CLASSIFICATION OF LEPROSY, II. THE VALUE OF FAT STAINING IN CLASSIFICATION

A. R. Davison, M.R.C.S. (Eng.), L.R.C.P. (London)
R. Kooij, M.D. (Amsterdam)[1]
and J. Wainwright, B.Sc., M.D. (Sheffield)

Westfort Institution
Pretoria, South Africa

In a previous article by Kooij (5) the results of a study of the same histologic preparations from cases of leprosy by a number of histopathologists in several countries were reported. There were great differences in opinion, showing that the diagnoses of the histopathology of leprosy is very difficult. It was suggested that fat staining might be helpful in the evaluation of the histologic picture.

It is now generally accepted that the Virchow lepra cells contain fat in their cytoplasm. Mitsuda (6) found the fat substance to be of lipoid nature of unknown but possibly very complex constitution. Herxheimer (4) carried out a detailed investigation of the fat of the Virchow cell by means of various methods of fat staining. His view was that the leprosy bacilli cause a lipoid degeneration of the Virchow cells. He concluded that the fat in the Virchow cells is a mixture of cholesterin, glycerol and fatty acids.

De Souza and Alayon (8), Campos (2), and Azulay and de Andrade (1) have used fat staining for differentiation of the histologic structures of leprosy. Their results will be discussed later.

MATERIAL AND METHODS

We have carried out fat staining in the histologic specimens of 118 patients with leprosy who have been under close observation for periods of one to three years and who were classified according to the Madrid criteria (3). The fat staining was done on frozen sections, using a saturated solution of a mixture of equal parts of Sudan III and Sudan IV in Herxheimer's solvent (equal parts of acetone and 70% alcohol). In addition, acid-fast bacilli in the regular histologic specimens were stained by the Ziehl-Neelsen technique, using 5 per cent sulfuric acid as decolorizing agent and passing the sections rapidly through alcohol. Control sections of lepromatous tissue were always stained.

Skin smears for bacteriologic examination were taken, stained and evaluated as described in the first article of this series (3).

A total of 129 cases was involved in this study, but not all of them are included in all of the tables. First we deal with a group of 118 which had been long enough under observation for the classification to be definite. There was an additional group of 11 cases not so definitely classified, of which the biopsy specimens were examined for fat and bacilli, and the total of 129 is used in the tables showing the correlation of the findings of those two elements.

[1] Now at the Groote Schuur Hospital Laboratory, Capetown, U. of South Africa.

RESULTS

The results of the fat staining in the frozen sections of the 118 patients who were classified with certainty are shown in Table 1.

TABLE 1.—*Fat staining in histologic sections of specimens from 118 well-classified patients with various forms of leprosy.*

Type of case	No. of	Results; degrees of positivity					
		4+	3+	2+	1+	±[a]	Neg.
Indeterminate	3	0	0	0	0	1	2
Lepromatous	30	2	11	12	3	1	1
Tuberculoid	22	0	0	0	2	5	15
T, reaction	42	2	3	2	14	8	13
Borderline	21	2	5	5	5	3	1

[a]Trace.

It will be seen from this table that the examination of sections for fat generally gives negative results in tuberculoid cases except those which are in reaction, whereas the results are usually positive in the sections of specimens from lepromatous and borderline cases. Among the 42 cases of tuberculoid in reaction, the result of fat staining was 4+ in 2, 3+ in 3, 2+ in 2, and 1+ in 14; 8 showed traces, and only 13 were entirely negative.

It appeared that the sections which gave strongly positive results with the fat staining came from patients with tuberculoid leprosy who clinically were suffering very strong reactions. Case reports of the five patients of clinical tuberculoid leprosy in reaction showing 4+ and 3+ fat staining are given in the annex to this report.

The correlation of the results of staining for both fat and acid-fast bacilli in the entire group of 129 patients is shown in Table 2. It can be seen that there is a good correlation between the degree of fat staining and the number of acid-fast bacilli in the histologic specimens of this group of 129 patients.

TABLE 2.—*Correlation of results of staining for fat and for acid-fast bacilli in sections of specimens from 129 patients.*

Bacilli	No. of cases	Fat					
		4+	3+	2+	1+	±[a]	Neg.
4+	34	4	9	9	6	3	3
3+	32	2	7	11	8	3	1
2+	13	0	2	3	5	2	1
1+	10	0	1	2	1	0	6
Vse	3	0	0	0	0	0	3
Neg.	37	0	0	1	7	10	19

[a]Trace.

In Table 3 is shown the correlation between the results of fat staining of sections and the grading of bacilli in smears from this same group of 129 patients.

TABLE 3.—*Correlation of results of fat staining of sections and the bacteriologic positivity of skin smears from 129 patients.*

Bacilli in smears	No. of cases	Fat in sections					
		4+	3+	2+	1+	±[a]	Neg.
4+	34	4	9	9	6	3	3
3+	32	2	7	11	8	3	1
2+	13	0	2	3	5	2	1
1+	10	0	1	2	1	0	6
Vsc	3	0	0	0	0	0	3
Neg.	37	0	0	1	7	10	19

[a]Trace.

DISCUSSION

The results here reported show that, in general, fat-staining of the histologic specimens from lepromatous and borderline patients gives positive results, while in the specimens from tuberculoid patients—other than tuberculoid in reaction—the findings are negative or at most very weak. A few tuberculoid patients who were suffering from very severe reactions were strongly fat-positive. These cases are of the type for which we need a means of differentiation between the borderline and lepromatous forms of leprosy on the one hand, and tuberculoid leprosy in reaction on the other hand. It is clear from our results that fat staining fails to help in just these cases.

Our results are not in accord with those of Azulay and de Andrade. They studied 1,053 histologic specimens from leprosy patients stained by Sudan III and Scarlet R in frozen sections. They stated that

> The research of lipid was 100% negative in the simple inflammatory and the tuberculoid (quiescent, reactional or borderline) types; at the moment that a borderline case becomes lipid-positive one already classifies it as a lepromatous one.

These authors found 84 per cent of their lepromatous cases to be positive for lipoids, 33 per cent of their undifferentiated cases, and only 17 per cent (3 out of 18) of their reactional tuberculoid cases. Most of their lipoid-positive cases of the undifferentiated form were old lepromatous cases. Campos, on the contrary, found the fat-staining positive in every one of his 7 cases of tuberculoid in reaction. The quiescent tuberculoid cases were fat-negative, and nearly every lepromatous case was positive.

Although not all of our cases of tuberculoid in reaction were fat positive, only 12 out of 42 cases were completely negative; the remaining 30 showed degrees of fat staining varying from trace (±) to 4+. In most of our cases of tuberculoid in reaction a correct classification diagnosis could easily be made on clinical grounds, but in those showing 3+ and 4+ degrees of fat-positivity the clinical classification was difficult. In addition, these cases histologically showed a lepromatous structure. However, they quickly became negative for bacilli. Probably the lepra

cells in the two types differ in their capacity to break down the leprosy bacilli. Perhaps by histochemical methods one might find differences [see Pepler, Loubser and Kooij ([7])].

It was also found that the results of fat staining generally run parallel with the numbers of acid-fast bacilli in the histologic specimens (Table 2), and even with the results of the bacteriologic examination of the skin smears (Table 3). Therefore, fat staining usually does not give any more information for classification than does the staining for bacilli.

It has repeatedly been noticed that when the fat staining in a section is heavy, then bacilli may stain less readily. That was observed, for example, in the second biopsy specimen of Case 9882 of the annex to this article. It is possible that in these cases the bacilli are fragmenting, and consequently retain the stain less well.

One could object that the five severe cases of tuberculoid leprosy in reaction with strong fat staining (details in the annex) actually belonged to the borderline group. In our opinion this is not the case, chiefly because the prognosis proved to be good.

SUMMARY

In the 129 patients with different forms of leprosy studied—118 well classified and another 11 less thoroughly followed up—fat staining of frozen sections of biopsy specimens was performed, as well as bacillus staining of regular histologic sections.

The result of fat staining was generally positive in the sections from the lepromatous and borderline patients, and generally negative in tuberculoid cases, with the exception of those of tuberculoid in reaction.

A few tuberculoid cases with very strong reactions were strongly fat positive. These are just the cases for which we need a means of differentiation from borderline and lepromatous leprosy, and just here the fat staining failed to help.

It was also found that fat staining usually does not give more information for classification than the staining of the bacilli in the histologic specimens.

RESUMEN

En los 129 enfermos con diversas formas de lepra estudiados—118 bien clasificados y 11 seguidos memos cuidadosamente—se llevó a cabo la lipocoloración de cortes congelados de ejemplares biópsicos, e igualmente la tinción de bacilos en cortes histológicos de los habituales.

El resultado de la lipocoloración fué en general positivo en los cortes de los enfermos lepromatosos y limítrofes, y en general negativo en los casos tuberculoideos, exceptuados los de reacción tuberculoidea.

Unos pocos casos tuberculoideos con reacciones muy poderosas fueron intensamente lipopositivos. Estos son precisamente los casos para los que necesitamos un medio de diferenciación de la lepra limítrofe y la lepromatosa, y ahí precisamente fué que no ayudó la lipocoloración.

También se observó que la lipocoloración no aporta por lo regular más información para la clasificación que la tinción de los bacilos en los cortes histológicos.

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ANNEX; ILLUSTRATIVE CASES

The following are detailed reports of five cases of tuberculoid in reaction in which fat in the sections was graded 4+ or 3+. These case reports will also serve to illustrate the clinical and histologic features of this classification group as it is discussed in the previous article of this series ([3]).

Case No. 12677. Bantu female, aged 51. Onset, 1954. Admitted November 1955.

Condition on admission.—General condition, fair. Suggestion of infiltration of face. On trunk and limbs, symmetrically disseminated pea-sized plaques. An annular tuberculoid lesion, red and swollen, on left upper arm. Density of eyebrows, 2. Infiltration of earlobes, 1. Anesthesia, none. Contractures, 5th fingers of both hands. Lepromin reaction: 48 hours, 2 mm.; 28 days, 2 mm. Classification: Tuberculoid in reaction.

Histopathology.—November 1955. Biopsy of tuberculoid lesion on left upper arm. Report: Lepromatous. Confluent infiltration of pale-staining histiocytes with focal collections of lymphocytes and groups of Virchow cells. Acid-fast bacilli, 4+. Fat, 3+.

December 1956. Biopsy of hypopigmented plaque of right thigh. Report: Probably tuberculoid. Predominantly perineural infiltration, with groups of epithelioid cells and more widespread lymphocytic plasma cell and eosinophil infiltration. Acid-fast bacilli, negative. Fat, trace.

Course.—In February 1956, small plaques with hollow centers. July 1956, plaque-like eruption on face, trunk and limbs; pain in limbs. August 1956, swollen arms. September 1956, branny desquamation of face; face worse. October 1956, marked subsidence of lesions of face, trunk and limbs. December 1956, very marked subsidence, lesions

paler and only slightly elevated, except plaque on the left upper arm; pain limbs. January 1958, declared arrested.

Bacteriology.—Positive, 1+ to 3+, in 4 of 6 sites examined in November and December 1955. Negative in all of 5 sites, January and February 1956. Positive, very scanty or 1+, in 4 sites April-July 1956. Negative thereafter (except very scanty in one site in January 1957) in 13 examinations (45 sites) to December 1957.

Comment.—This case is considered to be tuberculoid in reaction because: (1) Clinically there were some tuberculoid features. (2) The second biopsy showed tuberculoid features. (3) The bacilli disappeared rapidly.

Case No. 12334. Bantu boy, aged 16. Onset, 1954. Admitted December 1954.

Condition on admission.—General condition fair. Spongy nodules with clear-cut margins on face. Disseminated pea- to marble-sized nodules on trunk and limbs. Tuberculoid lesions with healing centers on back of neck and outer surface of right thigh. Tuberculoid lesions with raised centers on back of right thigh and outer surface of left thigh. Density of eyebrows, 1. Infiltration of earlobes, 0. Anesthesia, lesions of right leg anesthetic to light touch (cotton wool). No contractures of hands or feet. Lepromin reaction: 48 hours, 0 mm.; 28 days, 0 mm. Classification: Tuberculoid in reaction.

Histopathology.—January 1955. Biopsy of a nodule of right calf. Report: Lepromatous. Periadnexial and perivascular infiltrations of pale-staining histiocytes which are becoming confluent with focal collections of lymphocytes. Subepithelial clear zone present. Acid-fast bacilli, 3+. Fat, 4+.

Course.—DDS and atabrin. In January 1955, lesions scarlet and infiltrated. February 1955, lesions on face and neck more reactive; hardly able to open right eye. April 1955, lesions on face dusky and spongy; right eyeball just visible. October 1955, lesions of face flat and dusky; lesions on right leg slightly erythematous. December 1955, dusky plaques on legs. April 1956, declared arrested.

Bacteriology.—Strongly positive (3+) in 2 of 4 smears made in December 1954. Entirely negative thereafter (9 examinations, 35 smears) to February 1956.

Comment.—This case is considered to have been tuberculoid in reaction because: (1) Clinically there were tuberculoid features. (2) The bacilli disappeared rapidly.

Case No. 12656. Bantu girl, aged 14. Onset, 1955. Admitted October 1955.

Condition on admission.—General condition, fair. Marked infiltration over forehead, slight over malars surrounded by normal-looking skin. Raised plaques on trunk and limbs surrounded by normal-looking skin. Density of eyebrows, 1. Infiltration of the earlobes, 1. Anesthesia, none. Lepromin reaction: 48 hours, 0 mm.; 28 days, 0 mm. Classification: Tuberculoid in reaction.

Histopathology.—January 1956. Biopsy of plaque on right buttock. Report: Lepromatous. Periadnexial and perivascular infiltration of pale-staining histiocytes, with Virchow cells. Subepithelial clear zone present. Acid-fast bacilli, 2+. Fat, 3+.

Course.—In December 1955, plaques very raised and erythematous. September 1956, plaques subsided. December 1956, declared arrested.

Bacteriology.—In October 1955, 4 of 5 smears were positive (2 of them 4+); very scanty in the single smear made in November. Completely negative thereafter, in 9 examinations (33 smears) made between January and March 1956.

Comment.—This case is considered to be tuberculoid in reaction because of the rapid disappearance of the bacilli.

Case No. 12301. Bantu male, aged 44. Onset, 1954. Admitted November 1954.

Condition on admission.—General condition, fair. Diffuse infiltration, face; some areas elevated and red. Hypopigmented lesions on trunk and limbs, distribution asymmetrical. Lateral loss of eyebrows. Infiltration of earlobes, 1. Anesthesia, none. Con-

tractures, both hands. Lepromin reaction: 48 hours, 3 mm.; 28 days, 2 mm. Classification: Tuberculoid in reaction.

Histopathology.—January 1955. Biopsy of a red plaque on back. Report: Lepromatous. Periadnexial and perivascular infiltration of pale-staining histiocytes with focal collections of lymphocytes. Acid-fast bacilli, 3+. Fat, 3+.

Course.—In May 1955, patient undergoing an acute reaction; acute infiltration face, also areas of normal skin; plaques on trunk and limbs raised, red and desquamating; some lesions show healing centers. July 1955, reaction subsided. September 1955, dusky staining, face; infiltration minimal. Redness of plaques on trunk and limbs has disappeared. June 1956, declared arrested.

Bacteriology.—In November 1954, 3 of 4 smears positive (2 of them 4+). Positive findings on 2 of 3 occasions early in 1955. Entirely negative thereafter, in 8 examinations (35 smears) between July 1955 and June 1956.

Comment.—This case is considered to be tuberculoid in reaction because of the rapid disappearance of the bacilli.

CASE NO. 9882. Bantu female, aged 36. Onset, 1946. Admitted January 1947.

Condition on admission.—General condition good. Pale hypopigmented lesions on face, trunk and limbs with flat margins and healing centers. Anesthesia, left hand and ulnar aspect of right hand. (No further data available.) Classification: Tuberculoid leprosy. Discharged April 4, 1949, after treatment with chaulmoogra, intradermal and intramuscular.

Readmission.—Readmitted March 1955. General condition poor. Raised erythematous infiltrations of face, neck and both arms. Multiple pale flat lesions on trunk. Density of eyebrows, 3. Infiltration of the earlobes, 1. Lepromin reaction: 48 hours, 5 mm.; 28 days, no report.

Histopathology.—November 1955. Biopsy of plaque posteriorly on right upper arm. Report: Lepromatous. Diffuse zone of pale-staining histiocytes, in areas of spindle-cell type with Virchow cells. Subepithelial clear zone present. Acid-fact bacilli, doubtful. Fat, 2+.

August 1956. Second biopsy of the same lesion. Report: Nonspecific. Focal perivascular and periadnexial histiocyte and lymphocyte infiltration. Acid-fast bacilli, negative. Fat, 3+.

Course.—In July 1955, lesions very reactive. October 1955, subsiding; lesions spongy and glistening on face, hypopigmented and flat on trunk; infiltration arms, particularly left arm. December 1955, infiltration almost completely gone. January 1956, lesions flat and pale. May 1956, lesions clinically healed.

Bacteriology.—The record is voluminous, with 28 examinations (6 times only 1 smear, the others 4 or more) between March 1955 and July 1958. In the first examination 2 of 4 smears were 4+, and in the second done in the same month all 4 were 2+. From then to April 1958, wholly negative findings were interspersed with a few weakly positive smears, they entirely "very scanty" after July 1956. The last 3 examinations (12 smears) were entirely negative.

Comment.—This case is considered to be tuberculoid in reaction because of the rapid disappearance of the bacilli.