A LIPOLYTIC ENZYME IN REACTIVE HISTIOCYTES OF GUINEA PIGS WITH EXPERIMENTAL ENCEPHALOMYELITIS*

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PLATES 18 AND 19

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Encephalomyelitis, often accompanied by demyelinization, can be readily induced in several species of laboratory animals by the subcutaneous or intramuscular injection of an emulsion of brain tissue and adjuvants, as much work has shown (1–8). The similarity of the changes to those seen in the more acute phases of multiple sclerosis has been repeatedly noted, though their precise pathogenesis remains unknown. Recently it has been found that demyelinization similar to that occurring naturally in multiple sclerosis can be produced in living rabbits by means of pancreatic lipase injected intracerebrally or intravascularly (9). Hence it seemed not inconceivable that a lipolytic enzyme might also be responsible for the demyelinization that characterizes experimental encephalomyelitis. As a first step in probing the possibility a search has been made for lipolytic enzymes in various tissues of guinea pigs that had been given brain and adjuvants to induce the experimental disease. The findings will now be reported.

Materials and Methods

The experimental encephalomyelitis was induced in guinea pigs by means of intramuscular injections of brain emulsified with adjuvants, according to the method previously employed by others (1–8) and described in some detail below. The sites of injection, the lymph nodes draining them, and the spleens of the diseased animals were then studied histologically, with particular reference to the character of the inflammatory response and to the presence of lipase in reactive histiocytes, as demonstrated by the cytochemical method of Gomori (10–12); control observations were made of tissues with inflammation produced by a variety of means in guinea pigs devoid of the experimental disease. Additional tests—to be described in a later section—were made of the ability of suspensions of the various tissues to hydrolyze olive oil *in vitro*.

To produce the disease, 24 adult market-bought guinea pigs were injected intramuscularly in the right thigh with 2 cc. of an emulsion of human brain tissue and adjuvants in the following proportions: 2 parts of a 5 per cent suspension of human brain tissue in physiological saline, 2 parts of Bayol[1] containing 2.5 mg. of heat-killed *Mycobacterium butyricum* per cc.

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[1] Bayol is a light paraffin oil manufactured by Stanco, Inc., (Standard Oil Co.) of New York City.

(autoclaved at 18 pounds pressure for 20 minutes), and 1 part of Falba.[2] Seventeen of the 24 guinea pigs developed paresis or paralysis of the hind legs during the 3rd and 4th weeks following injection. The brains and spinal cords of these animals all showed focal areas of perivascular lymphocytic infiltration and demyelinization, and the same proved true in 4 additional animals that had not manifested neurological signs.

For control purposes 5 groups of animals were formed and treated as follows: 6 adult guinea pigs were injected intramuscularly in the right thigh with 2 cc. of the test emulsion lacking *M. butyricum;* 6 adult guinea pigs were given the emulsion lacking brain tissue; each of 3 guinea pigs was injected intramuscularly with a suspension of 0.2 gm. of sterile talcum in 2 cc. of physiological saline; 3 guinea pigs were injected intramuscularly with a suspension of 0.2 gm. of aluminum hydroxide in 2 cc. of physiological saline; and 12 adult guinea pigs served as normal controls. Neither weakness nor paralysis developed in the animals of the control groups, and on microscopic examination the brains and spinal cords of these animals showed neither inflammation nor demyelinization.

When animals of the experimental group manifested the characteristic weakness or paralysis they and a proportionate number of control animals were killed by exsanguination under light ether anesthesia. With aseptic technique tissues were taken from the region of the injection, from the inguinal and periaortic lymph nodes, and from the spleen, liver, pancreas, brain, and spinal cord. Representative blocks of each tissue were fixed in Zenker's solution and in 10 per cent formalin, and were stained either by hematoxylin-eosin or eosin–methylene blue; sections of spinal cord and brain were also stained for myelin by the Loyez method.

Additional blocks of each tissue were examined for lipase by the histochemical method of Gomori (10–12). In this procedure thin slices of tissue were fixed for 24 hours in chilled acetone, dehydrated at room temperature in two changes of absolute acetone for 24 hours each, and imbedded in paraffin. Sections were cut at 8μ mounted, and run through xylene and graded alcohols to distilled water. The mounted sections were incubated at 37°C. for 18 hours in a freshly prepared mixture composed of 25 parts of stock solution I (glycerin, 150 cc.; 10 per cent calcium chloride, 50 cc.; maleic acid–sodium hydroxide buffer at pH 7.4, 50 cc.; and distilled water to make 1000 cc.) and 1 part of a 5 per cent aqueous stock solution of a stearic ester of polyglycols, "product 81" (Onyx Oil and Chemical Co.). Following incubation the tissues were rinsed in three changes of distilled water and transferred to a 2 per cent solution of lead nitrate for 15 minutes; then thoroughly rinsed in a stream of distilled water and placed for 5 minutes in a dilute aqueous solution of ammonium sulfide. The deposition of lead sulfide constituted a positive histochemical test for lipolytic enzymes. The sections were examined microscopically both before and after counterstaining with hemotoxylin and eosin.

Granulomatous Response to the Injected Material

The inflammatory tissue at the injection sites in animals that had received brain tissue and adjuvants was characterized histologically by numerous tubercle-like granulomatous masses formed of compactly arranged epithelioid histiocytes that were infrequently multinucleated. Within and about these tubercles there were a moderate number of granulocytes and a few lymphocytes. An identical and extensive granulomatous reaction with tubercle formation was regularly present in the inguinal and periaortic lymph nodes. (Fig. 1.) When the injected material was composed of the adjuvants alone (without added

[2] Falba is an absorption base composed of a mixture of cholesterols and oxycholesterols derived from lanolin. It was procured from Pfaltz and Bauer, Inc., New York City.

brain tissue) the histologic character of the inflammation was altered in only minor respects: tubercle formation, although present, was less conspicuous particularly in the lymph nodes, and multinucleated giant cells were somewhat more numerous. By contrast, the elimination of *M. butyricum* from the inoculum resulted in a pronounced alteration in the character of the inflammation. This was markedly lessened in degree and tubercle formation was conspicuously absent. The reaction was formed of leukocytes and solitary histiocytes with vacuolated cytoplasm that were often grouped about lacunae where lipoid globules had been dissolved. The regional lymph nodes were hyperplastic and contained an occasional lipoid globule, but were devoid of granulomatous tissue (Fig. 2).

Histologic Demonstration of Lipolytic Enzyme in the Reactive Histiocytes of Guinea Pigs with Experimental Encephalomyelitis

In all 24 of the guinea pigs that had received brain tissue and adjuvants, lipolytic enzyme was regularly demonstrated by means of the histochemical method already described. This was present in stainable amounts in approximately one-tenth of the large cells that comprised the granulomatous tissue at the injection sites, and in up to one-half of similar cells in the regional lymph nodes. Counterstains disclosed that these cells were histiocytes, as was manifest from their large size, eccentrically placed ovoid nuclei, and abundant eosinophilic cytoplasm. In lymph nodes the enzyme-containing cells were present in greatest numbers within and about the tubercles, but they could be found throughout the parenchyma and in the peripheral sinusoids as well (Figs. 3 and 4). In addition, lipolytic enzyme was found in cells that were regularly distributed throughout the germinal follicles of the spleen; on counterstaining these were seen to have small eccentric nuclei and an abundant pale staining cytoplasm (Fig. 5). The histiocytes of talcum powder and aluminum hydroxide granulomas, as well as the related cells in the lymph nodes and spleens of normal guinea pigs, failed without exception to give the histochemical test for lipolytic enzyme.

As already mentioned, the experimental disease did not develop in the animals receiving brain tissue mixed with Bayol and Falba but without *M. butyricum*, and the same proved true when the injected emulsion contained adjuvants without brain. Under these circumstances the paucity of lipolytic enzyme in the inflammatory tissue at the injection site was noteworthy; in general the enzyme was present in stainable amounts in less than one histiocyte per high power microscopic field, while in the animals that had received the full emulsion and hence had much more pronounced local inflammatory reactions as well as the experimental disease, the granulomatous tissue often displayed 10 or more enzyme-containing cells per high power field. The contrast was even greater in the lymph nodes. For in the control animals the enzyme-

containing cells were even less numerous in the lymph nodes than in the inflammatory tissue, though the reverse was true in the diseased animals. Enzyme-containing cells were not to be found in the spleens of the non-diseased controls.

TABLE I

Lipolytic Activity of Lymph Nodes and Reactive Inflammatory Tissue of Guinea Pigs with Experimental Encephalomyelitis

Experimental groups	Lipolytic activity of lymph nodes*		Lipolytic activity of granulomatous tissue from injection site*	
	Range	Mean	Range	Mean
(*a*) 12 normal guinea pigs	0.0–0.2	0.1	‡	‡
(*b*) 15 guinea pigs with encephalomyelitis resulting from injection of adjuvants and brain	1.8–4.3	3.3	0.5–1.9	1.1
(*c*) 6 guinea pigs injected with adjuvants alone—none had encephalomyelitis when killed	0.2–0.5	0.3	0.5–1.6	0.8
(*d*) 6 guinea pigs injected with brain and adjuvants without *M. butyricum*—none had encephalomyelitis when killed	0.0–0.5	0.2	0.0–1.5	0.9
(*e*) 3 guinea pigs with talcum granulomas	0.0–0.4	0.2	0.1–0.5	0.3
(*f*) 3 guinea pigs with aluminum hydroxide granulomas	0.1–0.2	0.1	0.0–0.8	0.4

* Expressed as cc. of 0.01 N NaOH required to neutralize the acid formed by the interaction of 1 gm. of ground tissue and olive oil during 3 hours at 37°C.

‡ Lipolytic activity of subcutaneous tissue and fat from the normal guinea pigs gave values ranging from 0.1 to 0.4 with a mean of 0.3.

Chemical Tests for Lipolytic Activity in Tissues of Guinea Pigs with Induced Encephalomyelitis

It seemed important to learn if possible whether the lipolytic enzyme could be demonstrated by direct chemical means in the granulomatous tissues of guinea pigs with the experimental disease.

Weighed portions (approximately 0.5 gm.) of the inflammatory tissue procured with aseptic precautions from the sites of injection and from the regional lymph nodes of 15 guinea pigs with the experimental disease (as manifested by paralysis and by encephalitis proved microscopically) were ground individually with sterile sea sand and enough 0.9 per cent saline to make a 20 to 25 per cent tissue suspension. In a similar manner, tissue suspensions were prepared from 12 normal guinea pigs and from 18 animals of the 5 control groups. In each case, approximately 2 cc. of fresh tissue suspension was thoroughly mixed with 3 cc. of olive oil and incubated in a small Erlenmeyer flask at 37°C. for 3 hours. Blank controls formed of 2 cc. of saline and 3 cc. of olive oil were incubated simultaneously. The amount of 0.01 N NaOH required to alkalinize each solution to the end-point of phenolphthalein was determined, and the lipolytic activity was then calculated as the amount of 0.01 N NaOH in cubic centimeters (minus the value for the blank control) that was required to neutralize the acid

formed by the interaction of 1 gm. of ground granulomatous tissue or lymph node with olive oil.

In a further control experiment, mixtures were made containing 2 cc. of tissue suspension and 3 cc. of olive oil; when the mixtures were titrated without incubation, the results were comparable to those obtained with the blank controls.

The chemical assays confirmed the histologic observation that in guinea pigs with encephalomyelitis induced by the injection of brain and adjuvants lipolytic enzyme was regularly present in the granulomatous tissue at the injection sites and in even greater concentrations in the regional lymph nodes. It is noteworthy that the nodal tissue of guinea pigs with encephalomyelitis manifested lipolytic activity that was on the average eleven times greater than that exhibited by the lymph nodes of control animals. Although greater enzymatic activity was regularly manifested by the granulomatous tissue of animals that had encephalomyelitiis, the quantitative differences between the effects exhibited by the granulomatous tissue of diseased and control animals were not so pronounced. In this relation the point deserves emphasis that chemical analyses for lipolytic enzyme performed on such tissues from intramuscular and subcutaneous regions are made less accurate by the presence normally of a small amount of lipase in connective tissue fat, as demonstrated histochemically (10) and confirmed by chemical assay (footnote (‡) to Table I).

COMMENT

The relationship between inflammation and the formation or destruction of lipolytic enzymes has heretofore received but little attention in the literature. The normal pancreatic and hepatic parenchymal cells in guinea pigs and other mammals are known to contain large quantities of lipolytic enzyme, and this is also present in lesser quantities in adipose tissue and in the epithelial cells of the gastric, enteric, and bronchial mucosa (10). Inflammation in these organs when unaccompanied by necrosis does not alter their lipase content, while necrotic tissue, regardless of its origin, contains little or no lipase. It has been demonstrated by cytochemical methods that lipolytic enzyme is present in small quantities in the epithelioid cells of tubercles in cases of active tuberculosis in human beings and laboratory animals (11). An enzyme capable of hydrolyzing fat is formed in culture media in which tubercle bacilli are growing, and the same is true of *M. butyricum* and various strains of staphylococci (13). The accumulation of lipolytic enzyme in the cells of lymph nodes and spleen as a response to a focal inflammatory process has not been previously described.

Several observations indicate that the factors conditioning the inflammatory process for the induction of encephalomyelitis are the same as those affecting the accumulation of lipolytic enzyme in the reactive histiocytes. The development of encephalomyelitis in its acute form depends upon the presence in the inoculum of brain tissue and killed acid-fast microorganisms, and recent studies

have shown that the disease results only when brain tissue and the bacilli are deposited together in the same injection site (14). Under the conditions that induce encephalomyelitis the inflammatory tissue at the injection site is regularly abundant and comprised of tubercle-like granulomas, while the regional lymph nodes are greatly enlarged owing to the development within them of similar tubercles. By cytochemical methods and by chemical assays, lipolytic enzyme has been demonstrated regularly in the inflammatory tissue of the diseased animals; it was most abundant in the lymph nodes and was regularly present in the spleen also. The omission of brain tissue from the inoculum altered the histologic appearance of the inflammation in only minor respects, though the omission of killed *M. butyricum* resulted in a marked decrease in the granulomatous tissue and a conspicuous absence of tubercles. Under these conditions the experimental disease did not develop and the inflammatory tissues contained but little lipolytic enzyme, as determined both by the cytochemical method and by direct chemical assays; furthermore the lipolytic enzyme was practically absent from the lymph nodes of the non-diseased controls, and it was not to be found in their spleens.

In light of the demonstration that demyelinization can be induced in rabbits by the intracerebral and intravascular injection of purified lipase (9), the findings as a whole would seem to suggest that the lipolytic enzyme present in the reactive histiocytes may be a factor in the pathogenesis of the experimental encephalomyelitis.

SUMMARY

A lipolytic enzyme has been demonstrated by means of a cytochemical technique and by direct chemical assay in granulomatous tissues of guinea pigs with encephalomyelitis and demyelinization resulting from the injection of an emulsion comprised of brain tissue and adjuvants, including *Mycobacterium butyricum*. Combined histologic and cytochemical studies showed that the lipolytic enzyme was present in the cytoplasm of a large proportion of the reactive histiocytes in the granulomatous tissue around the site of injection in the diseased animals, and that the enzyme-containing histiocytes were even more numerous in the inflamed regional lymph nodes. In control experiments, when emulsions lacking either brain tissue or *M. butyricum* were injected in previously normal guinea pigs, the experimental condition did not develop; under these circumstances the lipolytic enzyme was found in only a small proportion of the cells of the granulomatous tissue around the injection sites, and it was almost negligible in the regional lymph nodes of these animals. It was absent from the cells of the lymph nodes of normal animals, and from the cells of talcum and aluminum hydroxide granulomas produced experimentally in guinea pigs.

The lipolytic enzyme may be a factor in the pathogenesis of the experimental encephalomyelitis and demyelinization.

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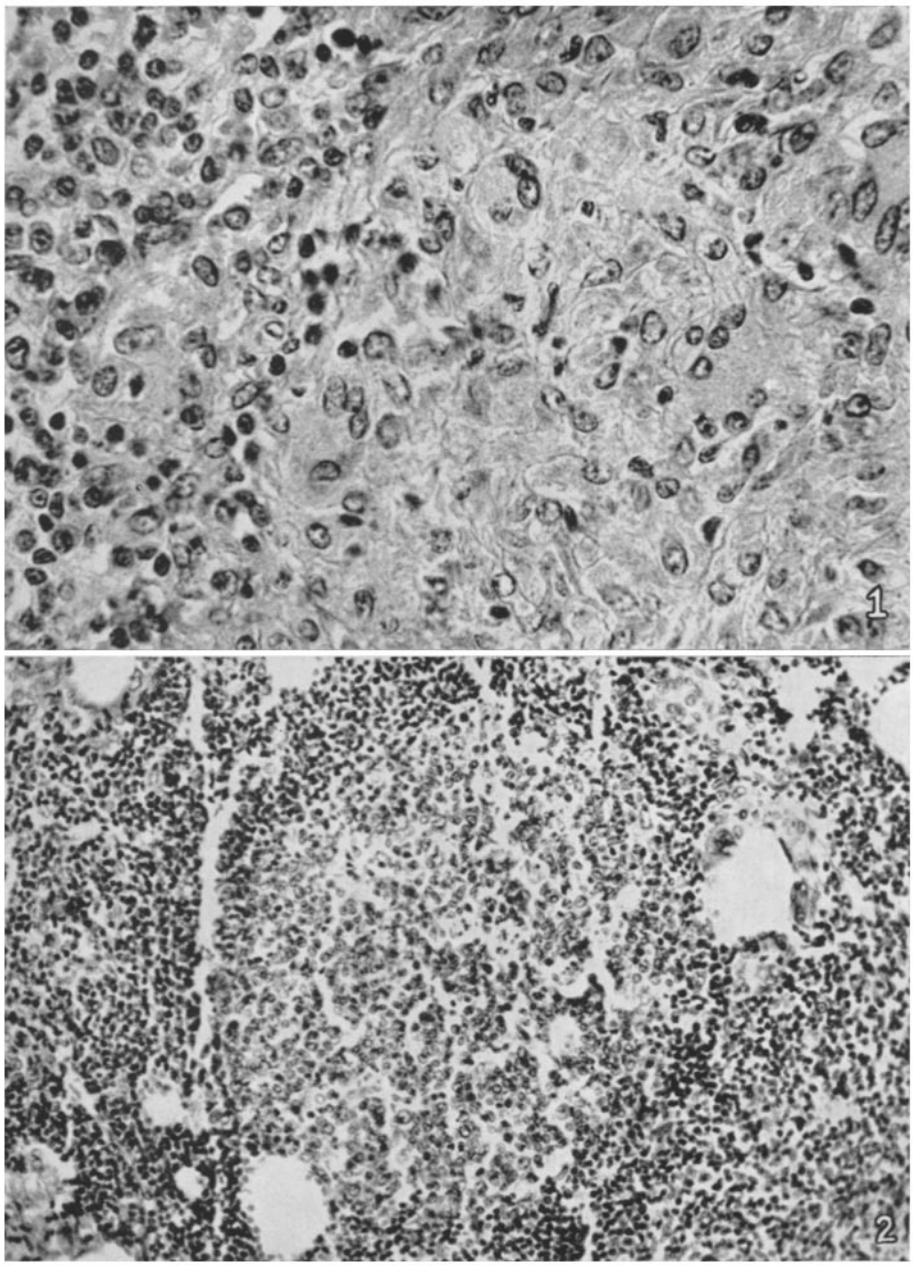
EXPLANATION OF PLATES

PLATE 18

FIG. 1. An inguinal lymph node from a guinea pig with experimental encephalomyelitis induced by the intramuscular injection of an emulsion of brain tissue and adjuvants in the thigh. The granulomatous reaction is characteristic and identical with that present at the injection site. It is formed of numerous tubercle-like masses of large epithelioid cells. Hematoxylin and eosin stain. × 500.

FIG. 2. An inguinal lymph node from a guinea pig injected intramuscularly in the thigh with the brain-adjuvant emulsion lacking *M. butyricum*. The lymphoid hyperplasia is conspicuous and at least two lacunae can be identified from which lipoid globules have been dissolved; but neither tubercles nor other granulomatous tissue can be seen. Hematoxylin and eosin stain. × 280.



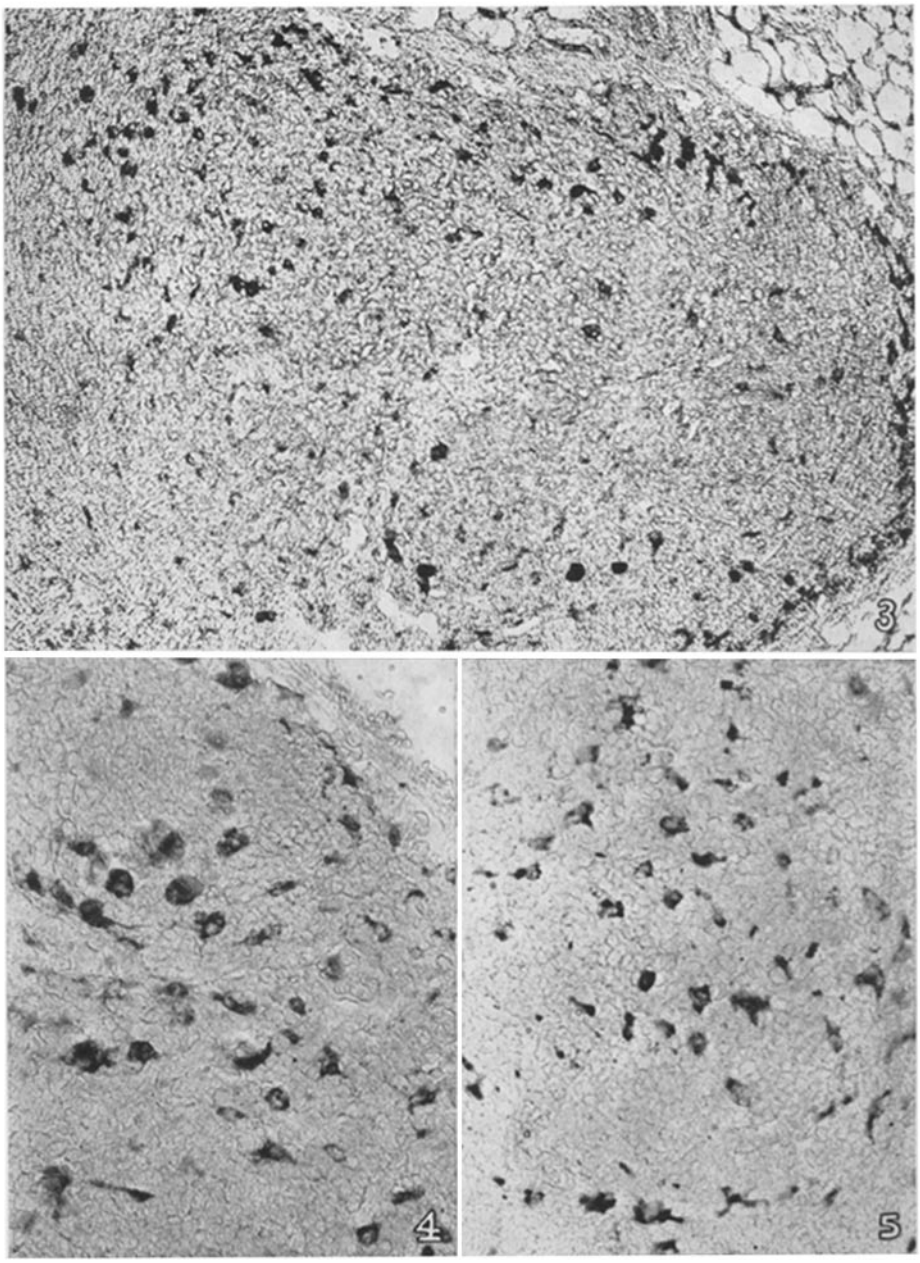
(Vogel: Lipase in experimental encephalomyelitis)

PLATE 19

FIG. 3. A lymph node procured from a guinea pig with experimental encephalomyelitis, stained for lipase by the histochemical method of Gomori. The enzyme-containing cells, black in the picture, were actually dark brown owing to the deposition in them of lead sulfide at the sites of lipolytic activity. Counterstains of this section with hematoxylin and eosin after photography showed that the enzyme-containing cells in the left upper corner are histiocytes comprising part of a granulomatous lesion. Other histiocytes containing the enzyme are present throughout the node and in the peripheral sinuses. × 120.

FIG. 4. A higher magnification of cells in the lymph node of Fig. 3. × 200.

FIG. 5. The spleen of a guinea pig with experimental encephalomyelitis, stained for lipase. Reticulo-endothelial cells in a germinal follicle show a positive cytochemical reaction for lipolytic enzyme. × 200.



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