when this was not grossly demonstrable.

As early as 1886 Chiari (29) found in the testicles of 15 variolous children and in 85 per cent of adults with smallpox, typical foci with a central area of necrosis surrounded by an area of cellular infiltration. He regarded these lesions as being due to the direct action of the virus. Similar necrotic specific changes in the bone marrow and testes have been found by MacCallum (31) in alastrim. MacCallum mentions the intense cloudy swelling and focal necrosis in the liver, and the exten-

sive degenerative changes in the kidneys. In most cases there was an acute bronchitis, and in many there occurred a rather severe lobar pneumonia.

Material and Methods

The rabbits used were mostly of the common gray variety; some, however, were of the albino type. No essential difference between them could be detected. The age of the animals appeared of no significance.

The animals studied were grouped and treated as follows:—

A) Twenty-one rabbits were injected intracutaneously with neurovirus plus testicle extract. Twelve of these died as a result of the infection. The others were killed when in a more or less serious general condition. All of them were autopsied during the course of extensive specific lesions in the skin, the area involved often occupying the whole flank and abdomen.

B) Eight rabbits were injected intravenously with either 1 or 2 cc. of neurovirus diluted to 1:10 or 1:20, alone or mixed with rabbit testicle extract. Two of the animals died; the others were killed.[1]

C) Two rabbits were injected in the testis, two more in the brain and one directly in the liver.

The disease resulting from the injection of neurovirus plus testicle extract when inoculated together into the skin has already been described (1).

Gross Lesions

At the postmortem examination of the animals dying from vaccinia the following changes were observed:

A markedly edematous zone of hemorrhagic character widely surrounded the borders of the vaccinial lesion in the skin. The lymph nodes of the drainage area were very much congested and enlarged. There was marked congestion of the splanchnic organs—kidneys especially; the liver was enlarged. The peritoneal cavity frequently contained a few cubic centimeters of viscid hemorrhagic fluid. The muscles of the abdominal wall occasionally showed discrete punctate hemorrhagic areas. There was uniformly congestion of the testes with some fluid in the tunica vaginalis. The most obvious gross changes were found in the lungs where

[1] The virus was prepared as described in other papers. This particular sample was of a uniform activity, giving positive takes in the skin at a dilution as high as 1:2.000.

the lesions consisted of foci of congestion and patches of lobular pneumonia. We are fully aware of the doubtful significance of such lesions in the rabbit lung. The pleurae and pericardium were occasionally involved, the serous cavities being distended by fluid.

In the animals killed the intensity of the lesions bore a comparable relationship to the gravity of the clinical condition.

Pocks in internal organs, as described by Douglas, Smith and Price, were never encountered. Only in the suprarenals and ovaries the existance of clean-cut and more or less hemorrhagic foci with superficial elevation of the gland surface enabled one to make the diagnosis of vaccinal infection macroscopically. The testicles likewise showed these foci, but less clearly. Skin generalization was observed but very rarely. In more than 200 rabbits with simple or enhanced cutaneous or testicular lesions, typical pocks were encountered with some regularity, although in small numbers, in the tongue and lips alone.

It must be kept in mind that we used the same strain of virus that Douglas, Smith and Price used, so that evidently idiosyncrasy in the rabbits is of paramount importance as regards the generalization and character of lesions produced by the neurovirus.

Microscopical Findings

Material was fixed in Zenker's fluid and thin sections—some 4 micra in thickness—were stained in Mallory's eosin-methylene blue, phloxine-methylene blue, or by Wolbach's modification of the Giemsa stain. Lesions regarded as specifically vaccinal were found by histological examination in lung, liver, spleen, lymph nodes, bone marrow, adrenal, ovary, and testis; one doubtfully specific lesion was encountered in the kidney. Changes considered as accessory and not definitely specific were found in spleen, lymph nodes and kidneys. In the specific visceral lesions nothing of the nature of Guarnieri bodies were found after long search, save perhaps in the ovary, where, in two animals, structures were present in Graafian follicle cells adjacent to an active vaccinal necrosis, seemingly cytoplasmic inclusions answering the histological criteria for the Guarnieri body. Both the characters and the distribution of the vaccinal lesions leave little doubt as to their specific nature. The characters are obvious from the subsequent descriptions. The distribution is not in agreement with that of any known type of accessory streptococcic invader since the organs affected mostly are those generally shunned by the blood-born streptococcus. Furthermore, a check on the vaccine content of the organ by subinoculation and a comparison between the organ content of virus and the blood content in the same animal would apparently eliminate any doubts.

Liver: Liver lesions occur in diverse forms. In the earliest foci the walls of the liver sinusoids are damaged and an exudate of precipitated albumen and fibrin occupies the space between endothelium and liver parenchyma. One might be

led to infer from these early capillary lesions, which are promptly followed by the appearance of fibrin plugs within the vessels, that the subsequently appearing, abscess-like foci are of thrombotic origin. However, before capillary thromboses occur even in the earliest lesions, one finds one or more punctate liver cell necroses, and it is the punctate character of these necroses which forces one to eliminate thrombosis as a causative factor in their genesis. The lesion is primarily parenchymatous. These necrotic liver cells undergo cytoplasmic hyalinization; they stain deeply pink with eosin-methylene blue or Giemsa stains; their nuclei become pyknotic or gradually fade out, the chromatin being reduced to a fine dust-like deposit. Neither nuclear nor cytoplasmic swelling ("ballonartige") has been seen.

As the lesion enlarges the capillaries become plugged over a wider area and the thrombosis may even extend into larger vessels. It is particularly in this case that one may be led to regard the lesion as thrombotic; however analysis forces us to abandon this suggestion since in several instances it has been possible to trace these wide-spread areas of hyalin necrosis and to follow their formation as fusions of originally punctate single cell necroses such as those shown in Fig. 1. They occur in rabbits grossly free from evidences of coccidiosis or verminous cysts. One noteworthy feature is the very low grade reactive process; a few polymorphonuclear leukocytes may appear in the thrombosed sinusoids and may invade the necrotic liver cells, but the appearance is that of a paralyzed, insignificant type of reaction; the leukocytes present exhibit marked fragmentation of their nuclei which rapidly degenerate into nuclear dust. Fibroblastic proliferation is absent in the acute stage. Guarnieri bodies have never been seen.

Suprarenal: The lesions of the suprarenal are quite similar to those of the liver, save that the broad zones of hyaline necrosis were but once encountered. The early foci are either of the nature of sharp punctate single cell necroses or isolated hemorrhagic foci of varying size. (Fig. 2). The parenchymatous cells undergo rapid hyaline degeneration; a fibrin exudate is thrown out about the capillaries; fragmented polymorphonuclear leukocytes invade the necrotic cells. The reaction may become fairly intense, whereupon the appearance is that of an abscess with intense necrosis. (Fig. 3). In the early stages there is evidence of stimulation of the suprarenal parenchyma and several mitotic figures per oil immersion field may be encountered in pink-staining, damaged cells. (Fig. 5). Ballooning degeneration has not been seen and no Guarnieri bodies were to be found. These lesions are not due to secondary bacterial invaders; they appear in animals injected intravenously or intratesticularly or directly into the liver—in animals without skin lesions to serve as a source for secondary microbic invasion.

Ovary: The ovarian lesions are widely distributed throughout all anatomic divisions of the organ. They are to be found in the stroma, they involve the interstitial cells, theca interna and externa, and corona radiata. They are of the usual hemorrhagic, necrosing, degenerative type (Fig. 4) with much fibrin deposit, and may call forth a considerable polymorphonuclear and monocytic reaction. In the ovary alone of all the viscera have structures been encountered which

answer all the histological requirements of the Guarnieri bodies. These cytoplasmic inclusions are pictured in Figure 6. One must emphasize, however, the difficulty of really interpreting these structures; necrosis is so acute and so extensive, cell fragmentation so wide-spread, bits of broken-up acidophilic cell detritus are so readily washed from place to place during the mere technical handling of material, that appearances may become very deceptive. We feel therefore some doubt as to the nature of the structures which answer the morphological requirements of Guanieri bodies, especially in view of the fact that in no other viscus have they been certainly encountered.

Testis: Testicular lesions involve tubules, interstitial tissues, and in one instance the tunic was affected, with the production of an acute vaccinal periorchitis. These specific lesions in no way differ from those of the ovary or suprarenal. Specific cell inclusions could not be identified. In addition, in many animals in which no specific testicular pustules were found, spermatogenesis was nevertheless in abeyance, a certain amount of isolated punctate sex cell necrosis was observed and although we are unable to state that these lesions are specific, they nevertheless are undoubtedly related to the vaccinia or to the generalized intoxication accompanying the disease. In the summary showing percentage incidence of lesions in various organs, these minor testicular findings have been regarded as non-specific and are therefore not included. Slight sex cell necrosis has been encountered in controls.

Lungs: In the lung the vaccinal lesions may readily be confused with those of rabbit pneumonia. They differ from the common pneumonic lesion in several respects: they are more sharply focal; they are not primarily associated with bronchi but rather with vessels. They occur as a pronounced inflammatory, necrosing process in the vessel wall, with edema, thick perivascular deposition, invasion of the vessel wall by polymorphonuclear leukocytes, greatly fragmented and largely necrotic. From the vessel the lesion spreads to the neighboring alveoli but is apparently slow in giving rise to exudates within the bronchi. The nature of the process is shown in Figure 9. Occasional localized pleural and subpleural lesions of the same type exist. The typical lung lesions have been but rarely encountered. In one animal an almost tubercle-like, sharply focal necrosing lesion was found, a necrosis with practically no reaction on the part of inflammatory cells and with no involvement of the bronchi. Trachea and esophagus have been uniformly negative.

Spleen: The splenic lesions do not partake of the pustular character of the typical vaccinal foci encountered in the lungs, liver, adrenal, ovary, testis, nor the dermal pocks. The most striking thing about the spleens is the very marked lymphocytic exhaustion. The Malphigian bodies are scanty and small; the pulp lymphocytes are reduced in number; occasionally germinal centers are seen without any surrounding zone of small lymphocytes. There may be necrosis of splenic capillaries with thick perivascular fibrin deposits (Fig. 7). Isolated cell necrosis,—of lymphocytes, polymorphonuclear leukocytes, and large phagocytic cells may

be found. Hemorrhage is common. Where necrosis has been present the splenic lesions have been classified as vaccinal; in spleens where the only feature has been the pronounced lymphocyte exhaustion the lesion has been regarded as accessory only. Numerous sub-inoculations with spleen might be required before these doubtful points could be decided. Lymph nodes contain lesions similar to those of the spleen and have been similarly regarded. Fig. 8 indicates the extensive necroses which may be encountered in the lymph nodes.

Bone Marrow: We have studied but one bone marrow. This marrow showed an extensive necrosing vaccinal osteomyelitis with destruction of all marrow elements. Very little reaction was present in comparison with the intensity of the necrosis. The reacting polymorphonuclear leukocytes were greatly fragmented.

Kidney: No specific lesions have been encountered in the kidneys examined. Nevertheless acute degenerative changes in the convoluted tubules and collecting tubules are very common. These changes consist in swelling, albuminous degeneration, the presence of tubule casts, and in one instance a rather puzzling lesion, provisionally interpreted as an infarct, but nevertheless a peculiar one, for instead of a sharp zone of necrosis with surrounding reaction, the necrosis was poorly outlined and isolated necrotic tubules extended in fan-like processes far outward from the zone of major necrosis into the surrounding region of normal tubules. Leukocytic reaction was conspicuous by its absence. It is therefore possible that this lesion was specific.

Percentage Incidence of Visceral Lesions

In rabbits bearing enhanced skin lesions the percentage incidence of visceral lesions is summarized in Table I.

TABLE I

Specific Necrotic Lesions in Rabbits with enhanced skin lesions

Organ	Number Examined	Number with Specific Lesions	Percentage
Ovary	4	3	75.0
Suprarenal	11	8	72.7
Lymph Nodes (Vicinity of Lesion)	11	6	54.5
Testicle	13	7	53.8
Spleen	15	6	40.0
Lung	21	4	19.4
Bone Marrow	1	1	100.0
Kidney	15	1*	6.6
Brain	8	0	0.0

* Doubtful.

Accessory lesions in rabbits with enhanced skin lesions have been found in 10 cases in the spleen (66 percent), (lymphatic depletion), in 13 cases in the lung (60 percent), and in 9 cases in the kidney (60 percent). It must be added that both specific and accessory lesions were present in practically 100 percent of rabbits dying of vaccinal infection. The lowering of this percentage is due to the introduction of figures from rabbits purposely killed, some of them while in a good general condition.

The results in animals injected intravenously, intratesticularly or into the liver are summarized in Table II.

TABLE II

Specific Necrotic Lesions in Rabbits Injected into Liver, Testis, and Brain

Organ	Number Examined	Number with Specific Lesions	Percentage
Ovary	1	1	100.0
Liver	7	3	42.9
Testicle*	10	4	40.0
Suprarenal	8	2	25.0
Spleen	8	2†	25.0
Lung	9	0	0.0
Kidney	10	0	0.0
Brain	1	0	0.0

* In the case of intratesticular inoculation, the lesions of the opposite testicle only have been taken into consideration.

† One doubtful.

Accessory lesions in these rabbits have been found in 6 cases in the lung (66 percent), 6 cases in the kidney (60 percent), and 5 cases in the spleen (63 percent), (lymphocytic depletion). In two rabbits injected into the brain and dying 3 days later, very wide-spread accessory lesions were found in the kidney and also in the intestine.[2]

In a total of nine rabbits, of which 5 were injected intravenously and killed after 4, 6, 7 and 9 days, in two animals dying with enhanced skin lesions and in two others dying 6 days after intratesticular injection, the organs were tested for the presence of the virus by applying

[2] The lymph nodes were never examined as there was no gross indication of any lesion whatsoever.

the various organs to the scarified skin of normal rabbits. That virus could be recovered from internal organs is shown in Table III.

TABLE III

Recovery of the Virus

Organ	Number Examined	Number from which Virus Recovered	Percentage
Ovary	1	1	100.0
Kidney	9	5	55.6
Testicle	8	4	50.0
Suprarenal	9	4	44.5
Liver	9	4	44.5
Lung	9	3	33.3
Spleen	7	2	28.9
Blood	9	1*	12.2

* Very weak eruption.

The histological examination of the same fragment of the organ which was tested for its virus content was in general agreement with the result of the inoculation. Nevertheless, there were some discrepancies, for virus was recovered from lung and spleen in three cases, where no lesions, specific or accessory, were found, and in three other instances lesions in liver, kidney and suprarenal were found, whereas no virus was recovered. Either the inhibitory effect of the organ or the possible antibody content may explain the apparent discrepancy. In three of the rabbits of injected intravenously, the neurovirus was inoculated after mixing with 2.0 cc. of rabbit testicle extract prepared as described in a previous paper (1). A very careful study of the organs of these rabbits from both the histological and the virus content point of view did not show more infection than in animals injected with the virus alone. Testicle extract therefore does not appear to enhance the action of intravenously injected virus.

DISCUSSION

It would be quite apparent that any statements as to organ susceptibility to vaccine virus must take several factors into consideration. If we test for the virus content of an organ by applying the organ to

the scarified skin of a normal rabbit we obtain a certain result. However we may be in error since the enhancing or inhibiting action of various normal organ extracts is so marked that these activities may be of more importance in influencing the take in the normal animal than is the actual virus content of the tested organ. Again in all probability when testing an organ for virus content we are inoculating a mixture of virus plus antibody; the antibody content may not be the same in all organs; the rate of antibody formation is perhaps quite different. Were one to separate by cataphoresis methods the virus from the antibody in the various organs, then quite a different organ virus content might be ascertained. However that may be, the simple application of the suspected organ to the scarified skin of the normal rabbit may give one index of so-called organ susceptibility. Were it possible to show that the enhancing or inhibiting effects of different organs are paralleled by the multiplication or suppression of virus within the living cells of these organs then one might adopt quite a different standard of organ susceptibility. Finally, the presence of specific histological lesions in different organs would seem to offer still a third criterion for estimating specific organ affinities.

Our charts as well as the results of Douglas, Smith, and Price demonstrate that nowhere does vaccine virus exhibit any clear-cut, selective affinity for organ groups of similar embryological origin. Every organ reacts to vaccine virus in its own peculiar manner. An organ may appear insusceptible so far as the actual presence of specific histological lesions is concerned, and yet, extracts of it enhance the action of vaccine virus when mixed therewith *in vitro* and injected into the skin of a normal rabbit; another organ may manifest specific vaccinal lesions, yet, *in vitro*, a similar normal organ may inhibit the action of the virus; an organ may contain virus but exhibit no histological lesion or may present a specific histological lesion but prove negative on sub-inoculation. Of all organs studied the testis alone yields a high percentage of specific lesions, gives a high percentage of takes on sub-inoculation, and has a marked enhancing action when its extract is mixed with vaccine virus and inoculated into the skin of normal rabbits. It is by far the most active of the organs we have tested.

SUMMARY

Rabbits bearing very intensive skin lesions resulting from the intracutaneous injection of neurovirus plus testicular extract show typical histological alterations in the gonads, suprarenals, liver, spleen, lung, lymph nodes, and bone marrow.

Similar but less wide-spread alterations are found after intravenous injection of neurovirus.

Although testicle extract injected intracutaneously with neurovirus has a marked enhancing action upon the activity of the latter, the same mixture injected intravenously yields no sign of any such enhancement.

The significance of these observations as regards the question of the ectodermotropism of vaccine virus is discussed, and the doctrine of specific organ affinities is considered.

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EXPLANATION OF PLATES

PLATE 16

FIG. 1. Liver. Zenker; phloxine-methylene blue. Focal hyaline liver cell degeneration. × 200.

FIG. 2. Suprarenal. Similar fixation, staining and magnification. Specific necrosing, hemorrhagic lesion.

FIG. 3. Suprarenal. Similar fixation and staining. × 53. Multiple specific, abscess-like, vaccinal lesions.

PLATE 17

FIG. 4. Ovary. Zenker; Giemsa. Hemorrhagic, necrosing lesion involving Graafian follicle and theca. × 110.

FIG. 5. Suprarenal. Zenker; Giemsa. Early necrosing lesion showing four mitotic figures as evidence of stimulation. × 220.

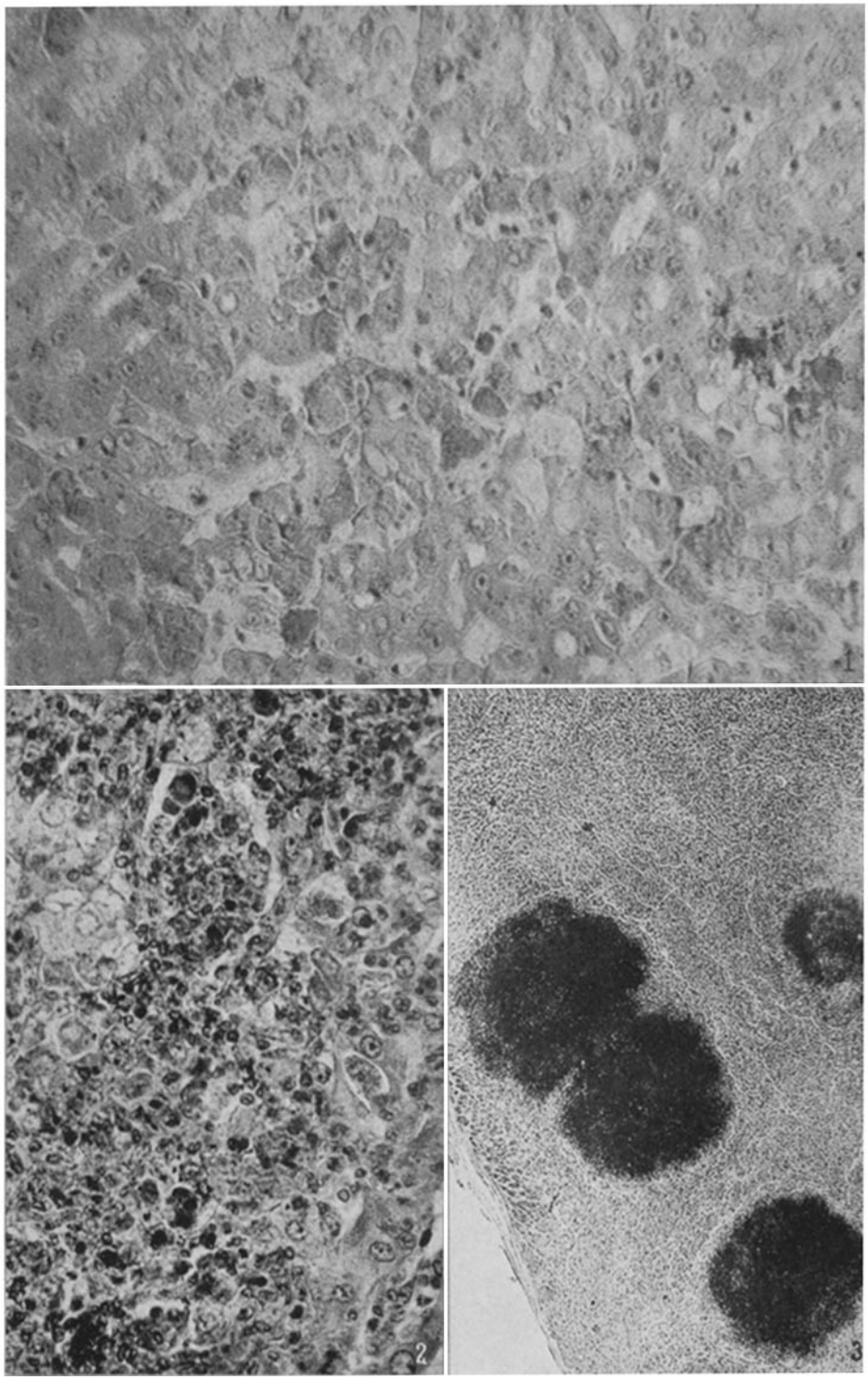
FIG. 6. Ovary. Zenker; Giemsa. Probable Guarnieri bodies. × 440.

PLATE 18

FIG. 7. Spleen. Zenker; phloxine-methylene blue. Diffuse fibrin deposit about vessels; marked diminution in lymphocytes. × 110.

FIG. 8. Lymph node. Zenker; eosin-methylene blue. Diffuse necrosing lesion. Node taken from drainage area of an enhanced skin lesion. × 53.

FIG. 9. Lung. Zenker; phloxine-methylene blue. Vascular and perivascular lesion interpreted as specific. × 200.



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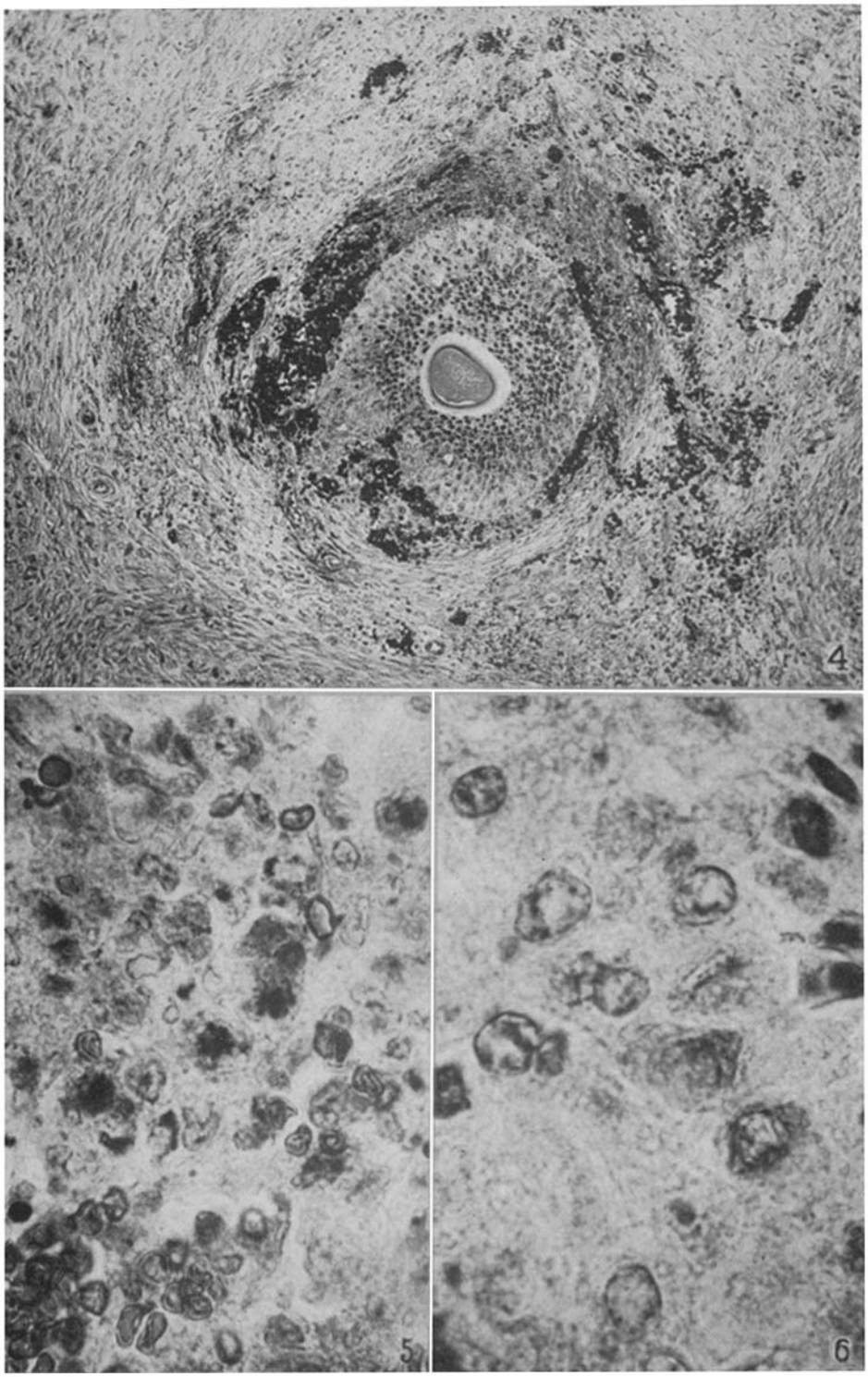

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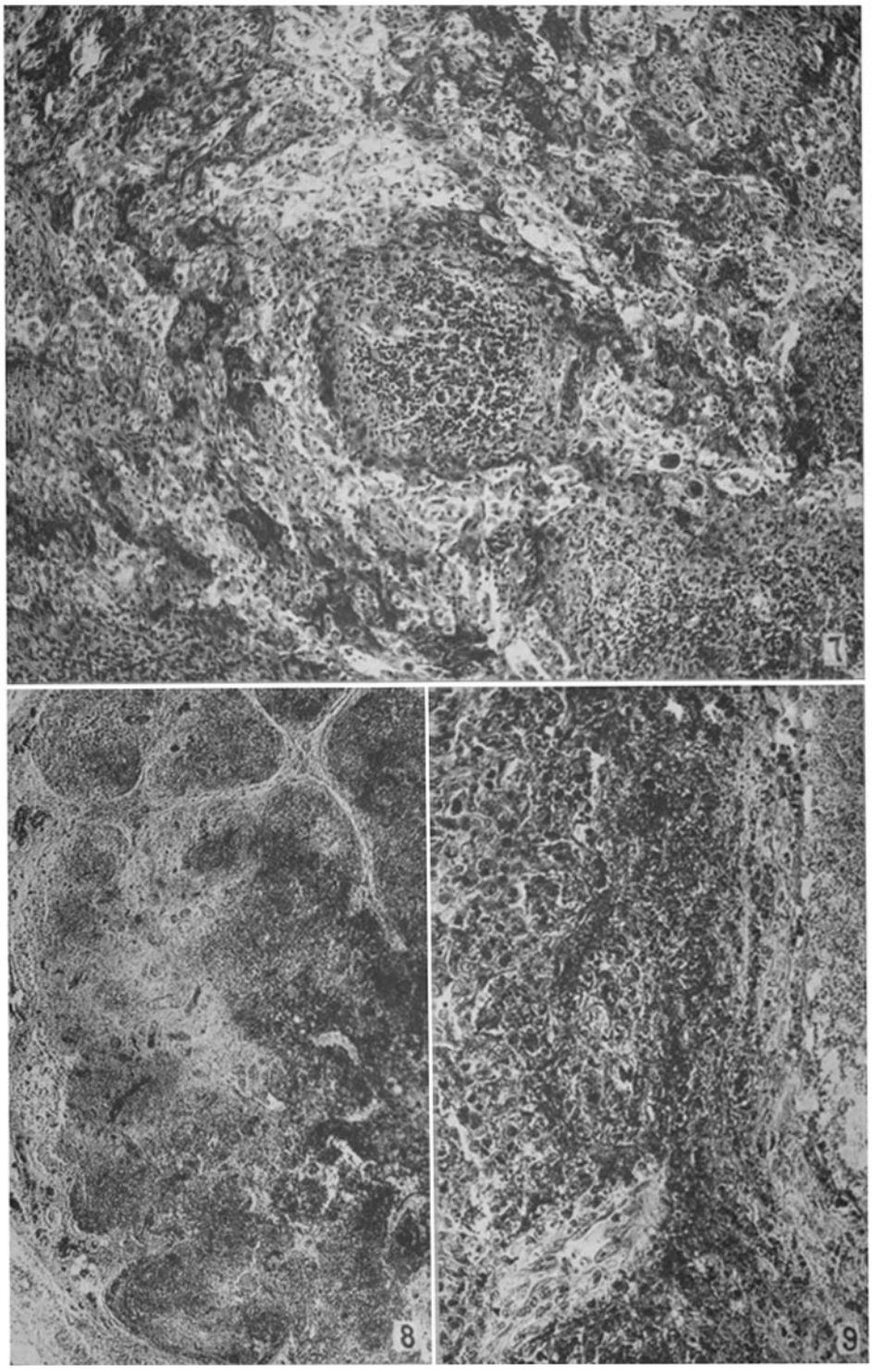

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