THE HISTOPATHOLOGY AND HISTOCHEMISTRY OF ERYTHEMA NODOSUM LEPROSUM[1,2]

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In reviewing the literature, we have found only two articles on the histochemistry of leprosy ([6,8]). Neither has included observations on erythema nodosum leprosum (ENL). Reports touching on the histopathologic changes of this reactional manifestation in leprosy have not mentioned a comparison between its acute and involuting phases. In continuing our interest in ENL, this study was undertaken to provide additional information on the nature of erythema nodosum leprosum by histochemical and histopathologic methods, and to compare the changes taking place in early lesions with those in subsiding ones.

In an attempt to explain some features of ENL in its early and late stages, attention was focused on the following points: (a) localization of the lesion; (b) cellular composition of the inflammatory infiltrate; (c) alterations in the ground substance; (d) specific changes in the connective tissue stroma (collagen, elastic tissue, and reticulum), and (e) changes occurring in the blood vessels.

MATERIALS AND METHODS

This study utilized biopsy specimens obtained from 27 patients at the Eversley Childs Sanitarium, Cebu, Philippines, by one of us (J.G.T.). Each patient had two biopsies. The first represented the early lesion of erythema nodosum leprosum, and the second the older or involuting stage. To obtain these, two representative lesions appearing at the same time in a patient with acute lepra reaction were selected and marked for excision. The early lesions ranged from 12 to 96 hours in duration and the older lesions from 9 to 12 days.

A total of 53 skin specimens fixed in neutral formalin were received by the Armed Forces Institute of Pathology. Material from eight patients was initially used in a study comparing the histopathologic features of ENL with those of classical erythema nodosum. The findings were reported and discussed at a symposium on research in leprosy sponsored jointly by the Leonard Wood Memorial and the Johns Hopkins University in Baltimore, Maryland ([7]).

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TABLE 1.—*Clinical and bacteriologic status, duration of leprosy and history of ENL attacks, according to sex.*

	Number of patients		
	Total	Male	Female
Clinical status			
Slight (L1)	—	—	—
Moderate (L2)	14	7	7
Advanced (L3)	13	11	2
TOTAL	27	18	9
Bacteriologic status[a]			
5.00 (40)	6	4	2
4.50-4.99 (36-39)	10	7	3
4.00-4.49 (32-35)	4	2	2
3.50-3.99 (28-31)	5	3	2
3.00-3.49 (24-27)	2	2	—
TOTAL	27	18	9
Mean bacteriologic score: 4.41 Standard error of mean: 0.11			
Duration of leprosy prior to admission			
1-3 years	10	6	4
4-6 years	7	5	2
7-9 years	6	4	2
10 years and over	4	3	1
TOTAL	27	18	9
Mean duration of leprosy: 5.52 Standard error of mean: 0.59			
History of ENL attacks			
First attack	3	3	—
Multiple attacks (2-4 times)	3	1	2
Recurrent attacks (unrecalled number of times)	21	14	7
TOTAL	27	18	9

[a] Bacteriologic scores for 8 sites, 2 nasal and 6 skin, were determined by giving each smear an arbitrary numerical rating, as follows: very scanty (V.S.) were given a grade of 1, those marked 1+ a grade of 2, 2+ a grade of 3, 3+ a grade of 4, and 4+ a grade of 5, thus ranging from 1 (v.s., 1 site) to a possible 40 (4+, 8 sites). Total scores were added and divided by 8 to denote approximate degree of bacteriologic advancement of each patient.

Complete data were available for each patient, including results of laboratory examination and of the lepromin test and history of previous sulfone treatment. Among the selected patients with ENL, 18 were male and 9 female. One male patient refused a seecond biopsy, so that the total of older lesions was 26. All were well-documented lepromatous cases, classified clinically as moderate (L2) or advanced (L3). They were bacteriologically positive and lepromin (Mitsuda)-negative. Two were new admissions and had never received sulfone treatment. Of the 27 lepromatous patients, 3 were experiencing their first attack of ENL, and the rest were having multiple or recurrent attacks. The duration of the patients' underlying disease ranged from 2 to 13 years. Tables 1 and 2 summarize the clinical status, bacteriologic status, duration of leprosy, history of ENL episodes, previous sulfone therapy, and age of early and older lesions, according to sex.

TABLE 2.—*Previous sulfone therapy and age of early and older ENL lesions at the time of biopsy, according to sex.*

	Number of patients		
	Total	Male	Female
Previous sulfone therapy (total DDS taken prior to biopsy)			
None (new admission)	2	2	—
Less than 10 gm.	6	5	1
10-19 gm.	3	1	2
20-29 gm.	9	3	6
30-39 gm.	5	5	—
40-49 gm.	2	2	—
50 gm. and over	—	—	—
TOTAL	27	18	9
Mean total DDS therapy: 21.34 Standard error of mean: 2.48			
Age of early ENL lesion at time of biopsy			
12 hours	1	1	—
24 hours	4	4	—
48 hours	6	3	3
72 hours	8	4	4
96 hours	8	6	2
TOTAL	27	18	9
Age of older ENL lesion at time of biopsy			
9 days	4	2	2
10 days	8	4	4
11 days	12	10	2
12 days	2	1	1
TOTAL	26[a]	17	9

[a] One male patient refused a second biopsy.

TABLE 3.—*Degree and character of the inflammatory reaction in early ENL lesions.*

Early ENL (hours)	No. of pts.	Degree and character of inflammation (per cent)			
		Chronic	Subacute	Slight (+) acute	Moderate to marked (++ and +++) acute
12	1	—	—	—	100.0
24	4	—	—	25.0	75.0
48	6	—	16.6	—	83.3
72	8	—	12.5	37.5	50.0
96	8	—	50.0	50.0	—
12 to 48	11	—	9.1	9.1	81.8
72 to 96	16	—	31.3	43.8	25.0
Total	27	—	22.2	29.6	48.1

Sections stained with hematoxylin and eosin and by the Fite-Faraco method were examined in each case. In histochemical examination, sections from formalin-fixed, paraffin-embedded tissues were made and stained according to the following procedures

referred to in the second edition of the Armed Forces Institute of Pathology Manual of Histologic and Special Staining Technics ([12]):

Colloidal iron stain, with and without hyaluronidase digestion.—The Rinehart and Abul-Haj modification of the Hale iron stain was used with a van Gieson counterstain.

Alcian blue stain.—The short method of Dr. F. B. Johnson was employed, using a 1 per cent solution of alcian blue in 3 per cent acetic acid (pH adjusted to 2.5 and 1.7) and a Kernechtrot (nuclear-fast red) solution as counterstain.

Periodic acid-Schiff reaction, with and without diastase digestion.—The AFIP modification of the McManus PAS reaction was followed with a hematoxylin counterstain.

Aldehyde-fuchsin stain.—The technic described by Gomori was used with a Metanil yellow counterstain.

Elastic tissue stain.—Hart's modification of the Mallory procedure for elastic stain was used.

Reticulum stain.—Spillan's modification of the Snook reticulum stain was followed.

RESULTS

1. *Histopathologic characteristics of ENL.*—(A) Hematoxylin and eosin stain. No significant epidermal changes were noted during either the early stage or the involuting phase of ENL. In most specimens, the upper dermis and mid-dermis showed the usual lepromatous histiocytic infiltrate distributed around blood vessels and adnexal structures. The superficial dermis was not involved by the acute inflammatory process present in the leprotic tissue located in the lower corium and adjacent subcutaneous fat. On the basis of Ridley's ([22]) classification, which divides ENL into "superficial," "deep," and "necrotic" lesions, 9 of the 27 early lesions were considered "superficial," i.e., involving only the lower dermis, and 18 were in the "deep" category, involving both the lower dermis and subcutaneous tissue. None of the lesions exhibited the "necrotic" type, which is characterized by a necrotizing angiitis and a tendency to ulcerate.

The criteria of grading used by Pepler *et al.* ([20]) in grouping the inflammatory reaction into three categories according to the cellular consistency were followed. Thus, an acute inflammation consisted predominantly of polymorphonuclear leucocytes; a subacute inflammation, of approximately equal numbers of neutrophils, lymphocytes, and plasma cells; and a chronic inflammation, mainly of lymphocytes, plasma cells, and histiocytes. The acute inflammatory process was further subdivided into slight (+ acute) and moderate to marked (++ and +++ acute), depending on the number of polymorphonuclear leucocytes. For older lesions, the chronicity of the inflammation was grouped into slight (+ chronic)and moderate to marked (++ and +++ chronic), according to the severity of the inflammation. Tables 3 and 4 show the degree and character of inflammation in early and older ENL lesions, respectively.

As seen in Table 3, the earlier the lesion at the time of biopsy (12 to 48 hours), the more acute and intense is the inflammatory reaction. At 72 to 96 hours, the inflammation has partially subsided and is less acute or subacute in character. In Table 4, most of the older lesions at 9 to 12 days show a chronic type of inflammation.

TABLE 4.—*Degree and character of the inflammatory reaction in older ENL lesions.*

Older ENL (days)	No. of pts.	Degree and character of inflammation (per cent)			
		++ and +++ chronic	+ chronic	subacute	acute
9	4	—	100.0	—	—
10	8	25.0	62.5	12.5	—
11	12	8.3	75.0	8.3	8.3
12	2	50.0	50.0	—	—
Total	26[a]	15.4	73.1	7.7	3.8

[a] One male patient refused a second biopsy.

Of 13 early lesions with ++ and +++ acute inflammation, 8 had micro-abscesses either in the lower dermis or in the hypodermis or both. In the "deep" lesions, the more superficial subcutaneous fat was generally involved in an acute panniculitis; in only one specimen was fat necrosis observed.

About the fourth day, the leucocytes characteristic of the acute phase had decreased in number, and more chronic types of cells such as lymphocytes, plasma cells, and histiocytes appeared. In regressing lesions 9 to 12 days old, the inflammation resembled that of healing granulation tissue with fibrosis and thickening of the interlobular fat septa of the adjacent subcutaneous tissue.

In determining the cellular composition of the inflammatory infiltrate in early and in older lesions of erythema nodosum leprosum, five oil-immersion fields of each lesion were selected by taking the first field in the central part of the lesion and the other four fields at different points around or near the center. All areas examined were limited to the deep dermis and subcutaneous fat that showed foci of active inflammation. The cells in each field were counted. Six cell groups were identified and listed. The cells included were neutrophils, eosinophils, plasma cells, lymphocytes, histiocytes, and mast cells. Fibroblasts and endothelial cells were excluded. The total numbers of cells counted in five fields were added, and the corresponding percentage values of each cell type from the total number of cells comprising five fields were computed. Table 5 gives the dispersion values, mean and standard error (S.E.) of mean of each cell type in early and older lesions of erythema nodosum leprosum.

Neutrophilic polymorphonuclear leucocytes and sometimes eosinophils dominated the picture in most of the early lesions. The presence of histiocytes at the outset could be explained by a preexisting lepromatous reaction. As the acute inflammation subsided, there was a significant decrease in the number of neutrophils and eosinophils. Mast cells, which were fairly numerous in the early lesions, decreased in the involuting stage but did not disappear completely. A corresponding rise in the number of lymphocytes and plasma cells was observed in the older lesions, indicating transition from the acute phase into the re-

TABLE 5.—*Dispersion values, mean, and standard error (S.E.) of mean of the cellular composition of the inflammatory infiltrate, according to cell type, in early and older lesions of erythema nodosum leprosum.*

Cellular composition of inflammatory infiltrate (per cent of lesion)	Dispersion			Central tendency	
	Values				
	Highest	Lowest	Range	Mean	Standard error of mean
Early ENL lesion (27 *patients*)					
Neutrophils	63.6	4.3	59.3	30.4	3.6
Eosinophils	6.6	0.8	5.8	2.3	0.2
Plasma cells	4.7	0.2	4.5	1.1	0.2
Lymphocytes	45.5	4.1	41.4	18.3	2.2
Histiocytes	65.9	17.8	48.1	45.9	2.6
Mast cells	5.4	0.6	4.8	2.0	0.2
Older ENL lesion (26 *patients*)[a]					
Neutrophils	13.1	0.0	13.1	1.6	0.6
Eosinophils	9.1	0.0	9.1	0.8	0.4
Plasma cells	13.5	1.1	12.4	5.0	0.6
Lymphocytes	57.4	18.6	38.8	40.1	2.2
Histiocytes	72.1	27.1	45.0	50.9	2.3
Mast cells	2.9	0.4	2.5	1.6	0.1

[a] One male patient refused a second biopsy.

gressing or chronic stage of erythema nodosum leprosum. A slight rise in the number of histiocytes was also noted.

Figure 1 shows a comparison of the cellular consistency of the inflammatory infiltrate in early and older lesions of erythema nodosum leprosum. Tests for significance[6] were made of the mean values for each cell type, comparing early and older ENL lesions, using the formula:

$$t = \frac{\overline{M_e} - \overline{M_o}}{\sqrt{\frac{\sigma_e^2}{N_e} + \frac{\sigma_o^2}{N_o}}}$$

where t = probability criterion
$\overline{M_e}$ = Mean, early lesion
$\overline{M_o}$ = Mean, older lesion
N_e = Number, early lesion
N_o = Number, older lesion
σ_e^2 = Variance in early ENL
σ_o^2 = Variance in older ENL

Endothelial swelling and edema of the blood vessels situated in the lower corium and adjacent hypodermis were generally the most consistent findings in the early stage of erythema nodosum leprosum. The veins, rather than the smaller arteries and arterioles, were more frequently involved. Slightly more than half of the 27 early lesions showed an angiitis aside from endothelial swelling and edema. Of 15 lesions exhibiting an angiitis, 3 conformed to an "allergic" type in which the vascular walls were infiltrated not only by neutrophils but

[6] Tests for significance and computation of mean values from statistical data obtained in the study were furnished by Mr. B. L. Parnell, Chief, Medical Statistics Section, Armed Forces Institutes of Pathology, Washington, D. C.

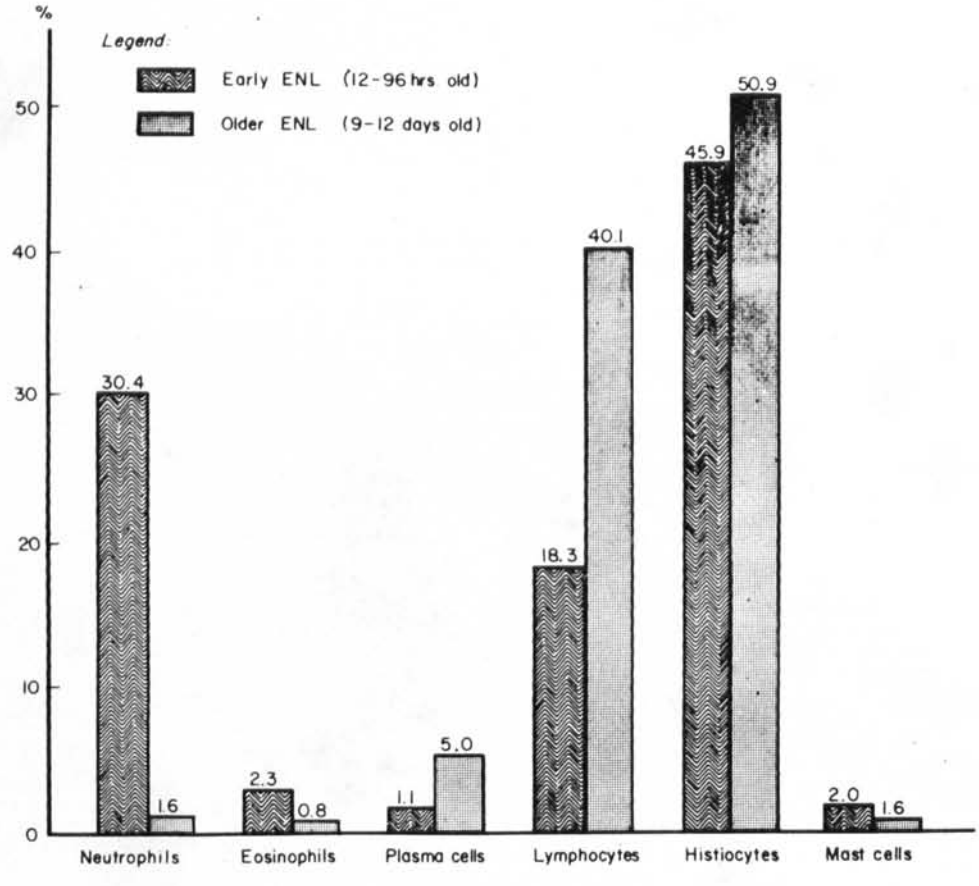

FIG. 1. A comparison of the mean percentages of cell types present in the inflammatory infiltrate in early and in older lesions of ENL.

also by numerous eosinophils. Endothelial swelling and edema were diminished in the older lesions, and in 10 the vessels did not show any remarkable change. In five subsiding lesions, however, a proliferative angiitis or obliterative endophlebitis or endarteritis was present. In these lesions, the affected venules or arterioles exhibited perivascular fibrosis, thickening of the wall, marked narrowing of the lumen, and infiltration of the perivascular zone by a variety of chronic inflammatory cells but with few or no granulocytes.

Table 6 demonstrates the vascular changes noted in early ENL lesions. Table 7 shows vessel alterations in older ENL lesions.

The small cutaneous nerves within the ENL lesion showed changes customarily seen in lepromatous leprosy, but did not appreciably share the inflammatory reaction seen in other tissues. Inflammatory cells were absent within neural tissue, although the perineural areas were infiltrated with many lepra cells containing acid-fast bacilli and few or

TABLE 6.—*Vascular changes observed in early ENL lesions.*

Early ENL (hours)	No. of pts.	Vascular changes (per cent)			
		Edema only	+ (slight or mild) edema and endothelial swelling	++ (moderate-marked) edema and endothelial swelling	Vasculitis plus edema and endothelial swelling
12	1	—	—	—	100.0
24	4	—	25.5	25.0	50.0
48	6	—	—	—	100.0
72	8	12.5	37.5	12.5	37.5
96	8	12.5	37.5	12.5	37.5
Total	27	7.4	25.9	11.1	55.5

TABLE 7.—*Vascular changes observed in healing ENL lesions.*

Older ENL (days)	No. of pts.	Vascular changes (per cent)				
		Normal	Edema only	Edema and endothelial swelling	Mild vasculitis, edema and endothelial swelling	Proliferative vasculitis or obliterative endarteritis
9	4	50.0	25.0	—	—	25.0
10	8	25.0	25.0	25.0	—	25.0
11	12	41.7	8.3	25.0	8.3	16.7
12	2	50.0	—	50.0	—	—
Total	26[a]	38.5	15.4	23.1	3.8	19.2

[a] One male patient refused a second biopsy.

very rare leucocytes. Lepra bacilli were found occasionally in phagocytes assumed to be Schwann cells.

(B) Fite-Faraco stain for acid-fast bacilli. The morphology of *Mycobacterium leprae* in erythema nodosum leprosum lesions was examined to a limited extent. Although this study is concerned with the histopathologic characteristics, we observed that most of the early ENL lesions showed predominantly granular and fragmented forms of acid-fast bacilli, a finding similar to that reported by Mitsuda, ([14]) Ridley, ([22]) Waters and Ridley, ([29]) and Job *et al.* ([10]) Lepra bacilli were frequently demonstrated in the swollen endothelial cells and occasionally in the interstices between smooth muscle cells of the vascular wall.

Another observation was the presence of apparently degenerating lepra cells, most of which contained broken-up globi or clumps of granular acid-fast bacilli in or around the zone of acute inflammation. With the hematoxylin and eosin stain, the mature or old foamy histiocytes appeared to have lost their cell boundaries or to have fused with other adjacent cells to form a picture of a "symplastic" arrangement. ([22])

Question arose as to whether some of the acid-fast granules assumed to be remains of bacilli were granules from degranulating or

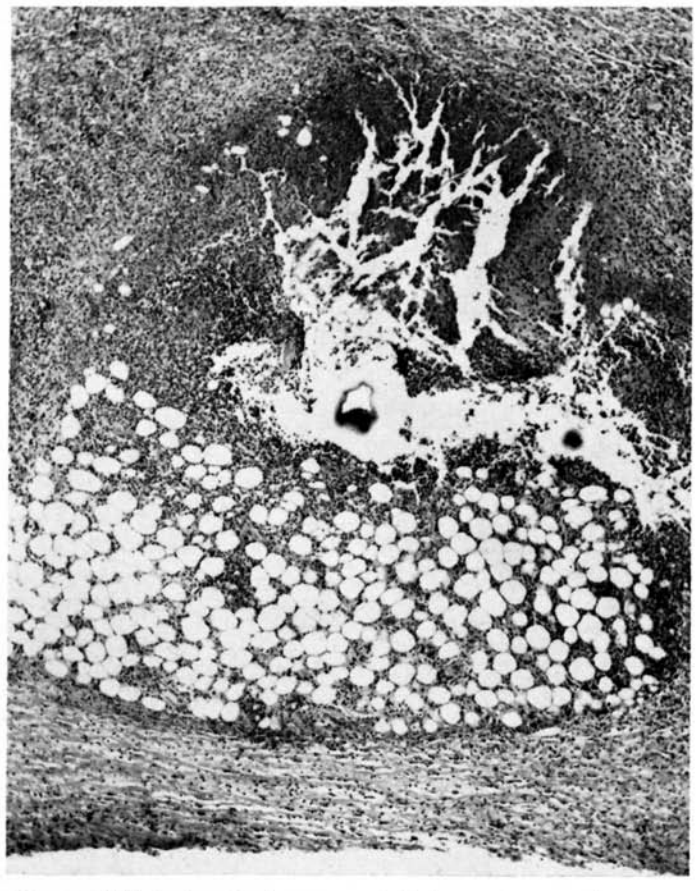

FIG. 2. A 24-hour ENL lesion showing an acute inflammatory reaction in the lower dermis and adjacent subcutaneous fat, as well as microabscess. The inflammatory infiltrate consists primarily of granular leucocytes. Hematoxylin and eosin. × 50. AFIP Neg. 64-4074.

disintegrating mast cells. An aldehyde-fuchsin stain to demonstrate the granules in mast cells confirmed our supposition that the Fite-staining (acid-fast) granules did not originate from mast cells but were in reality granular forms of the leprosy bacillus. *M. leprae* in its three forms (solid, fragmented, and granular) were not stained with aldehyde fuchsin. With the Fite stain, however, intracytoplasmic mast cell granules and those seen extracellularly stained a blue to blue-black.

2. Histochemistry of ENL.—(A) Periodic acid-Schiff reaction, with and without diastase digestion. Reticulum fibers tend to stain red after predigestion with diastase. PAS-positive intracytoplasmic granules were present in the cytoplasm of some foamy histiocytes and in mast cells. In the apparently degenerating fused lepra cells, "lakes" of broken-up globi containing innumerable granular forms of acid-fast bacilli also gave a weakly positive PAS reaction. Diastase predigestion did not remove the PAS-positive material observed in the cytoplasm of both mast cells and histiocytes and in the "lakes" of bacillary globi. Numerous bright magenta-colored granules similar to those seen within

histiocytic cytoplasm were scattered in the connective tissue stroma.

(B) Stains for acid mucopolysaccharides. (*a*) Alcian blue stain and colloidal iron stain, with and without hyaluronidase digestion. The reticular stroma of the lepromatous infiltration showed a moderate (++) to marked (+++ and ++++) positive reaction, indicating the presence of acid mucopolysaccharides. Occasional histiocytes also contained these substances. The faintest trace of a staining reaction was graded as +, and the most intense reaction was recorded as ++++.([11]) With the use of hyaluronidase digestion in specimens stained with colloidal iron, the acid mucopolysaccharide—presumably hyaluronic acid—was removed from the ground substance and cytoplasm of some of the histiocytes.

(*b*) Aldehyde-fuchsin stain. This stain was helpful in demonstrating sulfated acid mucopolysaccharides and related organic sulfur-containing substances in mast cells and elastic tissue.

The histochemical reactions in early ENL lesions did not differ appreciably from those described by Johnson and Helwig([11]) in normal skin except in those areas of the dermal ground substance occupied by foci of acute and chronic inflammatory cells. Nonsulfated hyaluronidase-labile acid mucopolysaccharides were slight or absent in the central zone of acute inflammation but were present in moderate quantities in the peripheral inflammatory zone, around existing lepromatous tissue, and in areas of reticulum-collagen genesis. The walls of blood vessels situated in the lower corium and subcutaneous tissue did not show an appreciably altered content of acid mucopolysaccharides. In older healing lesions, the acid mucopolysaccharide content of the ground substance showed a progressive decrease when compared to the amount in early lesions.

(C) Reticulum stain. Reticulum fibers were increased at the periphery of the acute inflammation, in and around collections of old and mature foamy histiocytes, in the periadnexal zones, and in areas adjacent to collagen formation. With the aging of the ENL lesion, reticulum fibers decreased in number and were more or less confined in the remaining foci of lepromatous infiltrate.

(D) Elastic tissue stain. Thin, delicate, short, or curled blue-black to black strands of what appeared to be broken or fragmented fibers of elastic tissue were scattered throughout the dermis between collagen bundles and around granulomata and sweat glands of the earlier lesions of ENL. The older lesions, 9 to 12 days old, showed fewer fragmented fibers, but there was a relative increase of the normally occurring longer, broader, and sometimes branched fibers of elastic tissue.

Table 8 summarizes the results obtained with various histochemical staining methods for mucopolysaccharides and connective tissue.

DISCUSSION

Early or new lesions of erythema nodosum leprosum generally

TABLE 8.—*A comparison of the staining reaction between early and older lesions of erythema nodosum leprosum obtained with various histochemical staining technics.*

Age of lesion and stain used	Number of patients	Amount or degree of positive staining reaction (per cent)			
		Scant or traces	Weak (+)	Moderate (++)	Marked (+++ to ++++)
Periodic acid-Schiff reaction (amount of neutral polymucopolysaccharide)					
Early	27	....	11.2	44.4	44.4
Older	26	....	57.7	30.8	11.5
Colloidal iron stain (amount of mucopolysaccharide)					
Early	27	....	....	25.9	74.1
Older	26	3.8	30.8	53.8	11.5
Alcian blue, pH 2.5 (amount of acid mucopolysaccharide)					
Early	27	....	....	25.9	74.1
Older	26	3.8	30.8	53.8	11.5
Reticulum stain (degree of reticular proliferation)					
Early	27	....	3.7	22.2	74.1
Older	26	....	34.6	34.6	30.8
Elastic tissue and aldehyde-fuchsin stain (degree of elastic fiber fragmentation)					
Early	27	3.7	29.6	48.1	18.5
Older	26	7.7	61.5	26.9	3.8

show an acute inflammatory infiltrate rich in neutrophils and sometimes in eosinophils. Lymphocytes, histiocytes, and plasma cells are present, but are more numerous in the subsiding phase. Mast cells are also relatively numerous. The inflammatory reaction is usually located in the lower dermis and adjacent subcutaneous tissue, and in some instances microabscesses are present. It is difficult to elaborate on the changes present in the deeper subcutaneous fat, since most of our biopsy specimens had an average depth of 6 to 8 millimeters. In the sections examined, however, subcutaneous fat necrosis and foreign body giant cells were rarely encountered, as would be expected in classical erythema nodosum. Acid-fast bacilli are scarce or absent in the central areas of acute inflammation.

The superficial dermis is seldom involved, but when it is, the deep-seated inflammatory process is usually severe. One lesion 9 to 12 days in duration, still showing an acute inflammation, exhibited epidermal vesiculation and an abscess in the superficial dermis, apparently an extension of the inflammatory reaction in the lower half of the dermis. Vesiculation, abscess formation, and ulceration of ENL lesions have also been mentioned by other investigators. ([10, 19, 22, 29, 32])

Preexistent lepromatous tissue reaction in the upper corium and midcorium has a pericapillary and periadnexal distribution; it is seldom diffuse. The histiocytic infiltrate in these areas is composed of imma-

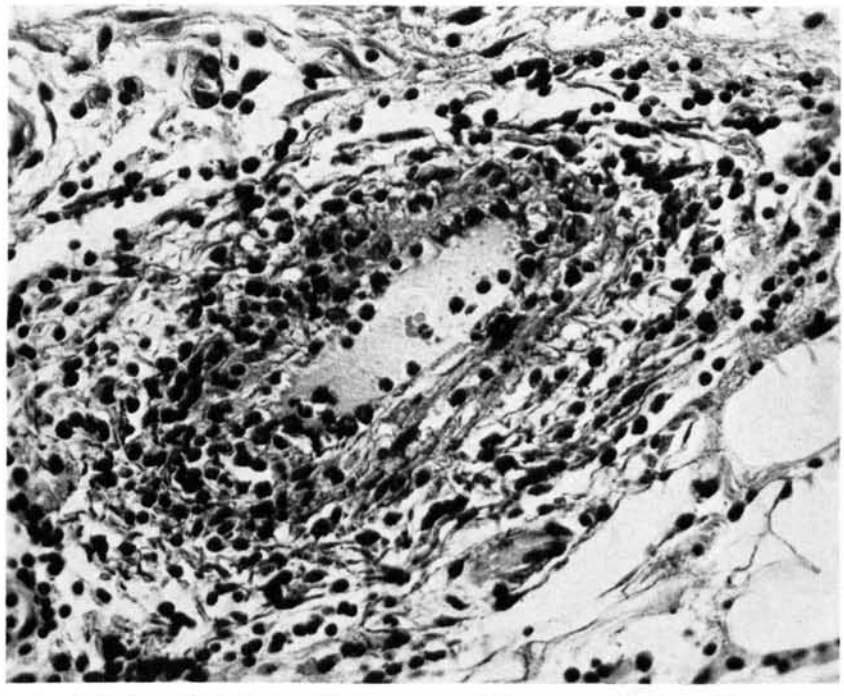

FIG. 3. A 24-hour ENL lesion illustrating an "allergic type" of angiitis. The vascular wall is infiltrated by acute inflammatory cells, and endothelial swelling and edema of the wall are present. Hematoxylin and eosin. × 300. AFIP Neg. 64-1407.

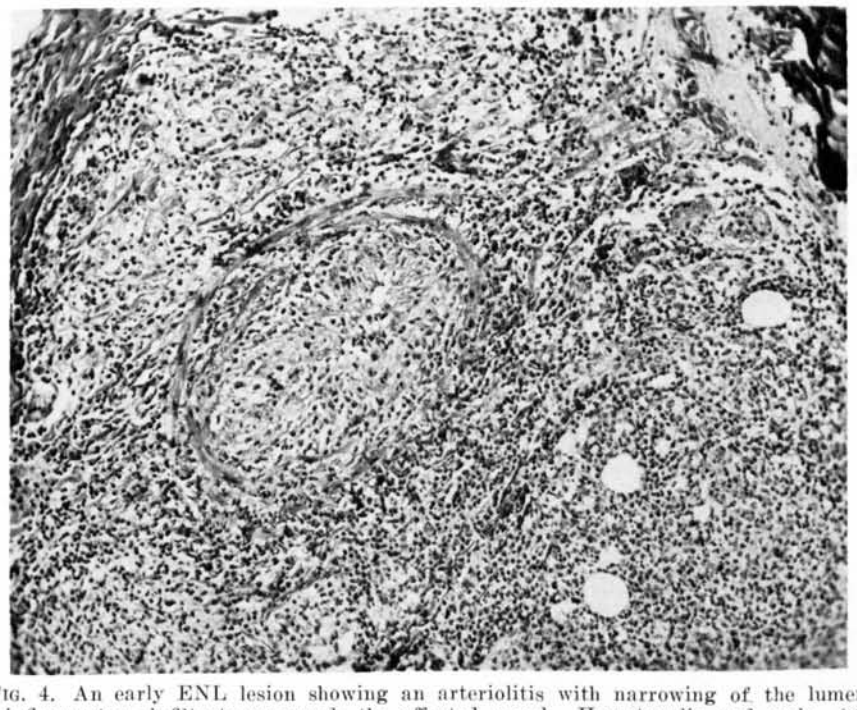

FIG. 4. An early ENL lesion showing an arteriolitis with narrowing of the lumen. A dense inflammatory infiltrate surrounds the affected vessel. Hematoxylin and eosin. × 115. AFIP Neg. 64-1412.

ture-appearing cells that are younger than those found in the lower dermis and hypodermis. Old and mature histiocytes showing the characteristic foamy appearance abound in the deeper portions of lepromatous skin. Some of the "aged" histiocytes present in the areas of acute inflammation show degenerative changes manifested by loss of cell boundaries or fusion of adjacent cells, which Ridley([22]) calls "symplasm." Our observations also indicate an association of degenerative changes occurring between *M. leprae* and its host cell, the foamy macrophage. Broken-up globi containing fragmented or granular forms of acid-fast bacilli are more often seen within the cytoplasm of older histiocytes than in the younger nonfoamy histiocytes.

The presence of old and mature foamy histiocytes in ENL lesions in various stages of degeneration, i.e., lipoid degeneration or symplastic formation, was also observed earlier by Stein, ([26]) Mitsuda, ([14]) and Ridley. ([22]) Stein pointed out that the lepra cells in the superficial dermis of erythema nodosum leprosum lesions contained scanty lipoid material when compared with the cells in the deeper layer. Ridley was of the opinion that formation of symplasm by degenerating foamy cells

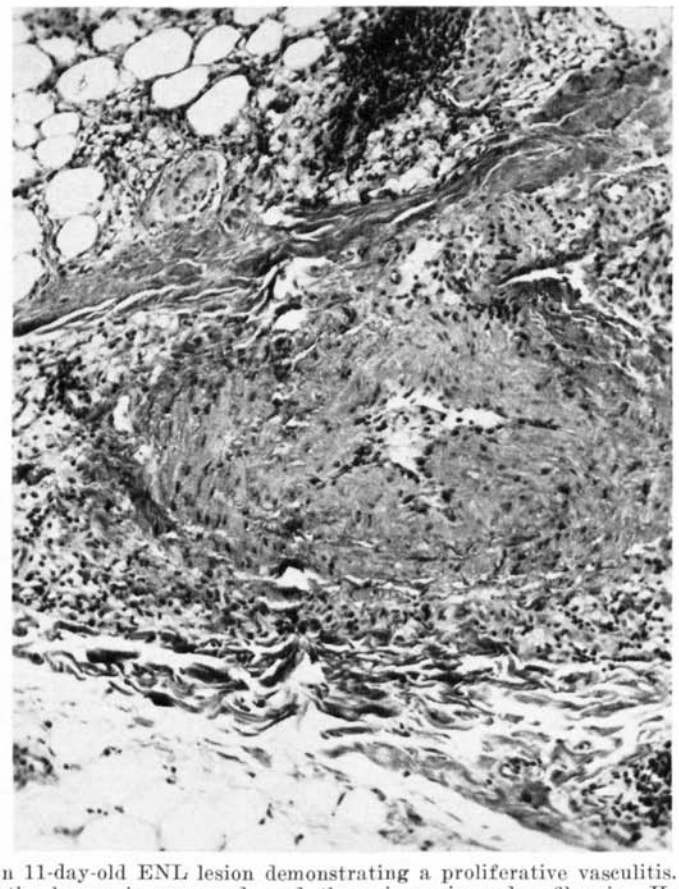

FIG. 5. An 11-day-old ENL lesion demonstrating a proliferative vasculitis. The vascular wall is thick, the lumen is narrowed, and there is perivascular fibrosis. Hematoxylin and eosin. × 85. AFIP Neg. 64-1413.

and granulation of bacilli were both indicative of aging and lessened activity, and that the latter change in the bacilli was more closely associated with the onset of erythema nodosum leprosum than with the appearance of symplasm.

The question arises as to why the inflammatory reaction in acute ENL originates in the deeper tissues of lepromatous skin and not in the superficial corium. Stein ([26]) proposed the idea that the supposedly new lepra reaction lesions were not new at all but an activation of old, nonevident, deeply situated lepromas by a "hyperergic" type of inflammation. Judging from our observations, the most probable explanation is that most of the histopathologic changes of ENL lesions occur in the lower corium and adjacent hypodermis of inapparently infiltrated skin of lepromatous patients undergoing a lepra reaction. First, one must consider the appearance of the histiocytic infiltrate in the upper and lower portions of dermis. There is a preponderance of older and mature histiocytes, some of which show degenerative changes in the deep dermis, in contrast to the scattered younger, healthy- looking nonfoamy histiocytes present in the superficial dermis. Secondly, there appears to be a preparation of the deeper tissue, in which an allergic type of inflammation may occur, i.e., presence of an unusually large number of mast cells, degenerative changes in both lepra cells and acid-fast bacilli, and alteration of the histochemical environment of the ground substance and connective tissue stroma.

The authors concur in the finding of Hollander and Sommers([8]) and of Ghosh *et al.* ([6]) on the presence of an unexpected number of mast cells in lepromatous tissue. Mast cells are fairly numerous in early lesions of erythema nodosum leprosum, but the number diminishes in older lesions. The gradual, but not complete, disappearance of mast cells from subsiding ENL lesions may be attributable to a decrease in vascularity or to the repair of tissue.

The experiments of Riley, ([23]) Riley and West, ([24]) West, ([30]) Mota *et al.*, ([15]) Fawcett, ([4, 5]) and Sir Henry Dale ([2]) gave credence to the presence of histamine in mast cells. There is reason to believe that mast cells may play a role in the production of erythema nodosum leprosum, on the assumption that liberation of histamine from degranulating or disintegrating mast cells incites the changes characteristic of an allergic type of inflammation. The release of histamine from mast cell granules may be effected by the action of hormones ([1]) or by certain substances acting as histamine liberators. ([16, 17]) Mota and Ishii ([16]) and Mota and da Silva ([17]) demonstrated that when antigen was applied to sensitized animal tissues and anaphylaxis induced, there was disruption of the mast cells and an immediate release of histamine. In this respect, we can only assume that if degenerating lepra bacilli presented antigen and acted as a histamine liberator, the production of lesions of erythema nodosum leprosum would be a combination of an

antigen-antibody response and the effects following liberation of histamine.

Presumptive evidence that acute ENL is of an allergic nature was demonstrated in 3 of 15 early lesions with angiitis, consisting of a severe inflammation of the vascular wall and seen as edema of the muscle layers, endothelial swelling, and infiltration of the wall not only by neutrophils but also by eosinophils. In most vessels in which edema and endothelial swelling are the only features, acid-fast bacilli can be demonstrated in the perivascular histiocytic infiltrate and within swollen endothelial cells. Melamed ([13]) mentioned that vascular alteration in leprosy reaction was determined by the penetration of the leproma into the vascular wall, setting up a "locus minoris resistentiae" in the altered vessel and converting it into an allergic "shock organ" for the bacillary antigens of the leprous granuloma and their corresponding antibodies circulating in the blood stream. Likewise, Job *et al.*,([10]) in a study of 36 cases with erythema nodosum leprosum, reported that 16 cases (44.4%) had vasculitis and that the over-all histologic picture of ENL was that of an allergic inflammatory reaction in which polymorphonuclear leucocytes, including eosinophils, infiltrated lepromatous skin. The resultant nodule was therefore a composite of an acute inflammatory process, tissue edema, and vascular proliferation. Other workers, including Wade, ([28]) Wolcott, ([32]) Reiss, ([21]) and Helwig, ([7]) have stressed the importance of vascular changes in the corium and subcutaneous tissue of ENL lesions. Muir, ([18]) however, has a dissenting opinion and offers Selye's general adaptation syndrome to explain the pathogenesis of erythema nodosum leprosum.

On the basis of the observation of Dunphy and Udupa ([3]) on healing of wounds, we may consider the interval from 12 to 24 hours in the age of the ENL lesion as a period of "shock" in which, in addition to cellular autolysis and phagocytosis and to absorption of debris and dead cells, there is increased production and accumulation of tissue mucopolysaccharides in the intercellular substance as a step towards fibroplasia of reticulum and collagen in the healing stage of ENL. Since the superficial dermis is only minimally involved in ENL lesions, residual cicatrization after healing is seldom seen.

The older lesions of erythema nodosum leprosum represent a healing phase in which replacement fibrosis predominates in areas showing the vestige of an acute inflammation. The interlobular fat septa become thickened as a result of the increase of fibrous connective tissue. A decrease in the amount of mucopolysaccharides occurs in foci showing collagen formation. The subsiding stage of an ENL lesion can be compared to the normal healing of wounds either by primary intention or by granulation tissue formation. The observations of Dunphy and Udupa ([3]) and of Taylor and Saunders ([27]) demonstrated the accumulation of large amounts of metachromic intercellular substance, believed to be an acid mucopolysaccharide, in the early stage of repair, which

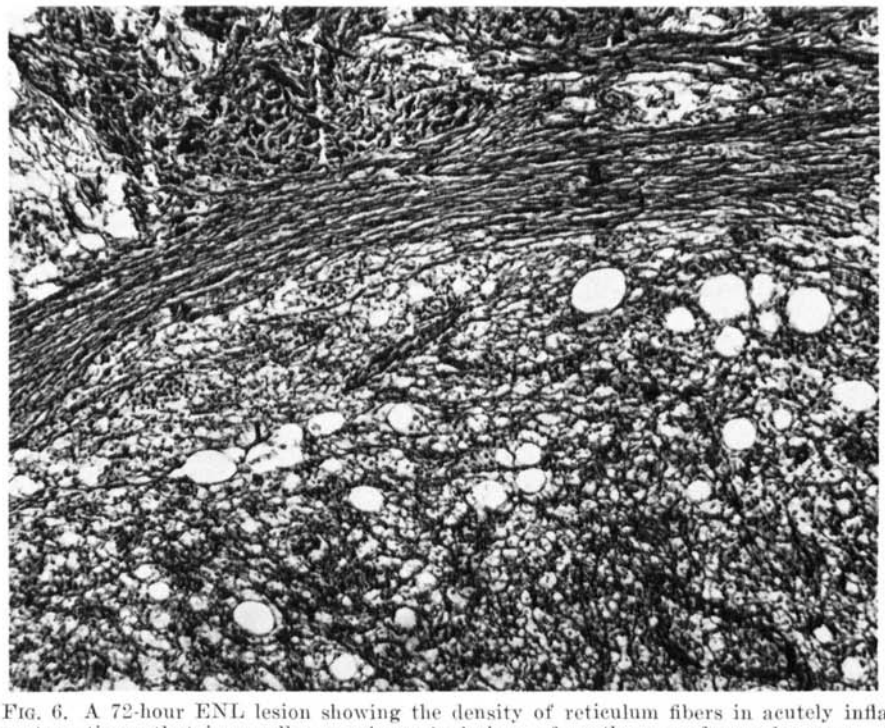

FIG. 6. A 72-hour ENL lesion showing the density of reticulum fibers in acutely inflamed lepromatous tissue that is usually seen in early lesions of erythema nodosum leprosum. The areas of reticulum proliferation show a positive reaction with PAS, colloidal iron, and alcian blue stains, denoting the presence of increased amounts of mucopolysaccharides in the tissue. Spillan's modification of the Snook reticulum stain. × 50. AFIP Neg. 64-4077.

reaches a peak at about 5 to 6 days and starts to diminish when the first collagen fibrils or precursors of collagen appear. Toward the end of the period of fibroplasia, the degree of metachromaticity and the intensity of the colloidal iron stain return to levels seen in normal skin and subcutaneous tissue.

In involuting ENL lesions, the blood vessels show either a return to the normal state or minimal changes. Only when the acute lesion has progressed into a chronic one can marked changes be seen in the vessels, as represented by a proliferative vasculitis or obliterative endarteritis or endophlebitis with perivascular fibrosis. This finding, in many instances, is associated with a persistent chronic granulomatous panniculitis, which Rodriguez ([25]) called erythema induratum leprosum.

Hollander and Sommers, in a study of nonreactive lepromatous tissue ([8]) and other cutaneous granulomas, ([9]) attributed the paucity of reticulin proliferation to a deficiency of neutral mucopolysaccharides in an acid environment that inhibited fibril formation as a precursor to fibrosis. They added further that the slow involution of leprous granulomas was ascribable to this form of alteration of the histochemical environment. In our study of ENL, however, tissue mucopolysaccharides were present in the affected tissues, and fibroplasia was dem-

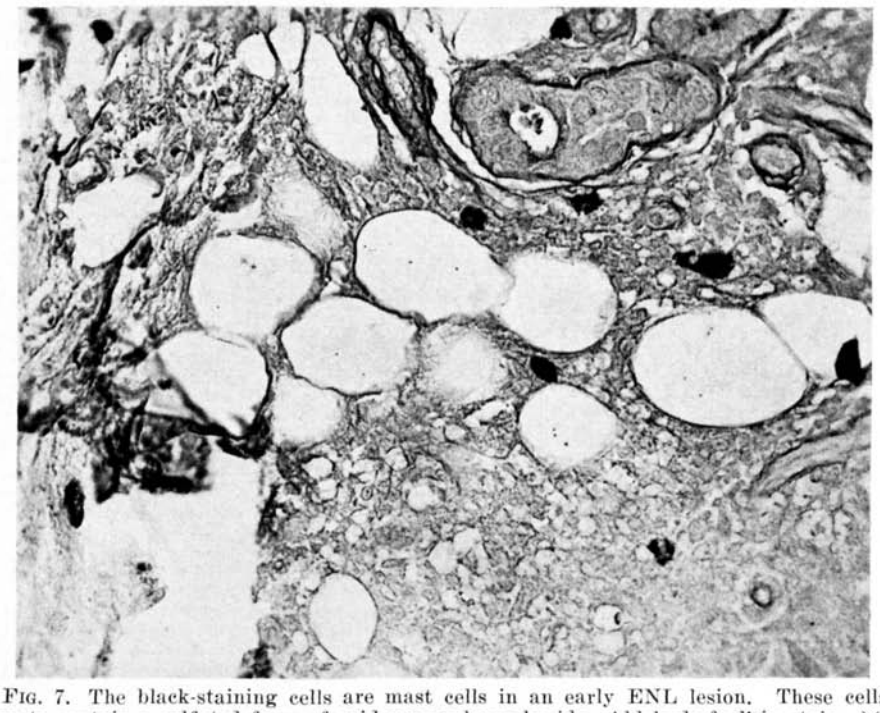

FIG. 7. The black-staining cells are mast cells in an early ENL lesion. These cells are known to contain a sulfated form of acid mucopolysaccharide. Aldehyde-fuclisin stain. × 300. AFIP Neg. 64-3047.

onstrated in healing and older lesions. Perhaps the occurrence of ENL lesions in patients with lepra reaction reverses the histochemical properties of the ground substance in lepromatous skin, thus enhancing connective tissue fibroplasia and involution of lepromata.

Mention was made earlier of finding PAS-positive material in intracytoplasmic granules of many histiocytes as well as in "lakes" of broken-up globi. The origin of this material, which is present in lepra cells, can not be ascertained. It is similar to the granular material present extracellularly or within the cytoplasm of mast cells and may represent phagocytosis of such material. In their extensive study of mast cells, Wegelius and Asboe-Hansen ([31]) commented that macrophages under certain circumstances have been seen to take up mucopolysaccharide material from the surrounding tissue, such as granules of disintegrated mast cells. We agree with Ghosh *et al.* ([6]) that lepra bacilli contain a mucopolysaccharide that can be demonstrated by the PAS reaction. In our study, however, the PAS-positive material in *M. leprae* was seen chiefly in the granular forms and, to a less extent, in the fragmented forms, but not in the solid, evenly stained forms. We presume that the mucopolysaccharide content of the leprosy bacillus is demonstrable only in its degenerative or unevenly stained forms. Bacillary globi and some histiocytic intracytoplasmic granules do not take up stains for acid mucopolysaccharides.

The aldehyde-fuchsin stain was helpful in refuting the argument

that mast cell granules instead of granular forms of lepra bacilli reacted to the Fite stain. Because of the affinity of this stain for sulfated forms of acid mucopolysaccharides, mast cell granules—whether present intracytoplasmically or extracellularly—were stained accordingly. Acid-fast bacilli were not stained by this method.

In the course of healing, neutrophils and eosinophils disappear from the scene of inflammation, and more chronic inflammatory cells such as lymphocytes, plasma cells, and liistiocytes make their appearance in the resolving tissue. No definite explanation can be given of the significant number of plasma cells in older ENL lesions, but it is construed to be an indication of a possible change that an acute lepra reaction has brought about in the physiologic and immunologic make-up of an otherwise anergic lepromatous patient. Lepra reaction probably offers the patient's system a chance to eliminate *M. leprae* through ENL and at the same time builds up his resistance through increased production of circulating immune bodies by proliferating plasma cells and cells of the reticuloendothelial system, if antibody production resides in these cells. Wolcott ([32]) offered the suggestion that the presence of erythema nodosum leprosum indicated an increasing resistance to the disease.

SUMMARY

A study is reported of the histopathology and histochemistry of early and regressing lesions of erythema nodosum leprosum observed in 27 essentially lepromatous patients confined at the Eversley Childs Sanitarium, Cebu, Philippines.

1. Acute or early ENL lesions showed an inflammatory infiltrate rich in granulocytes, which tended to disappear as early as the fourth day and were almost absent in the older lesions. The decrease in the number of neutrophils and eosinophils between early and older lesions of ENL was accompanied by a corresponding rise in the number of lymphocytes and plasma cells. Mast cells diminished in number, and histiocytes increased slightly.

2. The vascular changes noted in early ENL lesions were those of moderate to marked edema of the vascular wall and endothelial swelling. There was an angiitis in 15 (55.5%) of 27 early lesions; 3 of them were of an "allergic" type. In older lesions the vessels usually showed either minimal edema and/or endothelial swelling, or a return to the normal state, but 5 of the 26 older lesions showed a proliferative angiitis or an obliterative endarteritis or endophlebitis associated with a chronic histiocytic panniculitis.

3. Degenerative changes in both *M. leprae* (granulation and fragmentation) and histiocytes (loss of cell boundaries, cellular fusion) were demonstrable in most of the early lesions of ENL, and these changes were noted particularly in the lower half of the dermis and subcutaneous tissue, where an acute inflammatory process was taking place. The upper half of the dermis was uninvolved in the inflamma-

tion, except that it extended into the upper corium and epidermis, apparently when the intensity of the inflammatory reaction in the deeper tissues had reached a certain degree. A diffuse histiocytic infiltration was absent in the superficial dermis, and the many young or immature nonfoamy histiocytes present in the upper corium were distributed around capillaries and adnexal structures.

4. The numerous mast cells found in the early lesions suggested their importance in the development of erythema nodosum leprosum.

5. Mucopolysaccharides were present in ENL lesions stained with various histochemical methods. Acid mucopolysaccharides, especially in the early lesions, were abundant in areas of fibroplasia. The quantity of acid mucopolysaccharides in the inflamed areas showed a progressive fall when collagen fibers appeared in the process of healing.

6. Clumps of broken-up globi and histocytic intracytoplasmic granules stained positively with the periodic acid-Schiff stain. These were not digested with diastase, and therefore they were not glycogen but might be a mucopolysaccharide. "Lakes" of globi and granules of lepra cells were not stained with alcian blue or colloidal iron stains, a fact ruling out an acid mucopolysaccharide. The granules in the cytoplasm of some histiocytes staining positively for PAS may be granules of phagocytized mast cells.

7. Proliferation of reticulum fibers was a prominent feature in early ENL lesions and was associated with a large concentration of acid mucopolysaccharides in the ground substance adjacent to the areas of acute inflammation. The amount of acid mucopolysaccharides and the number of reticulum fibers diminished with the increase of fibroblastic activity and formation of collagen fibers, especially in the healing stage of ENL.

8. Fragmentation of elastic tissue was prominent in early lesions. Older lesions showed less fragmented elastic tissue fibers in relation to the longer, broader, and branched normal-appearing fibers.

RESUMEN

Se comúnica un estudio histopatológico e histoquímico de las lesiones tempranas y regresivas del eritema nudoso leproso, observadas en 27 pacientes esencialmente lepromatosos, confinados en el Eversley Childs Sanitarium, Cebu, Filipinas.

1. Las lesiones agudas o tempranas de ENL mostraron un infiltrado inflamatorio rico en granulocitos, el cual tiende a desaparecer tan pronto como al cuarto día y era casi completamente ausente en viajas lesiones. La disminución en el número de neutrófilos y eosinófilos entre las lesiones tempranas y antiguas del ENL, fué acompañado por un correspondiente aumento en el número de linfocitos y plasmocitos. Las celulas cebadas disminuyeron en número y los histiocitos aumentaron ligeramente.

2. Los cambios vasculares observados en las lesiones tempranas de ENL fueron de moderado a marcado edema de la pared vascular, y tumefacción endotelial. Hubo angeítis en 15 (55.5%) de las 27 lesiones tempranas; 3 de ellas fueron de tipo "alérgico." En las lesiones antiguas los vasos usualmente mostraron o un edema minimo o/y tumefacción endotelial, o un retorno al estado normal, pero 5 de las 26 lesiones antiguas mostraron una angeítis proliferativa o una endarteritis o endoflebitis asociada con una paniculitis crónica histiocítica.

3. En la mayoria de las lesiones tempranas de ENL, fueron demostrables cambios degenerativos en ambos *M. leprae* (granulación y fragmentación) e histiocitos (pérdida

de los límites celulares, fusión celular), y estos cambios fueron notados particularmente en la mitad inferior del dermis y del tejido subcutáneo, en donde un proceso inflamatorio agudo estaba ocurriendo. La mitad superior del dermis no estaba implicada en la inflamación, excepto en el corium superior y epidermis, aparentemente cuando la intensidad de la reacción inflamatoria en los tejidos profundos llegó a cierto grado. No se encontró infiltración difusa histiocítica en el dermis superficial, y los numerosos histiocitos inmaduros no-vacuolizados presentes en el corium superior, estaban distribuidos alrededor de los capilares y estructuras de los anexos.

4. Los numerosos mastocitos encontrados en las lesiones tempranas sugirieron su importancia en el desarrollo del eritema nudoso leproso.

5. Las lesiones de ENL coloradas por diversos métodos histoquímicos demostraron la presencia de mucopolisacáridos. Los mucopolisacáridos ácidos, eran abundantes en las areas de fibroplasia, especialmente en las lesiones tempranas. La cantidad de mucopolisacáridos ácidos en las areas inflamadas, mostraron una caida progresiva cuando las fibras colágenas aparecieron en el proceso de cicatrización.

6. Acúmulos de globos rotos y gránulos histiocíticos intracitoplasmaticos se colorearon positivamente con la coloración de ácido periódico-Schiff. Estos no fueron digeridos con la diastasa, y por lo tanto no eran de glicógeno, pero pueden ser un mucopolisacárido. Los "lagos" de globos y los gránuloes de las células leprosas no se colorearon con alcian blue o hierro coloidal, hecho que descarta un mucopolisacárido ácido. Los gránulos en el citoplasma de algunos histiocitos que se colorearon positivamente con el PAS, pueden ser gránulos o mastocitos fagocitados.

7. La proliferación de celulas reticulares fueron una imagen prominente en las lesiones tempranas de ENL y estaba asociada a la gran concentración de ácidos mucopolisacáridos en la substancia fundamental adyacente a las áreas de inflamación aguda. La cantidad de mucopolisacáridos ácidos y el número de fibras reticulares, disminuyeron con el aumento de la actividad fibroblastica y la formación de fibras colágenas, especialmente en los estadios de curación del ENL.

8. La fragmentación del tejido elastico fué prominente en las lesiones tempranas. Las lesiones mas antiguas mostraron menor fragmentación de las fibras del tejido elástico en relación con las fibras de apariencia normal, que son mas largas, mas anchas y ramificadas.

RÉSUMÉ

On rapporte ici une étude sur l'histopathologie et sur l'histochimie des lésions d'érythème nouex lépreux, précoces ou en voie de régression, telles qu'elles ont été observées chez 27 malades nettement lépromateux isolés à l'Eversley Childs Sanitarium, Cebu, dans les Philippines.

1. Les lésions aigües ou précoces d'ENL ont montré un infiltrat inflammatoire riche en granulocytes, qui a eu tendance à disparaître aussi précocement que le quatrième jour et qui était presque absent dans les lésions plus âgées. La diminution du nombre de neutrophiles et d'éosinophiles au cours du passage du stade précoce vers le stade tardif des lésions d'ENL a été accompagnée par une augmentation correspondante du nombre de lymphocytes et de plasmocytes. Les mastocytes ont diminué en nombre, et les histiocytes ont légèrement augmenté.

2. Les changements vasculaires notés dans les lésions précoces d'ENL ont été ceux d'un oedème d'intensité modérée à marquée dans les parois vasculaires ainsi qu'un gonflement de l'endothélium. Une angéite a été notée dans 15 (55.5%) des 27 lésions précoces; dans 3 de ces cas l'angéite était du type "allergique." Dans les lésions plus âgrées, les vaisseaux ont généralement montré soit un léger oedème, soit un gonflement endothélial, ou bien ces deux lésions à la fois. Dans d'autres cas on a noté un retour à l'état normal, mais 5 des 26 lésions tardives toutefois ont montré soit une angéite proliférative, soit une endartérite oblitérante, soit une endophlébite associée avec une panniculite chronique à histiocytes.

3. Dans la plupart des lésions précoces d'ENL, des changements de type dégénératif ont pu être démontrés à la fois dans *M. leprae*, sous forme de granulation et de fragmentation, et dans les histiocytes, sous forme de perte des limites cellulaires et de fusion

des cellules. Ces changements étaient notés particulièrement dans la moitié inférieure du derme et dans le tissu sous-cutané, lorsqu'un processus inflammatoire aigü était présent. La moitié supérieure du derme n'était pas affectée par l'inflammation, sinon que celle-ci pouvait s'étendre dans le chorion supérieur et dans l'épiderme, apparemment lorsque l'intensité de la réaction inflammatoire dans les tissus plus profonds avait atteint un certain degré. Le derme superficiel n'a pas montré d'infiltration histiocytique diffuse. Les nombreux histiocytes jeunes ou bien non spumeux et non encore arrivés á maturation qui étaient présents dans le chorion supérieur étaient distribués autour des capillaires et des structures annexes.

4. Les nombreux mastocytes trouvés dans les lésions précoces suggèrent que ces cellules sont importantes dans le développement de l'érythème noueux lépreux.

5. Des mucopolysaccharides ont été mis en évidence dans les lésions d'ENL lorsque celles-ci avaient été colorées par diverses méthodes histochimiques. Les mucopolysaccharides acides étaient abondants dans les zones avec fibroplasie, particulièrement au niveau des lésions précoces. Dans les régions enflammées, la quantité de mucopolysaccharides acides a progressivement diminué au cours de la cicatrisation lorsque des fibres collagènes sont apparues.

6. Avec le colorant périodé de Schiff on a pu colorer des agglomérats de globi fragmentés et des granules histiocytaires intracytoplasmiques. Ceux-ci n'étaient pas digérés par la diastase. Ils n'étaient dès lors pas constitués de glycogène, mais pouvaient consister de mucopolysaccharide. Des "lacs" constitués de globi et de granules provenant des cellules lépreuses n'ont pu être colorés avec du bleu alcien ou des colorants à base de fer colloidal, ce que met hors de cause un mucopolysaccharide. Les granules qui pouvaient être colorés positivement avec le PAS dans le cytoplasme de quelques uns des histiocytes pouvaient être des granules provenant de mastocytes phagocytés.

7. La prolifération des fibres réticulaires a constitué une caractéristique remarquable des lésions précoces d'ENL. Cette prolifération était associée à une grande concentration de mucopolysaccharides acides et le nombre de fibres réticulaires diminué lorsque l'activité fibroblastique et la formation de fibres collagènes a augmenté, surtout lors du stade de cicatrisation de l'ENL.

8. La fragmentation du tissu élastique a été extrémement nette dans les lésions précoces. Les lésions plus âgées ont révélé moins de fibres tissulaires élastiques fragmentées par rapport aux fibres d'apparence normale, plus longues, plus larges et avec bifurcations.

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