

TWO NEW CASES OF EPITHELIOMAS ORIGINATING IN LEPTOMAS¹

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This is not the first time that we have seen in Fontilles a patient with epithelioma and leproma occurring together. The two cases reported by Vilanova (³) and by Rubio (⁴) were from this institution. Since then no other case has been observed until within the past year, when the condition occurred in two female patients, both leptomatous. One had a spinocellular epithelioma in consequence of which she died in a short time after admission. The other, at present hospitalized in the sanatorium, has a basocellular epithelioma.

As pointed out by F. Contreras (¹), reported cases of epitheliomas on leptomas are relatively few. Nevertheless, the diagnosis of this association is of importance, so we are constrained to describe these cases. Their clinical features are given first, and then the histologic findings are discussed, beginning with a description of the epithelioma and leproma cells, their biology and interrelationship, and then passing on to the study of the connective tissue fibers and the amorphous basic substance.

CLINICAL STUDY

CASE 1. M.F., female, 63 years old, a meat vendor. Admitted to the sanatorium October 17, 1956, and died February 18, 1957. Married, with 4 healthy children. At the age of 39 she was diagnosed as having leprosy, and since then until her hospitalization she received home treatment with antileprol and DDS, which was well tolerated. At the age of 61 the patient noticed an ulceration on the left cheek, which increased in size and depth. The ulceration, which because of its appearance and bad odor was believed to be of leprosy origin, finally obliged the patient to enter the sanatorium.

At the time of hospitalization the patient was in bad general condition, with severe anemia. The skin, which was very pale, showed on the face and extremities erythematous infiltrations which in places were in regression because of treatment. These infiltrates were clearly leptomatous, and they yielded numerous bacilli, isolated or in globi. On both wrists and hands, and in some places on the face, there were dyskeratotic lesions. The whole cutaneous surface showed a state of gelatinous edema, most marked on the legs. A great ulcero-productive lesion on the face covered part of the forehead and the left eye, which was blinded, and extended over one-half of the face down to the upper lip (Fig. 1). As a whole the new formation was of cauliflower aspect, formed by large and medium-sized fleshy buttons of bright reddish color, continuously oozing fetid fluid and bleeding easily. The borders were elevated, thick, and irregular with many small,

¹ Translation from the Spanish, approved by the authors.

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friable nodulations. The nasal pyramid had disappeared. The patient complained of intermittent pain of moderate intensity, sometimes increasing at night.

The patient was immediately treated for the anemia and given vitamins and antibiotics; and locally a preparation composed of podophyllin, salicylic acid, ethanol and acetone was used as a palliative treatment of the tumor.

At the end of a month there was moderate general and local improvement. With this improvement one could observe the depth of the lesion, which extended to and perforated the soft palate.

The improvement lasted a month and one-half. Then the tumor invasion began to increase alarmingly, producing severe hemorrhages that were not easily controlled. The swallowing of food became very difficult, and the invasion extended to the right eye, causing total blindness. The patient became cachectic, with bronchopulmonary complications, and increased malnutrition not remedied by nutrient enemas, injectable preparations of amino acids, and glucose-saline hypodermoclysis. With intractable cardiovascular decompensation and generalized lymph-node metastases, the patient died four months after admission.

Necropsy could not be performed, and we were limited to obtaining ample fragments of the skin of the face, comprising lepromatous and tumor tissue, for histopathologic examination. A biopsy had previously been made, but with more limited material.

CASE 2. D.G., female, 63 years old, peasant. Admitted to the sanatorium July 24, 1957. She had had leprosy for 9 years, beginning with several "blisters" on the legs. Married, with three children, two of whom are still living and healthy: the other one died at the age of 24 of unknown cause. The father of the patient died at 65 of a tumor with metastatic involvement of the femur.

At the age of 35 the patient noted for the first time a lesion identical with the present one, at the external angle of the right eye but located slightly lower, on the cheek. This was cured by a topical medicament the name of which she could not remember, which at first caused severe local inflammation lasting 15 to 20 days before the lesion healed. Another similar manifestation on the same site appeared when the patient was 55, and this was healed in the same manner. The present lesion began at the age of 62 appearing like the previous ones as a small, elevated and whitish nodule which ulcerated and became covered with a crust. When this crust peeled off the ulceration became broader and repeated formation and peeling off of crusts caused the ulcer continuously to increase in size. She did not complain of pain. She had never received antileprosy treatment.

On admission the patient showed generalized lepromatous infiltration of the entire skin area of the face, neck and extremities. Scars of more or less recent ulcers were present on both forearms. On the backs of the hands and forearms there were several large-sized, highly-pigmented lentigos, but without signs of malignancy. On the external surface of the lower third of the right leg there was a large leprosy ulcer, and on the anterolateral surface of the left leg there was one which occupied almost the entire lower half. These ulcers showed somewhat thickened borders surrounded by a blackish pigmentation. The bases were partly smooth and partly granular, and they secreted an abundant, fetid serous discharge. There were abundant varicose veins on the thighs and legs. Both the cutaneous infiltrations and the ulcers were strongly positive for Hansen bacilli.

With respect to the ulcerative lesion of the face,³ it was located just beyond the external angle of the right eye, extending forward in a two-prong fashion to affect and destroy the upper and lower eyelids in different degrees, the lower one more than the

³ The picture supplied with the manuscript, very small, is not a photograph and cannot be reproduced. The lesion has the appearance of a typical rodent ulcer, with nothing unusual about it except perhaps its location.—EDITOR.

upper. In overall measurement, the lower limb of the ulceration was about 2 cm. long and the upper one 1.5 cm. The base was slightly irregular, slightly elevated and indented internally, whitish in color, and rather hard in consistency. The lesion was not painful to touch.

For histologic examination a biopsy specimen was obtained which included the border of the ulcer and some of the adjacent lepromatous infiltration area.

HISTOPATHOLOGIC STUDY

CASE 1

This description deals with a histologic preparation in which there are both a leproma and a spinocellular epithelioma, the cells of which are well differentiated.

The epidermal epithelium shows slight hyperkeratosis and atrophy of the papillae in the nontumor areas. At different points of the epidermis there is marked mitotic activity with evident nuclear abnormalities in the germinal and spinous strata of the mucous body of Malpighi. Extraordinarily numerous epithelial cords invade downward into the dermis, pointing in all directions (Fig. 2). The nuclei of the cells of these tumor cords are entirely atypical, and sometimes monstrous (Fig. 3). The cells show a tendency to keratinization (Fig. 4), which sometimes produces true cornified bodies. By oil immersion, the epithelial fibrils are seen to be well developed. In the nontumor part of the dermis there can be distinguished some areas which, because they have not been invaded by the leproma, are now showing defense or reaction against the epithelioma by means of a typical stroma reaction. On the other hand, lepromatous areas which have to react against the tumor have developed a stroma reaction with certain peculiarities. In parts remote from the tumor a typical conglomeration of small lepromas are to be found.

DESCRIPTION OF PLATES

FIG. 1. Case 1, M.F. Spinocellular epithelioma originating in lepromatous tissue.

FIG. 1. Case 1. Tumor cords invading the dermis, with stream reactions and lepromatous tissue. (Hematoxylin-eosin.)

FIG. 3. Case 1. A typical nucleus of the spinocellular epithelioma. (Hematoxylin-eosin.)

FIG. 4. Case 1. Tendency to formation of whorls of cornified cells in the tumor cords. (Hematoxylin-eosin.)

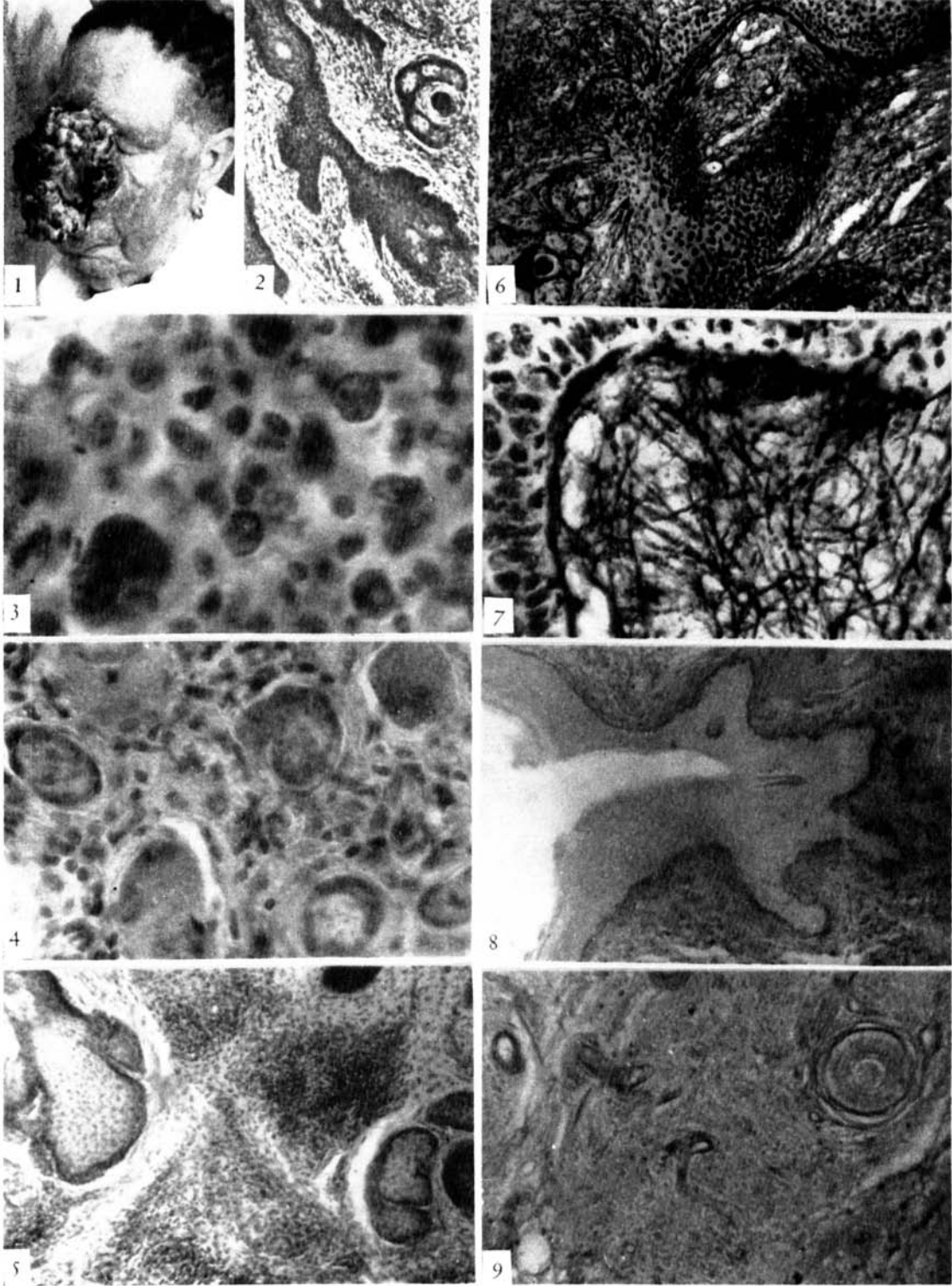
FIG. 5. Case 1. Stroma reaction in the lepromatous tissue. Round cells predominate; some polymorphonuclears are present. (Hematoxylin-eosin.)

FIG. 6. Case 1. Disorganized and broken reticular fibers, with new formation in places. (Double impregnation of Del Rio Hortega.)

FIG. 7. Case 1. A high-power photomicrograph of the section shown in Fig. 6.

FIG. 8. Case 1. Basal membrane of the epidermis, in an area where there are neoplastic downgrowths. Note the fidelity with which the membrane accompanies the epithelial growth. (PAS.)

FIG. 9. Case 1. A much-infiltrated area where the PAS reaction is practically negative. Note the difference of staining between these zones and those in which the collagen, even if not completely destroyed, appears darker in the photograph.



We will not undertake a description of the stroma reaction in the parts where there is no lepromatous tissue, since this process is well known. The most interesting parts are those where the stroma reaction occurs in lepromatous tissue (Fig. 5). These are areas of marked infiltration with round cells in absolute predominance. The connective tissue cells, in their attempt at defense, are seen in great activity, showing large numbers of fibroblasts of elongated form. The Virchow cells are the only ones which do not participate in the stroma reaction, maintaining their peculiar form and structure. Global cavities are not found, or foreign-body giant cells. By Ziehl-Neelsen staining bacilli are demonstrated, preferentially intracellular, but not in great numbers. By scarlet-red staining, which appears to have special selectivity for the lipoids of the Virchow cells, these are demonstrated in great quantity. Besides the lymphocytes, the connective-tissue cells, and the Virchow cells there are to be found some polymorphonuclears, the presence of which may be attributable to the vascular phenomenon occurring in these areas, or else to the ulcerative phenomena. With respect to the irrigation of these areas, there is found at some points a distinct new formation of capillaries, although most frequent are moderate degrees of simple endothelial reaction and—in vessels of larger sizes—infiltration of the vessel walls.

Those other parts, remote from the tumor, which have not reacted to it, are composed of small lepromas which merge together to give rise to infiltration of the deep part of the dermis and to some extent of the subcutis.

Collagen.—This substance practically does not exist. The dermis is so crowded with cells that, simply for reason of space, there is no longer room for the collagen. There are, however, some areas of collagen residues. These are elemental collagen fibrils which are fragmented and short, as though crushed by the pressure of the cellular mass. They no longer stain maroon with silver, except in tiny areas; the majority stain black, thus resembling reticular fibers, although differentiated from them by their being thicker, and straight.

Reticulin.—The reticular fibers of the basal membrane in those places that border on the tumor projections are destroyed. At certain points where one gains the impression of an attempt of regeneration they seem completely disorganized, because instead of being grouped normally in skeins more or less parallel to the epidermal epithelium they are now seen torn and loose, going in all directions for short distances (Figs. 6 and 7). In the remnants of the connective tissue there is now formation of reticulin, the fibers of which are mixed with the residue of the collagenous material described. It is only in the adipose tissue that reticular fibers appear very clearly, surrounding the adipocytes.

Elastica.—The elastic fibers have also suffered intense destruction, even more completely than the collagen. Although different methods of staining have been used, we have only been able to demonstrate tiny fragments, sometimes in the form of rods and sometimes as tiny balls.

In summary, it may be said that the fibrillar framework is integrated by persistence and new formation of reticulin fibers, by destruction of collagen fibers, with perhaps new formation of fine, elemental collagen fibers, and by scarce remnants of destroyed elastic fibers.

Amorphous basic substance.—For the study of the amorphous basic substance we used the Hotchkiss-Lillie-McManus method, which consists of treating the sections with the leucofuchsin of Schiff prior to oxidation with periodic acid (PAS). With this process we were able to demonstrate, among the epidermal cells, an intensely positive material which we believe coincides in nature and chemical composition with that described by Rodriguez Perez (⁶).

The zone pertaining to the basal membrane is clearly stained red, i.e., it is PAS positive. In some areas it continues closely with the tumor projections without increasing in thickness or in form in places where, by means of the silver methods, the reticular strands are seen to be disorganized. This supports the idea that it is not the reticular fibers that are PAS positive, but the amorphous substance which bathes them (Fig. 8). The rest of the connective tissue shows different PAS reactions, according to location.

In places where the collagen is only slightly destroyed, the PAS reaction is distinctly positive. The areas which are markedly affected, either by infiltration of the lepromatous granuloma, or by a stroma reaction, or by lepromatous granuloma with stroma reaction where the collagen is completely destroyed, give a practically negative reaction or at most a very weakly positive one (Fig. 9). In areas with moderate cellular content, whose collagen fibers show a slightly advanced stage of destruction, the PAS reaction is moderately positive. Lastly, the regions surrounding the glands, hair follicles, vessels and nerves, and the adipose tissue, which regions are not affected, are strongly PAS positive.

With the PAS procedure only some of the nuclei of Virchow cells are stained red. On the other hand, great numbers of the nuclei of tumor cells are stained brightly in that color. This shows that there is a great quantity of desoxyribose nucleic acid (DNA) in the nuclei of the tumor cells, and little when any in the nuclei of the Virchow cells, thus confirming the observation of Fite (⁷) on this matter.

We have also seen PAS-positive small round structures in tumor cells, inconstantly and in far from all cells, which we assume to be remnants of nuclear material and not glucogen inclusions, since these elements were found to be resistant to digestion by salivary amylase.

Desiring to identify the PAS-positive materials present in the connective tissue, we followed the procedure of Rodriguez Perez, who in turn followed that of Lillie (⁵). The sections were treated with pepsin and trypsin before applying the McManus technique, to eliminate the possibility that we were dealing with polypeptides. The preparations thus treated gave the same results, except for logical variations, as were obtained with the simple PAS reaction. To eliminate the possibility that the positive reactions might be due to the presence of polysaccharides or lipoids, the sections were subjected to the action of amylase and pyridin, respectively. The result was similar to that in the previous case.

We have also performed the Feijle test to eliminate the possibility that beta-hydroxy-alfa amino acids were involved, this resulting in negative preparations.

These observations led to the conclusion that we were dealing with mucopolysaccharides. To verify this we performed the McManus-Cason test, which, according to Rodriguez Perez, is specific for the identification of mucopolysaccharides or mucoproteins. The sections were first acetylated, blocking the OH and NH₂ groups, after which the PAS reaction gave practically negative results. Next we subjected the acetylated sections to the action of sodium hydroxide; unblocking the OH groups but leaving the NH₂ blocked; this gave preparations entirely similar to those obtained with the PAS reaction alone.

From all these experiments we conclude, with Sommers and Hollander (⁶), that the acid mucopolysaccharides in the lepromatous tissue suffer no change, adding that neither are they affected when the lepromatous tissue is in a stroma reaction in the presence of an epithelioma.

These complex connective-tissue zones, situated between the tumor cords and moderately invaded by the lepromatous infiltration, constituted by a fibrillar material (collagen which is more or less destroyed, and reticulin) and by the amorphous, PAS-positive basic substance, presented the problem of determining whether the PAS positivity is due to the basic substance, either formed or amorphous, or only to the latter. We believe that a solution to this problem can be obtained through what is described below.

As Ham (⁴) points out, some authors maintain the theory of the positivity of the PAS reaction in the fibrillar material and they believe that this is due to the presence of proteins with reactive aminoacids. The other and only possibility of substances capable of giving a positive PAS reaction is, in our case, the presence of mucopolysaccharides. Bowels [cited by Gay Prieto (³)] has demonstrated, by means of chromatography, that the only mucopolysaccharide that is combined with the fibrillar material is the PAS-negative chondroitin-sulfuric acid. The presence of proteins with reactive amino acids was ruled out in

our experiments because in the first step of McManus-Cason reaction (blocking of the OH and NH₂ groups) the PAS reaction was completely negative, and in the second step of the reaction (unblocking of the OH groups, leaving the NH₂ groups blocked) the result of the PAS reaction did not differ from that in preparations treated simply for tuberculoid reaction. There being, therefore, no proteins with reactive amino acids, and since the only mucopolysaccharide of the fibrillar material is PAS negative, it can be concluded that the positive PAS reaction of these areas is due solely and exclusively to the amorphous basic substance.

CASE 2

Sections of the basal-cell epithelioma which developed upon a diffuse lepromatous infiltration show, in the epidermal epithelium, rare papillae which are much deformed, and in some of which there is observed "cellular intranquility." In the prickle-cell layer the cells appear vacuolated, giving in general a spongy appearance. Some hair follicles are seen involved in clearly tumoral structures.

In the dermis there are abundant epithelial cords composed of rather fusiform cells with highly chromatic nuclei. (Fig. 10). The cords cross one another, giving a reticular aspect, and between them are poorly delimited areas occupied by a connective-tissue stroma in which occur the lepromatous granuloma and the stroma reaction. Much less frequent are well-differentiated epithelial cords. In them the marginal cells have larger nuclei, which are highly chromatic. The central cells of these cords show signs of degeneration, and sometimes the cytoplasm is vacuolated. On oil-immersion examination no epithelial fibrils are seen.

In many places the lepromatous and tumor areas are well delimited, although in other regions the tumor infiltration contains an admixture of elements of the lepromatous granuloma. In one instance we found various Virchow cells, with bacilli in their vacuoles, included in a cellular mass which was clearly of tumor nature. This observation is in accord with the idea of Rubio (7) that during its infiltrative growth the tumor mass encloses elements of the lepromatous granuloma, and while the Virchow cells persist the remaining elements are resorbed.

In this case, also, there is observed a clear stroma reaction of the connective tissue in which the Virchow cells do not participate (Fig. 11). On staining with scarlet red the Virchow cells appear with lipoid granulations in their cytoplasm.

With modifications of the Ziehl-Neelsen technique for staining acid-fast bacilli in the tissues, there are found scarce bacillary masses grouped in globi, and very scarce isolated bacilli. Most of the bacilli can be seen to be inside the cytoplasmic vacuoles of the Virchow cells.

With respect to the fibrillar basic substance, this will be studied separately.

Collagen.—Among the tumor cords there are seen some collagen fascicles. After silver staining these fascicles are seen to be in a stage of destruction, due in part to the lepromatous infiltration and in part to the tumor invasion (Figs. 12 and 13). This picture is very demonstrative, since on the one hand we can see loss of the maroon coloration by the silver, while on the other hand there is a fibrillar pattern similar to reticulin but of greater thickness. The fibers in the process of destruction, together with the reticulin, are arranged to form a fine mesh in the interstices of which the tumor cords are situated, some fibers being arranged in circular form around them. In areas where the lepromatous infiltrate is not very marked there is the same fibrillar entanglement, but with much more dense trabeculae. Lastly, in the areas where the infiltrate is minimal, the collagen is practically normal in form and staining. In short, it can be said that there is destruction of the collagen with the formation of a fibrillar network at the expense of the collagen material, and new formation of reticulin.

Reticulin.—In the stroma of the tumor is found, together with the destruction of collagen, a new formation of reticulin which can be distinguished by its tortuous arrangement and by the lesser thickness of the fibers. This network forms an active part of the fibrillar entanglement described.

Elastica.—There is an even greater destruction of the elastic fibers than of the collagen, leaving very scarce remnants of filamentous form (Fig. 14).

With respect to the study of the amorphous ground substance, we have applied the same methods as in the first case described. This led to the same conclusions, which goes to corroborate their veracity (Figs. 15-17).

SUMMARY

Two new cases of epitheliomas in leprosy patients are reported. One was of the spino-cellular type and the other basocellular, both develop-

DESCRIPTION OF PLATES

FIG. 10. Case 2. View of the cords of the basal cell epithelioma which developed on a lepromatous infiltrate. (Hematoxylin-eosin.)

FIG. 11. Case 2. Aspect of the lepromatous infiltrate in an area very close to the cords of the tumor. (Hematoxylin-eosin.)

FIG. 12. Case 2. Collagen in destruction and new formation of reticulin between the tumor cords. (Double impregnation of Del Rio Hortega.)

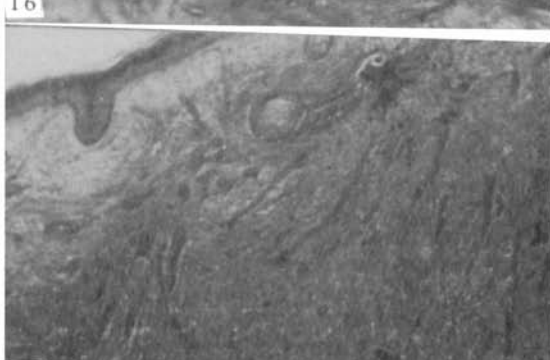
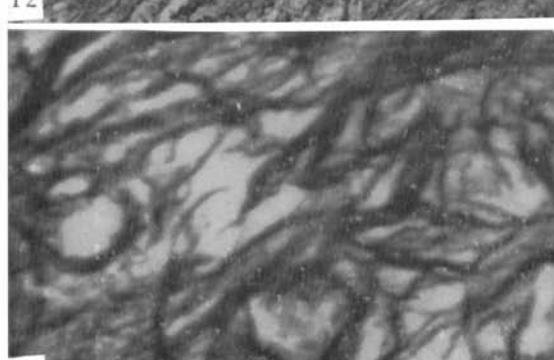
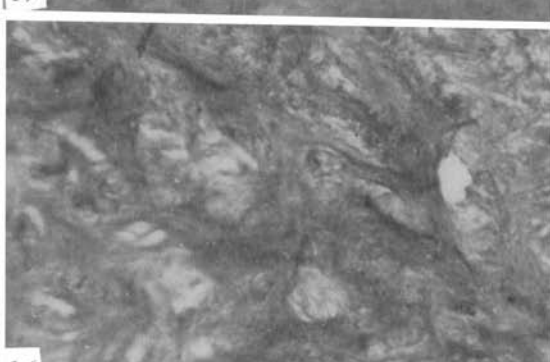
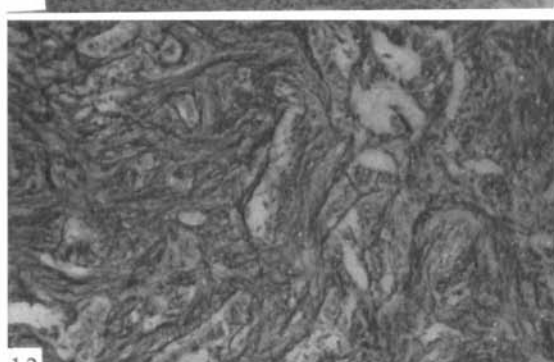
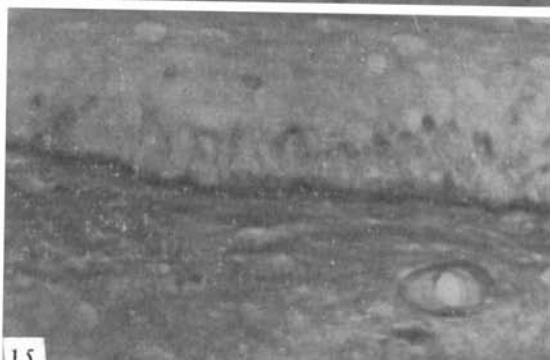
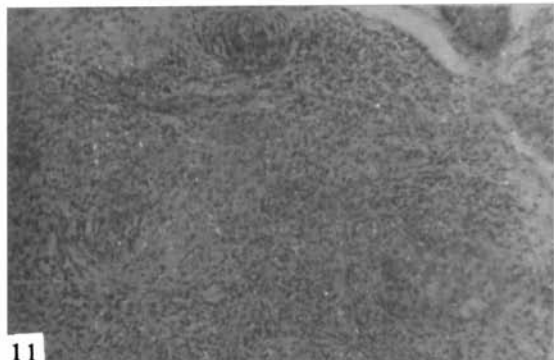
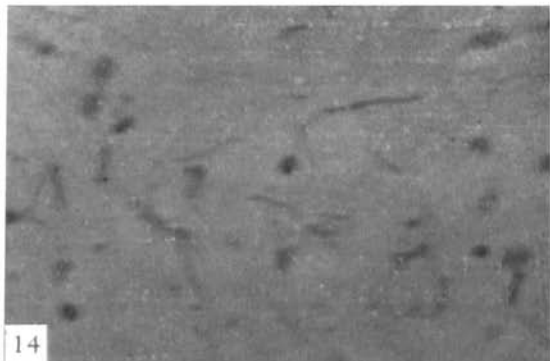
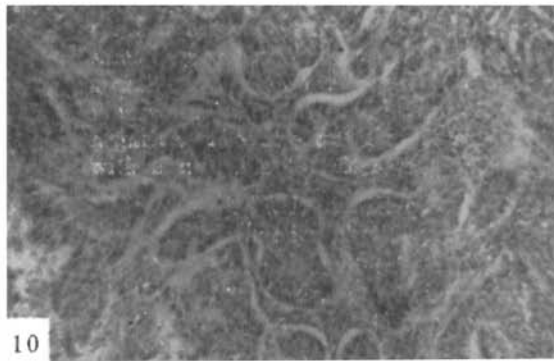
FIG. 13. Case 2. Showing the destruction of collagen. (Double impregnation of Del Rio Hortega.)

FIG. 14. Case 2. Remnants of elastic fibers, in filamentous form. (Orcein.)

FIG. 15. Case 2. Basal membrane of the atrophic epidermis. (PAS.)

FIG. 16. Case 2. PAS-positive zones between the cords of the tumor. (PAS.)

FIG. 17. Case 2. Areas near to the tumor, with intense lepromatous infiltration which gives a practically negative PAS reaction. (PAS.)



ing in lepromatous skin. The clinical features of the patients are described. The histologic findings are given, with special reference to the amorphous ground substance, and also to the fibrillar elements. The observations respecting the stroma cells coincide with those of Vilanova and Rubio.

In the study of the amorphous ground substance of the dermis, there are described zones which differ with respect to the PAS reaction, which seems to explain the greater or lesser destruction of the collagen. Qualitatively there was not seen any modification in the acid mucopolysaccharides.

Assuming that the histochemical techniques used are good, the conclusion is reached that only the amorphous ground substance is responsible for positivity to the PAS reaction, and not the fibrillar material.

RESUMEN

Aportación de dos nuevos casos de epitelomas en enfermos de lepra, uno de ellos espinocelular y basocelular el otro, asentando ambos sobre piel lepromatosa. Se describen las características clínicas de los dos enfermos y a continuación se hace el estudio histológico, especialmente el de la sustancia fundamental amorfa.

Se hace una descripción de las células del estroma, que coincide con las observaciones de Vilanova y Rubio. Se describe la trama fibrilar del estroma. En el estudio de la sustancia fundamental amorfa de la dermis, se describen zonas distintas reacción PAS, lo que parece explicarse por la mayor o menor destrucción de colágena. Cualitativamente no se encuentra modificación en los mucopolisacáridos ácidos.

Dando por buenas las técnicas histoquímicas empleadas, se llega a la conclusión de que en la positividad a la reacción PAS, solo es responsable la sustancia fundamental amorfa y no el material fibrilar.

REFERENCES

1. CONTRERAS, F. Secondary infections and neoplasms in leprosy patients. *Leprosy Rev.* **28** (1957) 95-957.
2. FITE, G. L. Leprosy from the histologic point of view. *Arch. Path.* **35** (1943) 611-644 (cited).
3. GAY PRIETO, J. *Dermatología*. Barcelona and Madrid: Ed. Científico-Médica, 4th ed., 1957.
4. HAM, A. W. *Histology*. Philadelphia: J. B. Lippincott Co., 2nd ed., 1953.
5. LILLIE, R. D. *Histopathologic Technic and Practical Histochemistry*. New York and Toronto: Blakiston Company, 2nd ed., 1954.
6. RODRIGUEZ PEREZ, A. P. Contribución al conocimiento histoquímico del epitelio cutáneo. *Actas Dermo-Sif.* **47** (1955-1956) 273-281.
7. RUBIO, J. Epitelioma sobre leproma. *Actas Dermo-Sif.* **36** (1944-1945) 668-676.
8. SOMMERS, S. C. and HOLLANDER, A. Histochemical study of mucopolysaccharides of leprosy of the skin. Symposium 5, XI Internat. Congr. Dermat., Stockholm, 1957.
9. VILANOVA, X. Epitelioma sobre leproma. *Actas Dermo-Sif.* **36** (1944-1945) 1004-1010.
10. VILANOVA, X., LLORENS RIBAS, M. and ALVARADO, L. Epitelioma sobre leproma. *Actas Dermo-Sif.* **42** (1950-1951) 39-43