# A SPECTROGRAPHIC STUDY OF LEPROUS LESIONS *

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These determinations were made with the idea that significant modifications might occur in Na, Ca, Mg, P, Fe and, perhaps, other elements in the lesions as compared with unaltered tissue of the same sort. It was thought that if a marked chemical change paralleled the development of distinctive lesions, something might be done to re-establish normal ratios between the elements that would be of therapeutic value. In this paper we wish to report a beginning in this direction made possible by the fact that histopathological studies on leprosy are being carried on in the same laboratory in which Dr. Gordon H. Scott and his associates are using the technique of histospectrography.[1-4] We are very grateful to Dr. Scott for his help and advice. We have made a spectrographic examination of leper lesions from 6 cases, the examination involving comparison with five "normal" skins taken to represent roughly normal conditions, and with three other such skins on which chemical analyses were run. All eight of the controls afford opportunity for semiquantitative estimates (of the "greater than" or "less than" variety as described by Scott and Williams [2]) of P/Ca, Na/Ca, Mg/Ca and Fe/Ca ratios in leper lesions as compared with normal skin; and we have worked out numerical values for the P/Ca and Na/Ca ratios based upon the spectrographic findings on three chemically analyzed normal skins.

## MATERIAL

The leper tissues were collected at biopsy from five lepers at the United States Marine Hospital, Carville, Louisiana, with the permission and cooperation of Dr. O. E. Denney and through the kindness of Major S. Simmons, at autopsy by Dr. E. DeCoursey of the Board of Health Laboratory, Ancon, Canal Zone. The former are referred to as L 11 to L 15. The lesion in each was divided into five pieces which were qualitatively alike as far as could be determined

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macroscopically. These were immediately fixed (1) in 10 per cent formalin in absolute alcohol, (2) 10 per cent formalin, (3) Regaud's fluid (3 per cent potassium bichromate 4 parts, and formalin 1 part), (4) formalin-Zenker's for histological examination and (5) frozen with dry ice and kept frozen until studied spectrographically in St. Louis. The latter, designated L 3 and L S 3, were fixed in the formalin-alcohol mixture and mailed to St. Louis therein. On arrival equivalent parts were used for histological and spectroscopic examination. It is necessary to specify the source and the histopathology of the particular lesions studied spectroscopically because others of different nature may have a different mineral content.

L 11. Patient P. A. 1049, Mexican, male, 42 years of age, mixed leprosy with cutaneous type predominating, 4 years duration with no treatment. *Biopsy:* removal of the ear lobules.

*Epidermis:* Slight hyperkeratosis; 35–75 $\mu$ thick, average 45 $\mu$. Considerable pigment. No leukocytic invasion. No epithelial pegs.

*Papillary Layer:* Almost obliterated owing to absence of pegs and flattening of dermal papillae. The remains of the layer constitute the subepithelial marginal band of Unna.[5] The thickness of this band varies from 36–40 $\mu$. It is made up of collagenic fibers with a few fibroblasts between them. The elastic fibers, normally present in the papillary layer, are much reduced in number and no traces remain of the sense organs of the dermal papillae in any of these leprous specimens. The band has a blood supply greatly reduced from that of the original papillary layer, but it is separated from the underlying reticular layer by a zone of moderately distended vessels. The relative immunity of this subepithelial marginal band to penetration by the leproma cells, emphasized by Unna, holds for this and other specimens in our series.

*Reticular Layer:* Much thickened by a leprous nodule, which is only partly divided into lobules by septa of connective tissue (seldom more than 75 $\mu$ wide), blood vessels (about normal in size), many sebaceous glands and hair follicles. The latter are slightly atrophic. The nodules are made up chiefly of histiocytes, foam cells, lymphocytes, fibroblasts, polymorphonuclear leukocytes, tissue mast cells and tissue eosinophiles, cited in order of frequency. The term "plasma cell" is used in the sense of von Marschalkó,[6] which is different from that of Unna, who employed it as synonymous with "protoplasmic cells," which clearly include histiocytes (macrophages, monocytes, epithelioid cells, and so on) as well. No giant cells like those illustrated by Wade's [7] Figure 12 were seen in this or in specimens from any of the other cases. Bacilli are numerous in the histiocytes as typical "cigar packs," or intracellular globi. Globi of the same sort are found in a few fibroblasts and occasionally in vascular endothelial cells. Bacilli, not clumped as globi, are occasional in the outer cells of sebaceous glands, duct cells of sweat glands and in the tissue fluid. Much larger, roughly globular masses of bacilli, which are not intracellular but intercellular and have been called "giant globi" by Unna, are rare. Denney has commented upon them in

a recent paper.[8] We hope to present in another communication our views as to their nature, relation to the small intracellular globi, and the whole very important question of lymphatic involvement. Here we are concerned only with their occurrence and size in an attempt to grade the tissues. In L 11 they were not numerous and the largest had a maximum diameter of 15 $\mu$.

Only a small amount of *subcutis* is included in the sections. Contrasted with other specimens, nodule formation is the least advanced, giant globi least frequent and the normal structure of the skin least disturbed. Conversely, L 11 exhibits more fatty areolar tissue, well formed sebaceous glands, and in the nodules a higher percentage of tissue mast cells and plasma cells than any of the others.

L 12. Patient W. M. 619, white, male, 30 years of age, mixed leprosy with cutaneous type predominating, 10 years duration under routine chaulmoogra oil treatment. *Biopsy:* removal of nodule on forearm.

*Epidermis:* Atrophic, tendency to hyperkeratosis; 30–135 $\mu$ thick, average 95 $\mu$. Little or no pigment. Slight leukocytic invasion. Epithelial cells swollen, increase in size approximately 33 per cent. No epithelial pegs.

*Papillary Layer:* Ironed out owing to lack of pegs and dermal papillae. The subepithelial marginal band is 10–150 $\mu$ wide, much less dense than in L 11 and limited internally by a zone of dilated vessels.

*Reticular Layer:* The leprous nodule is of fairly uniform consistence, since it is not broken up by sebaceous glands, hair follicles, fatty areolar tissue or large bands of connective tissue; for all these are absent. The sweat glands are atrophic but the blood vessels are not noticeably changed. The nodule is more cellular and less fibrous than in L 11. The connective tissue increases gradually as the subcutis is approached and becomes disposed in strata and whorls. In the nodule, intercellular spaces are marked, particularly near the external margin, suggestive of much tissue fluid, more indeed than in any of the other specimens. The most abundant cells in the nodules are histiocytes. There are many foam cells, a few lymphocytes and occasional polymorphonuclears. Plasma cells are rare and no tissue mast cells or tissue eosinophiles are seen. Bacilli are abundant as small intracellular globi in histiocytes and free in the tissue. Giant globi are fairly numerous. The walls of the lymphatics, which contain them, show more marked hyperplasia than in any of the other specimens except L 3, so that when cut at an angle they simulate giant cells. No true polykaryocytes are seen. The *subcutis* is not included in section.

L 13. Patient F. H. 971, Mexican, male, 36 years of age, with marked cutaneous leprosy of 5 years duration. Has had no treatment. *Biopsy:* removal of nodule on forearm.

*Epidermis:* 20–80 $\mu$ thick, average 35 $\mu$. In one area, about 1 mm. wide, it is distinctly atrophic being approximately 20 $\mu$ thick. The cells are largest and vacuolated where the epithelium is thickest. Much pigment. No leukocytic invasion. No epithelial pegs.

*Papillary Layer:* Almost flattened out by absence of epithelial pegs and great reduction in size of dermal papillae, which only penetrate epidermis to depth of 20 to 50 $\mu$ and are not numerous. The subepithelial marginal band is about half the thickness of the overlying epidermis. It is mostly collagenic, but in its outer lamina rather more elastic fibers are demonstrable by resorcin fuchsin than in the other cases. A few pigment cells are present in the band.

*Reticular Layer:* Thickened, by growth of leprous nodule which is not much separated into lobules by connective tissue or epithelial derivatives. No sebaceous glands or hair follicles. On the average there is only one duct of sweat gland per section, the lumen of which is almost closed. In the nodule, histiocytes are most numerous, plasma and foam cells are rare, the blood vessels are open with normal walls. No tissue mast cells or eosinophiles were seen but long search was not made. Bacilli are quite granular, but very numerous especially as small globi intracellular in histiocytes. There are many giant intercellular globi. The largest was 50 $\mu$ in diameter (measured in 10 per cent formalin-fixed material). Only one instance of proliferation of lymphatic endothelium leading to pseudo-giant cell formation was noted.

*Subcutis:* Only partly included in section. No fatty areolar tissue anywhere in specimen.

L 14. Patient F. V. 514, white, male, 35 years of age, mixed type of leprosy with cutaneous lesions predominating, 19 years duration, routine treatment with chaulmoogra oil. *Biopsy:* removal of nodule from nape of neck.

*Epidermis:* Atrophic and smooth; 25–40 $\mu$ thick, average 30 $\mu$. Small amount of pigment. No leukocytic invasion or pegs.

*Papillary Layer:* This is practically absent, because the pegs and dermal papillae are lacking. The subepithelial marginal band is narrower and less pronounced than in any other specimens of the series. It varies in width from 10–25 $\mu$. In some cases the cells of the nodule come into direct contact with the inner surface of epithelium. But in that portion fixed in alcohol-formalin the band is wider and more like tissue from the other cases.

*Reticular Layer:* Enlarged by nodule formation. Not broken up into lobules by hair follicles, sebaceous glands or sweat glands; for these are absent, except in the alcohol-formalin-fixed piece in which there are two small sebaceous cysts. It consists of histiocytes, foam cells in moderate numbers, a few lymphocytes, plasma cells, and occasional polymorphonuclears together with fibroblasts and connective tissue fibers disposed in thin bands. There are no tissue mast cells or eosinophiles. Bacilli are very numerous, free and in small globi. Giant globi are more frequent in the Regaud-fixed fragment than in specimens from any of the other cases, but in other pieces from this case they are not unusually abundant. No *subcutis* is available. No fatty areolar tissue or giant cells are seen.

L 15. Patient J. P. 1050, Mexican, male, 30 years of age, mixed type of leprosy with cutaneous lesions markedly predominant, 8 years duration with no treatment. *Biopsy:* removal of nodules from face.

*Epidermis:* Atrophic, 30–55 $\mu$ thick, average 40 $\mu$. It contains considerable pigment. Leukocytic invasion is very rare and there are no pegs.

*Papillary Layer:* Reduced by absence of pegs and dermal papillae. The subepithelial marginal band is 30–100 $\mu$ thick and chiefly collagenic. The most dilated venules in the series are just internal to the band but the number of vessels is not increased.

*Reticular Layer:* Greatly increased in thickness by nodule formation. The nodule is of fairly uniform consistence, being very little broken up by sebaceous glands and hair follicles which are distinctly rare. Connective tissue bands are but feebly developed and there is no fatty areolar tissue except in the fragment fixed in formalin-Zenker in which the included lesion is less severe. The nodule in all specimens of L 15 is made up of histiocytes, with plasma cells more numerous than in any of the other cases except L 11, lymphocytes, foam cells and a few tissue mast cells; but only one multinucleated giant cell much vacuolated and about 35 $\mu$ in maximum diameter was seen. Bacilli are numerous, free, in small globi and in giant globi. The latter, though not particularly frequent, are the largest seen in the series of leprosy specimens. One was oval in shape in section with maximum diameter of 125 $\mu$ and minimum of 108 $\mu$. In general, tissue from this case is rather like that of L 11.

## L 3. Autopsy No. 10563, Board of Health Laboratory, Ancon, Canal Zone.

*Epidermis:* 20–80 $\mu$ thick, average about 50 $\mu$. Heavy pigmentation. No leukocytic invasion. Inner surface is very uneven owing to extension of numerous short (20–120 $\mu$, average 70 $\mu$), pointed epithelial pegs — a feature more marked in this than in any of the other specimens.

*Papillary Layer:* Atrophied as compared with normal, but not ironed out, like many of the others, because dermal papillae alternate with the pegs. It contains more than the usual number of pigment-holding cells. The subepithelial marginal band is indistinct. Increase in collagenic fibers is only moderate and decrease in elastic fibers is limited to approximately the inner half of the reticularis. Moreover, the thickness of the band is variable (30–100 $\mu$, average 70 $\mu$) depending on the proximity of the nodules to the epithelium.

*Reticular Layer:* Contains layer of relatively small flattened nodules, each having a length (parallel to epidermis) of about 150–800 $\mu$ and maximum thicknesses of 40–300 $\mu$. Between them stretch bands of connective tissue, blood vessels, a few nerve fibers and the ducts of sweat glands. The nodules are backed internally by a feltwork of thick strands of connective tissue containing many elastic fibers. In the feltwork are nodules of the same and larger size which extend toward the subcutis as far as the section goes, namely 3–4 mm., and are accompanied by fatty aveolar tissue in amount only slightly less than normal. The composition of the nodules differs. Those of the outermost layer are made up for the most part of foam cells and histiocytes, the former being most numerous. Lymphocytes and plasma cells are scarce. Only two tissue mast cells and no eosinophiles were seen. In the deeper nodules there are with the foam cells, a fair number of lymphocytes and plasma cells, some fat, many giant globi attaining a maximum diameter of 50 $\mu$, and numerous giant cells whose maximum diameter is about 70 $\mu$ and whose largest number of nuclei in a 6 $\mu$ section is 10 per cell. In some cases, the giant cells are merely sections through hyperplastic

walls of lymphatics in which cell boundaries are lost. Bacilli in both superficial and deep nodules are granular and fragmented.

L S 3 is from the same autopsy as L 3, selected because on gross inspection it showed so few signs of leprous change that the results of spectrographic examination would be significant as compared with L 3.

*Epidermis:* Similar.
*Papillary Layer:* Similar but slightly more elastic tissue remaining and tissue mast cells distinctly more numerous.
*Reticular Layer:* Outer layer of small nodules similar. Deeper nodules less than half as large. The cytology of the nodules is similar, except that there are fewer giant globi and giant cells than in L 3.

The normal tissues consisted of pieces of skin removed from the upper left chest of five white cadavers, age about 60 years or more. These are known as N 1 to N 5 inclusive. In addition, skin from three individuals was analyzed chemically for P, Na and Ca by Miss C. C. Buhrmester, as well as studied spectrographically. The specimens were from the abdomen of a male and female cadaver of about the same age as N 1 to N 5 and from the chest of a male, aged 53, who died of empyema (our Department of Pathology Autopsy No. 6184), obtained through the courtesy of Dr. H. L. McCordock. These are called M, F and A, respectively. We are grateful to Dr. R. J. Terry for permission to collect skin from the cadavers.

## Spectrographic Analyses

### (*A*) *Handling of Material*

The method of obtaining the spectra of the materials has been fully described elsewhere.[1, 2, 3] It consists essentially of burning 2–4 cmm. (per spectrum of 15″ exposure) of tissue in a high frequency spark and photographing the emission spectrum with a small quartz spectrograph. The material to be burned was first cleansed of subcutaneous fat by scraping with a knife, then cut in strips about 2 mm. square, and up to a centimeter or two in length, in the case of normal skin. With the leprous lesions, however, the strips were necessarily somewhat shorter. They were held in small pyrex glass tubes for introduction into the spark. The portion of each leper lesion selected was always immediately adjacent to those examined histologically. With each of the normal skin cases M, F and A, the

strips for the spectrographic record were taken at three different places from the rather large area of skin (25 to 100 sq. cm.) used for the chemical analysis. The whole area of skin was cleansed of subcutaneous fat before the strips were cut.

The spectra, in general, show spectral lines characteristic of Ca, K, Na, Cu, Mg, Fe, P, Si, C and occasionally Ag. These lines are visible on the accompanying plates, which exhibit representative portions of the sets of spectra obtained for the materials employed. Beside the spectra are listed the designations employed in the description of material. Only the lines actually measured up are indicated — for the others, see previous papers.[1,2,3] Also very evident are the continuous bands due to the passage of the spark through the air.

The density (= $\log_{10}$ opacity) of one line each for P, Na, Ca, Mg and Fe was measured with an electrical densitometer [4] in each spectrum examined. The results were then averaged for each kind of tissue. For instance, the four spectra available for leper Case 11 yielded four values for the P line density, and the average of these gave the P line density characteristic of the case. The results of such treatment of all the densitometer readings are given in Table I, which indicates also the number of spectra measured for each material. All spectra were taken with the same exposure time (15 seconds) and on the same type of plate (Eastman 50).

### (*B*) *General Quantitative Estimates*

The working hypothesis is: If in the spectra of two similar tissues A and B, the Mg lines are of about the same density, while the P lines of A are much more dense than those of B, it can be concluded that the P/Mg ratio is greater for A than for B. Any other two elements can be taken, besides Mg and P. It is not sufficient to fix attention on the lines of only one element, since errors would ensue from the considerable variations in the rate of consumption of material by the spark. It is assumed that the spectra to be compared are taken on the same type of plate and with the same exposure time, and given as nearly as possible the same development. The spectra here considered do not very well satisfy the development requirement, since they were taken over an interval of some months without particular reference to the scheme of presentation now employed. However,

the relations to be pointed out are sufficiently pronounced not to be invalidated by this criticism.

The hypothesis requires that with a given general type of tissue (for example, skin, which burns brightly, as distinct from fat, which melts and sputters in the spark) the line intensity increases with increasing amounts of the element in the tissue. With the low metallic element concentrations encountered in nearly all tissues, and with the large amount of organic material present which tends to "ballast" spark phenomena against alteration due to changes in metallic content, self-reversal and quenching of the spectral lines of the elements of interest should be slight; consequently, the requirement should be satisfied.

The outstanding difference between leper and normal tissue seems, for the cases at hand, to concern calcium and phosphorus. From Table I the average Ca line density for leper Cases 11–15 is 0.65, while for the eight normals it is 0.76; but the P relations are in the other direction — leper 0.67, normal 0.44. Hence we conclude the P/Ca ratio is larger for leper lesions than for normal skin. This can be seen in comparing Figures 1 and 2 for relative P and Ca line strengths. Almost the same numerical relations exist for the Ca and Fe line densities, and hence the same conclusion follows for the Fe/Ca ratios, see again Figures 1 and 2. While inspection of the Ca and Mg densities indicates a tendency for the Mg/Ca ratio to be greater for the leper material, the differences are perhaps not large enough to establish anything in view of the large variations observed from sample to sample of the same type of material, and in view of possible errors arising from the fact that the spectra concerned were on various plates. Likewise, the Na/Ca ratios show only a tendency to be greater in the leper material.

Leper Case 3 is of special interest. Here we have spectra of two lesions of the same type but of different severity from the same patient on the same plate. Further, both samples were taken at autopsy. Thus the comparison is free from certain of the qualifications which apply to those already mentioned. In the first place it is very evident, not only from Table I, but also from Figure 1, that the P/Ca ratio is greater for the heavier lesion (L 3) than for the lighter one (L S 3). The Mg/Ca ratio shows again the tendency in that direction, as does the Na/Ca ratio. But the Fe/Ca ratio seems to be the same in both.

This last point, perhaps, accounts for the high Fe/Ca ratio in leper Cases 11–15 as compared with the normals; the former samples were taken at biopsy, the latter generally months after death, while both samples for Case 3 were taken at autopsy at the same time. Since the spectrum of whole blood (Scott and Williams,[2] Figure 4, Spectrum 1) has been found to be extremely rich in Fe from the hemoglobin, it is probable that in spite of the low vascularity of leper lesions enough blood was retained after biopsy to account for the

TABLE I

*Mean Line Densities for Spectra of Leper and Normal Tissues*

| Case | Number spectra | Line densities | | | | |
|---|---|---|---|---|---|---|
| | | Ca | Mg | P | Fe | Na |
| Leper L 11 | 4 | 0.73 | 0.51 | 0.62 | 0.76 | 0. 3 |
| L 12 | 4 | 0.54 | 0.51 | 0.66 | 0.51 | 0.82 |
| L 13 | 4 | 0.70 | 0.55 | 0.66 | 0.82 | 0.91 |
| L 14 | 3 | 0.58 | 0.51 | 0.66 | 0.66 | 0.82 |
| L 15 | 3 | 0.69 | 0.64 | 0.77 | 0.66 | 0.89 |
| Average | | 0.65 | 0.54 | 0.67 | 0.68 | 0.85 |
| Normal $N_1$ | 4 | 0.73 | 0.53 | 0.38 | 0.34 | 0.78 |
| $N_2$ | 4 | 0.79 | 0.46 | 0.36 | 0.41 | 0.74 |
| $N_3$ | 4 | 0.63 | 0.44 | 0.40 | 0.32 | 0.84 |
| $N_4$ | 4 | 0.67 | 0.34 | 0.27 | 0.31 | 0.70 |
| $N_5$ | 4 | 0.87 | 0.48 | 0.41 | 0.38 | 0.72 |
| M | 12 | 0.86 | 0.44 | 0.72 | 0.71 | 1.04 |
| F | 12 | 0.93 | 0.56 | 0.67 | 0.73 | 1.07 |
| A | 10 | 0.68 | 0.37 | 0.36 | 0.38 | 0.88 |
| Average | | 0.76 | 0.45 | 0.44 | 0.45 | 0.85 |
| Leper L 3 | 10 | 0.96 | 0.83 | 0.87 | 0.76 | 0.92 |
| L S 3 | 10 | 0.94 | 0.73 | 0.58 | 0.73 | 0.75 |

strong Fe lines in the leper spectra, particularly as the samples were immediately frozen in solid $CO_2$ rather than preserved in a liquid fixative. It should be remembered, also, that the normals were taken from the chest or abdomen in cadavers which had been hanging vertically for some time, and signs of extensive blood drainage into the lower portions of the bodies were evident.

Finally it is instructive to apply the above method of interpretation, for the P/Ca and Na/Ca ratios, to the normal skin cases M, F and A, for which these ratios were determined chemically (Table II, columns 5 and 7). Referring again to Table I, Ca (M) < Ca (F),

while P (M) > P (F); hence, P/Ca for M is greater than for F, which checks the chemical results. The comparison of A with either M or F is not quite of the same sort, and must be handled differently. We may note that the difference of the P and Ca line densities for A, (0.36 − 0.68 = −0.32) is less algebraically than the corresponding difference for either M or F (− 0.14 and − 0.26). Since the smaller the amount of P present in proportion to the Ca, the smaller should be this difference, we again check the chemical results. A similar treatment holds for the Na/Ca relation. The differences in Na and Ca line densities for M, F and A are respectively 0.18, 0.14 and 0.20 which are in the same sequence as the chemical findings 14.1, 10.5 and 18.1 for the Na/Ca ratios.

### (*C*) *Numerical Estimates*

In the foregoing we used the difference in the P and Ca line densities for a particular kind of tissue as an index for the P/Ca ratio in that tissue, and similarly for Na/Ca. The qualitative validity of this choice of index was shown by the agreement obtained for the normals M, F and A with the chemical findings. We shall now correlate the indices for M, F and A numerically with the chemical results, and by graphical methods find the P/Ca and Na/Ca ratios for the other materials concerned.

As previously remarked, the spectrographic plates employed differed somewhat in development, and possibly also in regard to the intensity (more specifically, number of sparks per second; the nature of each was probably constant) of the analyzer spark during the period in which they were taken. It was desirable to correct for these influences as much as possible before making numerical estimates. From consideration of the probable spark phenomena, and of the behavior of the characteristic (density-versus-intensity) curves for the photographic plates with varying degrees of development, it seemed plausible to fix upon the density of some particular portion of the airband system, averaged for all the spectra on a plate, as a means of such correction. An airband density was found, then, for each plate. The mean for all the plates concerned was determined. This mean value divided by the value for a particular plate gave a factor by which all line densities for that plate were multiplied. These factors were applied to give the corrected, and somewhat ab-

breviated, set of line densities shown in Table II, columns 1, 2 and 3.

The factors ranged from 0.77 for the plate with the M and F spectra to 1.31 for the N 1 to N 5 plate. No great claims are made for the precise accuracy of these corrections, but it is hardly to be doubted that they are in the right direction: they work proportionate increases in line densities for the fainter plates and proportionate

TABLE II

*Mean Corrected Line Densities for Spectra of Leper and Normal Tissues, and Computed Element Ratios. The Ratios Marked * were Determined Chemically*

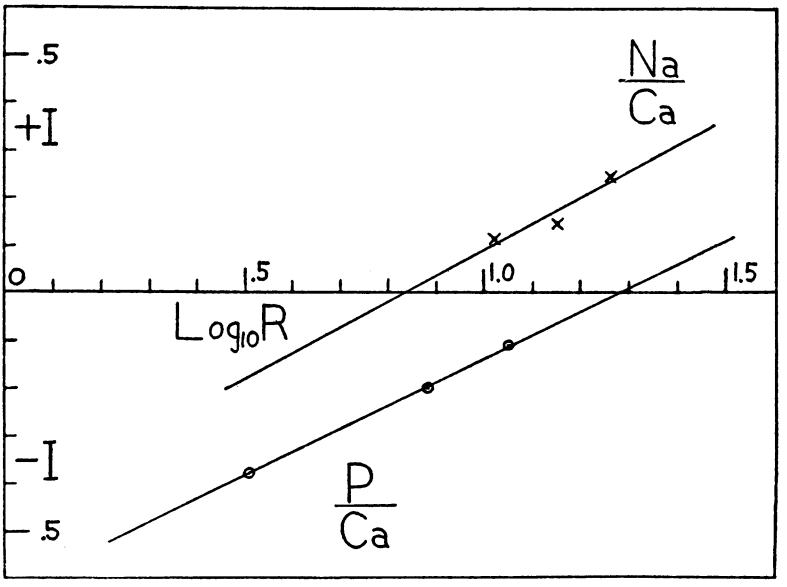
| Case | Corrected line densities | | | P/Ca | | Na/Ca | |
|---|---|---|---|---|---|---|---|
| | P | Na | Ca | $I_1$ | $R_1$ | $I_2$ | $R_2$ |
| Leper L 11 | 0.66 | 0.88 | 0.76 | −0.10 | 12.00 | 0.12 | 11.50 |
| L 12 | 0.69 | 0.86 | 0.59 | 0.10 | 30.90 | 0.27 | 21.40 |
| L 13 | 0.70 | 0.94 | 0.74 | −0.04 | 15.80 | 0.20 | 16.20 |
| L 14 | 0.68 | 0.85 | 0.60 | 0.08 | 28.20 | 0.25 | 20.00 |
| L 15 | 0.79 | 0.91 | 0.72 | 0.07 | 26.90 | 0.19 | 15.50 |
| Average | | | | | 22.70 | | 16.90 |
| Normal average $N_1$–$N_5$ | 0.47 | 0.99 | 0.96 | −0.49 | 1.90 | 0.03 | 7.95 |
| Normal M | 0.55 | 0.80 | 0.66 | −0.11 | *11.30 | 0.14 | 14.10* |
| F | 0.52 | 0.83 | 0.72 | −0.20 | * 7.55 | 0.11 | 10.50* |
| A | 0.44 | 1.06 | 0.82 | −0.38 | * 3.25 | 0.24 | 18.10* |
| Average | | | | | 3.95 | | 10.30 |
| Leper L 3 | 0.85 | 0.90 | 0.94 | −0.09 | 12.60 | −0.04 | 5.90 |
| L S 3 | 0.57 | 0.73 | 0.92 | −0.35 | 3.70 | −0.19 | 3.00 |
| Column | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

decreases for the blacker ones. Furthermore, the general quantitative estimates are unaltered by the corrections.

Column 4, Table II, gives the differences between the P and Ca corrected line densities for the various materials, which are taken as indices $I_1$ for the P/Ca ratios $R_1$ of the materials. For three of these materials (M, F and A) we know $R_1$ from chemical measurements. Now, to a rough approximation, spectral line density is proportional to the logarithm of the intensity of the light producing the line; and, for minute quantities of an element, the intensity should be proportional to the amount of the element entering the spark. Thus, to get

a curve for purposes of computation, $I_1$ is plotted against $\log_{10} R_1$ in Graph 1 for M, F and A, the points being shown as circles in the graph. A straight line is drawn as nearly through the three points as possible. Then the $R_1$ values for the other materials are secured by fitting their $I_1$ values to this straight line. For instance, $I_1$ for leper Case 11 is $-0.10$; the corresponding $\log_{10} R_1$ value is given by the graph as 1.08, and the antilog of this is $12.0 = R_1 = P/Ca$ for Case 11. The other cases are handled in the same way.

The indices $I_2$ for Na/Ca are given in column 6, Table II; and their $R_2 = \frac{Na}{Ca}$ values are computed as above from the upper Na/Ca line in Graph 1, and are given in column 7, Table II. The crosses are the points from cases M, F and A, which establish the line.




GRAPH 1. Differences (I) in corrected line densities in terms of element ratios (R).

For obvious graphical reasons an R value is likely to be most accurately determined when the degree of extrapolation is least, *i.e.* when its I values fall within the range covered by those of cases M, F and A. The corresponding R ranges for these 3 cases are about 3–12 for P/Ca, and 10–19 for Na/Ca; so, in general, more re-

liance should be placed on computed R values within, or close to, these limits than on values far outside them. However, the P/Ca calibration points are sufficiently separated to permit plausible extrapolation for, perhaps, all of the $R_1$ values given.

## Discussion

Herman Brown [9] has determined, among other things, P, Na and Ca for a series of normal human skins by chemical means. The samples were taken at autopsy, freed from subcutaneous fat by scraping and cover an age range from the fetal stage to 82 years. The location is not given. There are other figures on Na and Ca by the same author in an earlier paper [10] for skin "from the ventral region between the clavicles and the symphysis pubis." The ratio P/Ca from his figures ranges from 2.6 to 5.5 for the age group 60 to 80 years; our Table II gives 3.95 for the mean of the eight normals. For Na/Ca in the same age group Brown's figures range from 9.2 to 11.7; Table II gives 10.3 for the normal average. The P/Ca (but not the Na/Ca) for L S 3 also agrees with these chemical results.

These agreements with previously published findings fortify our P/Ca and Na/Ca results for leper Cases L 11 through L 15. The mean P/Ca for the 5 cases is 22.7; Brown's normal results for the age group (30 to 42 years) concerned range from 4.6 to 6.8 and average about 6.0. We may also note that $22.7/6.0 = 3.8$ happens to be comparable with $12.6/3.7 = 3.4$ for L 3 and L S 3. Thus it seems safe to conclude that the P/Ca ratio for the cases at hand is around three times higher in well developed leper lesions than in normal skin. As to Na/Ca, the average 16.9 for the leper lesions is within the range for the corresponding age group as found by Brown (12.0 to 20.0, average around 14.5). It should be noted that the small difference in Na/Ca ratios mentioned in (*B*), and further shown in column 7, Table II, was real enough for the samples at hand; it simply becomes of no consequence when the large variation of Na/Ca with age, as established by Brown's figures, is taken into account.

Consideration of the location of the leprous lesions brings in, however, a factor which we have been unable to check. In the five biopsies they were removed from the ear lobule, forearm, forehead, nape of the neck, and face. These are all exposed parts of the body in contrast with the areas ordinarily covered with clothes, from which we

removed our supposedly normal skin samples. We have not been able to find any data in the literature as to the presence or absence of a difference in the P/Ca ratio of exposed and unexposed parts of the body under normal conditions. Our own attempts to secure samples of normal skin at autopsy from the same exposed areas for spectrographic analyses have not been successful because of the mutilation involved in collecting them. But we think it very unlikely that the high P/Ca ratio in our leprosy cases is due primarily to a regional difference in chemical composition of the skin.

The available evidence points to the conclusion that the deviation from the normal of the ratio is related to the length of time which elapsed since the leprous condition was first diagnosed clinically. Reference to Table II, column 5, shows that this ratio is high in L 12, 14 and 15 in which the disease had been established for 10, 19 and 8 years and lower in L 11 and 13 of 4 and 5 years duration. But this may not mean so much because there is no assurance that the particular lesions studied were the first detected. In other words, those with unusually high P/Ca ratios may have developed comparatively recently in persons in which the disease had already been well established elsewhere. Brown has found considerable variation in ratio values for undiseased skins from cases of approximately the same age. However, an accidental correlation due to natural variability is improbable where 5 cases are concerned even though the correlation does not involve, for instance, a direct proportionality between ratio and duration.

A high P/Ca ratio signifies either an increase in P relative to Ca, or a decrease in Ca relative to P, or both. We accordingly attempted to discover whether a correlation exists between a high P/Ca ratio and a high percentage in volume of fatty aveolar tissue, sebaceous glands and true leprous nodule consisting of cells charged with bacilli or with products of their degeneration (foam cells) as compared with other components making up most of the remaining bulk of the skin, namely: epidermis, hair follicles, sweat glands, connective tissue, blood vessels and tissue fluid all grouped together.

Obviously the calculations leave much to be desired from the point of view of accuracy for they were based entirely on the impressions gained by repeated microscopic comparisons of the tissues. Another consideration must be mentioned that may detract from their value. The fifth piece of tissue, into which each specimen removed at bi-

opsy was divided, was frozen and again subdivided, part being used for spectrographic analysis and part for further histological control. The last named was not so well preserved for histological examinations as the four others intentionally fixed for this purpose and, since in L 14 and 15 there was a qualitative difference in the pieces, as already described, it is possible that the tissue burned was not always absolutely comparable to those on which our histopathological account is based.

However this may be, L 12 with highest P/Ca ratio (30.9) showed no fatty areolar tissue; whereas L 11, with lowest P/Ca ratio (12.0), had more than L 15; while L 13 and 14 possessed no demonstrable fatty areolar tissue. Lack of correlation with volume of sebaceous glands was likewise evident. No sebaceous glands were found in tissue from L 12. These structures were most marked in L 11. But the relative volume of leprous cells was noticeably greater in L 12 with the highest ratio than in L 11 with the smallest one. Moreover, in L 11 the bacilli were less numerous and giant globi less conspicuous than in other members of the series. The other cases (L 13 and 14) were difficult to grade and showed no definite correlation of the same sort. Our findings in tissues of L 3, which had to be kept separate from the others because they alone of the leprous tissues were removed at autopsy, suggest, however, the same correlation because the ratio was higher in L 3 with large deep nodules than in L S 3 with smaller ones.

Unfortunately there are no data in the literature on the actual richness in P of leprous nodules of small size dissected free of surrounding tissue. It is merely an assumption that they contain much P and that our correlation between large total volume of cells containing bacilli and their products and high P/Ca ratio partly explains the height of the ratio. We have examined frozen and alcohol-formalin-fixed specimens from the 5 cases by the method of micro-incineration, which is not useful for the demonstration of P, but reveals many mineral constituents including Ca, and have observed that the mineral residue left by the leprous cells is not very extensive. In blood serum Wooley and Ross [11] report, on the basis of chemical analyses, total amounts of Ca and inorganic P which average, for 47 cases of leprosy, well within normal limits as represented by 15 controls. The diffusible Ca, however, in 53 cases averaged considerably lower than in the 15 controls. In a later paper [12] these au-

thors mention the probability that "diffusible calcium" and "available calcium" are essentially synonymous, and advance the opinion that nerve, muscular and bone changes in leprosy may be in part due to this effective Ca deficiency. Perhaps Ca starvation of tissue may be aggravated in the actual foci of leprous infection and may contribute to the abnormally high P/Ca ratios that we have observed.

The absence in our results of a pronounced difference between leper and normal tissue in regard to Mg/Ca ratio is not surprising in view of the considerable chemical similarity of Ca and Mg. That is, a disease condition tending, say, to reduce Ca content might for this reason have an effect at least in the same direction on Mg. As to the Fe/Ca ratios, it might at first sight seem logically unsound to discount the high Fe/Ca ratio in leper tissue as compared with the normals simply on account of the difference in the way samples were taken and because of the behavior of one leper case (L 3).

## Summary and Conclusions

1. The P/Ca ratios in 5 leper cases studied are on the average probably three times those in normal skins from the same age group. The Na/Ca, Mg/Ca and Fe/Ca ratios show no notable variations from the normal.

2. A fair correlation is obtained in the 5 leper cases for P/Ca ratio with known duration of disease and volume of leprous cells in tissue analyzed spectrographically. It may be conditioned by increase in P, decrease in Ca, but probably by both.

3. The method of histospectrography, as developed by Scott and his collaborators, can evidently be used for the study of small pieces of tissue removed at biopsy which would be altogether insufficient in amount to permit of routine chemical analysis by ordinary methods. Once the spectrograms have been taken, essentially the same procedure is employed for the determination of ratios between several elements; whereas the chemical estimation of each element would be different and in some cases very involved.

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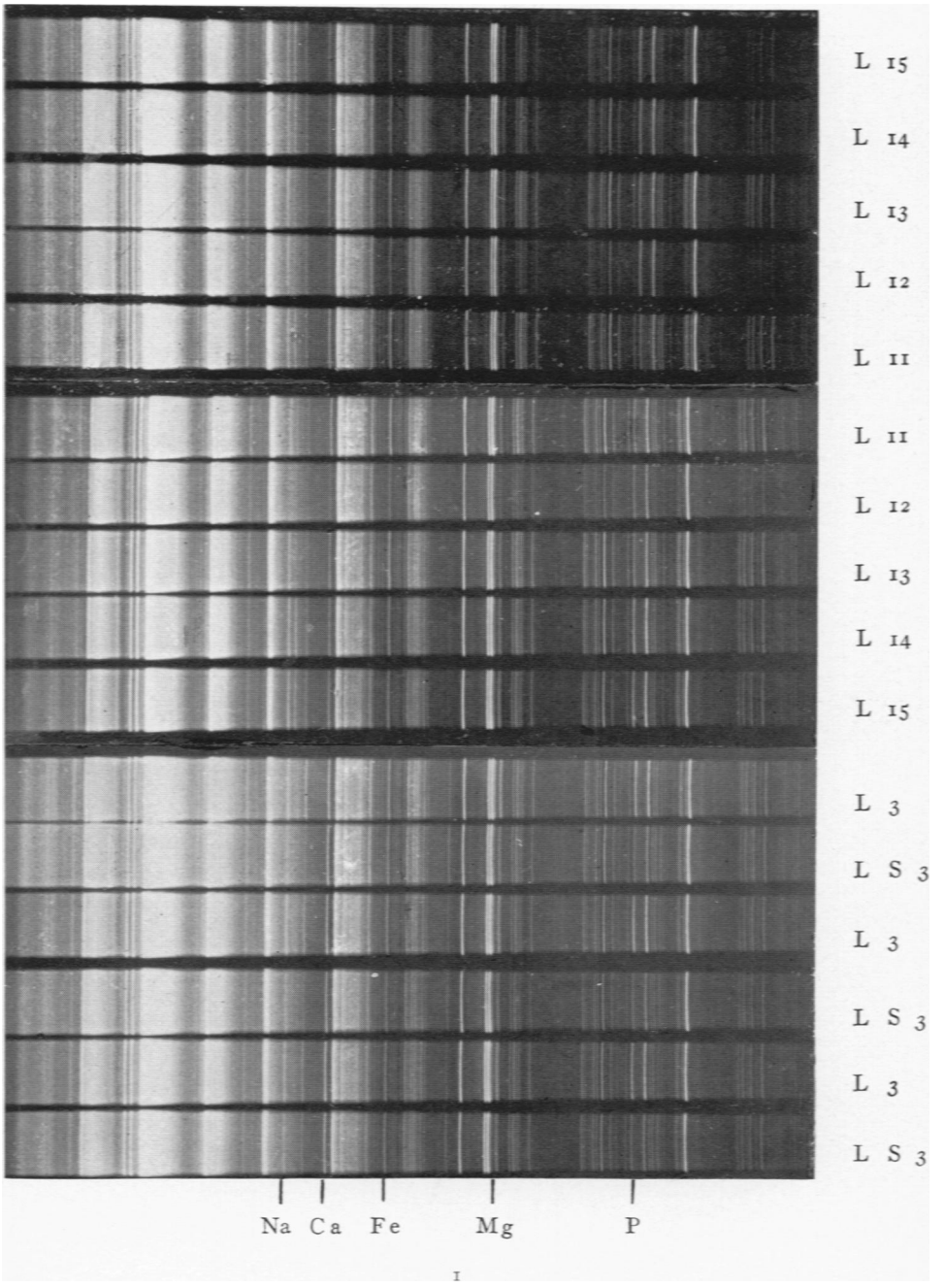
## DESCRIPTION OF PLATES

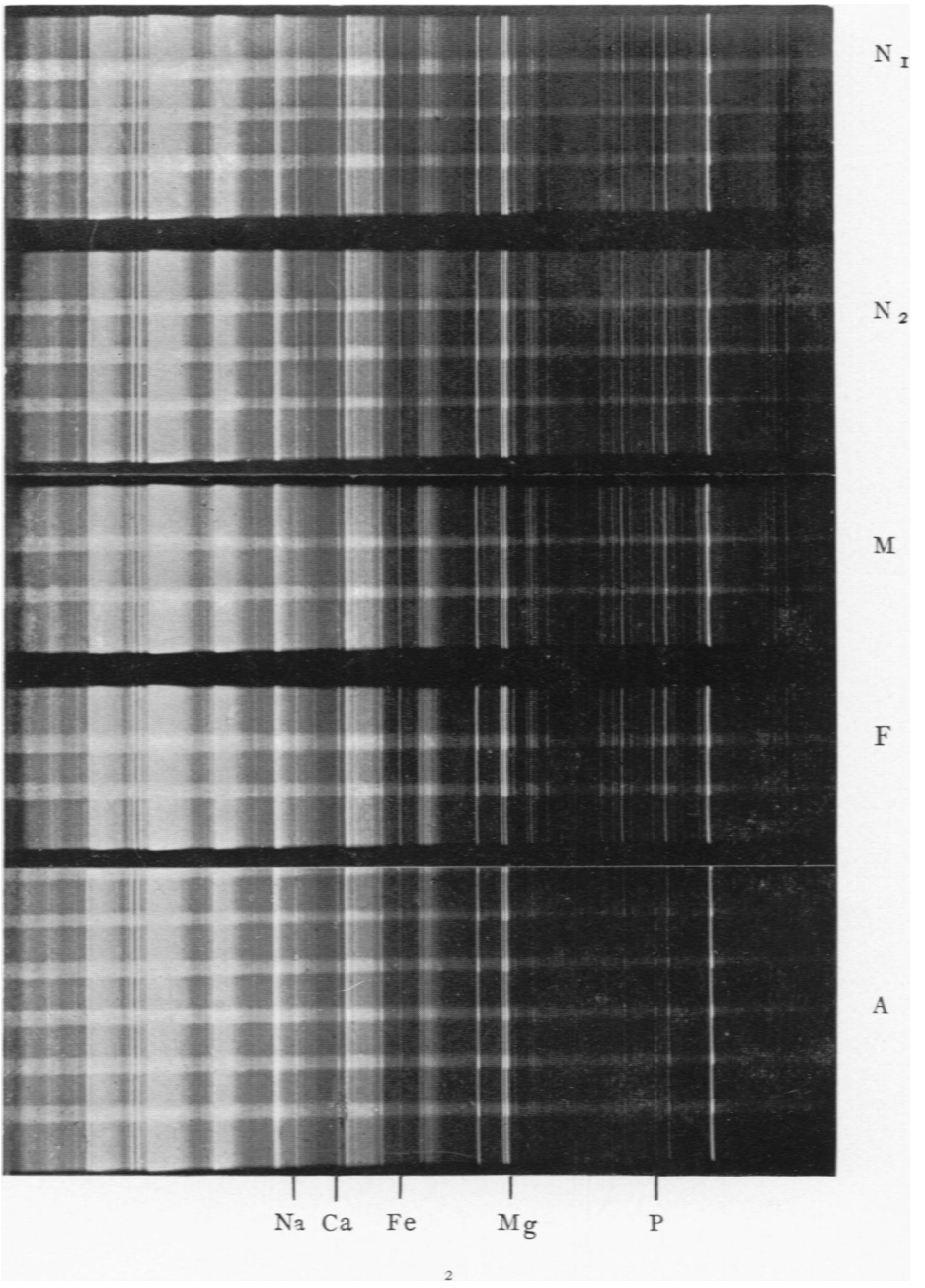
### PLATE 2

FIG. 1. Representative spectra (enlarged) of leprous tissues. The numbers of the cases are indicated in the margin and the lines measured for the particular elements studied are identified below. The two groups of spectra shown for L 11 to L 15 are reproduced from different plates. The spectra from Case 3 (L = heavy lesion and L S = light lesion) are from still another plate. The particular Mg line indicated is visible only with difficulty in these prints. It is immediately to the right of the two very bright lines.


L 15
L 14
L 13
L 12
L 11
L 11
L 12
L 13
L 14
L 15
L 3
L S 3
L 3
L S 3
L 3
L S 3
Na
Ca
Fe
Mg
P
I

## PLATE 3

FIG. 2. Representative spectra of normal tissues for visual comparison with the leprous ones illustrated in Figure 1. Case and line designations are arranged as in Figure 1. N 1 and N 2 are from one plate, M and F from another and A from a third. Note that the phosphorus lines, relative to the calcium lines, are weaker than in Figure 1. This is in accordance with the conclusion reached in the text that the Ca/P ratio is less in leper lesions than in normal tissue.


N 1
N 2
M
F
A
Na
Ca
Fe
Mg
P
2