HISTOLOGIC STUDIES ON THE PLANCHA OR INFILTRATION METHOD OF LEPROSY TREATMENT [1]

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INTRODUCTION

The so-called infiltration or "plancha" method of treating leprosy unquestionably has attained a position of importance. Though Rogers (17), using a solution of the chaulmoogra sodium salts, and McDonald and Dean (6), using the ethyl esters, made injections into lesions, this was done only incidentally, and the present position of the intradermal method has resulted from developments at the Culion Leper Colony. According to Wade [2] some of the patients at Culion were found in 1922 to be administering chaulmoogra preparations to each other by a method which they called "plancha", this consisting of multiple injections directly in the skin lesions. This practice was interdicted at the time to avoid interference with the evaluation of the new treatment materials then under trial, but later Rodriguez [3], importuned by the patients, undertook controlled observations of the method on a small scale and became so convinced of its value that he wrote the technic into the booklet "A Description of Leprosy" (20). When Lara resumed charge of the clinical work he pursued the matter actively and systematically, with the result that some six years ago the method, as improved by him, was finally adopted as the primary one for active treatment at Culion. Detailed reports were published in 1929 by Lara (3), Lara and Nicolas (4), and Velasco et al (19). Since then the method has been adopted by Muir (10) and others (e.g., Fraser 2), and it bids fair to be the method of choice in most cases of leprosy.

[1] Published with the approval of the Director, Bureau of Health.

[2] Personal communication from Dr. H. W. Wade, who served as Acting Chief Physician at Culion during the reorganization period, 1922 and 1923.

[3] Dr. José N. Rodriguez, Acting Chief Physician at Culion for two years from 1924 to 1926, during the absence of Dr. C. B. Lara, who had recently been appointed Chief Physician.

In view of its growing importance it is essential to obtain all possible information concerning the processes which are set up by this manner of injection. No record from elsewhere has been found concerning the histological changes in lesions after local infiltration. The writer undertook such studies several years ago, describing in 1929 the changes in the skin induced by antileprotic drugs in the course of treatment. Later, because of the observation that the leucocytes take an active part in the disposal of injected oils, the changes in the underlying nerves and in the regional lymphatic nodes were studied. The immediate effects of soluble hydnocarpus compounds were also investigated. The subjects studied were lepers, non-lepers, dogs and monkeys. Reports of the various phases of the work have been made in detail (11, 12, 13, 14, 15, 16), but the significant points and conclusions are here summarized and coordinated.

I. LESIONS INJECTED WITH IODIZED ETHYL ESTERS

In this study (11) three lots of biopsied human material were examined. One lot was from eight patients after ten months of the infiltration treatment with iodized (0.5 per cent) ethyl esters of *Hydnocarpus wightiana* oil.[4] The other two lots were from three patients, one after a single injection, the other after four or five. Before treatment was begun two apparently similar lesions were selected in each case; one was treated and the other kept as a clinical control, and both were examined histologically. This control was, admittedly, not as strict as if the same lesion could have been used for both purposes, but because of the apparent similarity of the lesions at the outset it is believed that the comparison is valid.

In the first lot, infiltrated for ten months, treatments were supposed to be weekly but the numbers actually given varied from 17 to 32. Intramuscular injections also were given during the same period. The lesions treated were bacteriologically positive; two were macules, three infiltrations, and three nodules. Marked clinical improvement occurred in the locally treated lesions, with but little apparent change in the untreated lesions.

The second lot—one patient with macular lesions, one with infiltrations, and one with nodules—were each given one infiltration treatment and tissue taken 24 hours later. The same three patients were then given four or five weekly infiltrations and second specimens were taken 24 hours after the last treatment. The tissues were fixed in Zenker's fluid, and paraffin sections were stained

[4] The biopsy material was supplied by Dr. C. B. Lara, Chief Physician, Culion Leper Colony.

with eosin and hematoxylin, Mallory's aniline blue, Mallory's phosphotungstic acid hematoxylin, and for acid-fast organisms.

HISTOLOGICAL FINDINGS

(a) *Lesions treated once.*—The lesions treated once and excised 24 hours later all showed polymorphonuclear and large mononuclear cellular exudate, matted in fibrin. Most of the exuded cells showed advanced degenerative changes, with darkly stained and fragmented nuclei. There was no degeneration in the leprotic tissues, and no yellowish globules were seen.

(b) *Lesions treated four or five times.*—Clinical improvement was noted only in the nodular case, where histologically the leprous tissue was found to be about one-half the thickness of the control. In the two other lesions there was no apparent reduction. The cellular reaction was very similar to that found in the first lot.

(c) *Lesions treated for ten months.*—Clinically, after ten months of treatment the three infiltrations and three nodules treated by local infiltration had all improved markedly, and three of them had apparently subsided completely. The three infiltrations and three nodules used as controls all showed little clinical change.

Histologically, the leprous tissue of the treated lesions was from one-fourth to one-half as thick as in the control sections. It was not in a continuous layer as in the latter, but was interrupted by coarse strands of connective tissue. In all the treated lesions there were large mononuclear leucocytes loaded with peculiar yellowish globules, hereinafter to be called "globulated cells." These were not found in the untreated lesions. Granulation of the bacilli was also a striking feature of the treated tissues.

The two control macules showed a tuberculoid picture. The treated macules, however, showed neither ordinary leprotic nor tuberculoid changes. Instead, both showed perivascular collections of the globulated cells. Again, these were not found in the control lesions. In neither control or treated lesions could acid-fast organisms be demonstrated.

Comment.—The fact that in the lesions treated by local infiltration for ten months with the iodized hydnocarpus ethyl esters the amount of leprous tissue was markedly reduced, and that the untreated lesions remained clinically unchanged although intramuscular injections of the same drug were also given, indicates clearly a local favorable effect of this method of therapy. The histologic picture of the locally treated lesions, in which the leprotic layer was comparatively thin and discontinuous, interrupted by strands of dense connective tissue, indicates that the leprous tissue has been partly absorbed and its place taken by connective tissue.

Walker and Sweeney (21) and Schöbl (18) demonstrated the peculiar bactericidal effect of chaulmoogra preparations on organisms of the acid-fast group. Muir (9) claims favorable effects from counter-irritation, which he believes stimulates a local reaction with

the result that the bacilli become granular and less resistant. That there is a direct and immediate irritation from the intradermal administration of the drug is shown in the first two lots of this experiment.

If counter-irritation has any therapeutic effect, and if the drug really has any specific action upon the microorganisms in the tissues, the present method of administering the drug must be doubly effective, for besides producing local irritation the drug is present in the lesion in far greater concentration than when brought from a distant point in the blood stream. The peculiar yellowish globules observed in the large mononuclear leukocytes in the sections treated for ten months, but not encountered in the untreated sections, presumably represent the injected drug, as is demonstrated in a subsequent section of this report.

CONCLUSIONS

1. The method of treatment by infiltration of the lesions with iodized hydnocarpus ethyl esters stirs up a mild local inflammatory reaction, and enables the injected drug to accumulate in the skin lesions in greater concentration than by other methods. This accumulation is in the form of yellowish globules in large mononuclear leucocytes (''globulated cells'').

2. The particularly rapid effect on the leprous lesions (reduction of lepromatous tissue, granulation of bacilli) is evidently due to local action, for though intramuscular injections were also given the patients at the same time the lesions not treated locally showed far less improvement.

3. Whether the irritant action or a specific effect of the drug upon the microorganisms is responsible for the diminution of leprous tissue is not apparent from the observations made.

II. IODIZED ETHYL ESTERS IN NORMAL SKIN.

To study the local changes induced in normal skin by local infiltration with the iodized hydnocarpus ethyl esters, and to control the occurrence of the yellowish globules—since these globules, being found in leprous subjects, might possibly be of leprous nature—experiments were made with three non-leper volunteers (15).

An area about 2 by 5 cm. was selected on each side of the back, about two inches below the inferior angle of the scapula. At irregular intervals, varying from one to four weeks, infiltrations of 1 cc. of the drug were made on one side; on the other infiltrations with salt solution were made as a control.

One of the subjects withdrew twenty days after the first and only injection because of iodism with fever, pain in the joints, and acneiform eruptions. The two others (C. G. and R. T.) received 14 and 15 local infiltrations, respectively, during a period of seven and a half months.

Tissues were excised[5] 20 days (C. G.) and 34 days (R. T.) respectively, after the last infiltration. In the first subject the control site was excised without any further injection; in the second the control site was infiltrated with the drug the day previous to excision (24 hours section). Tissue was also excised from the subject who developed iodism, 9 months after the injection, to see if the yellowish globules could be found after that length of time.

The area injected the day before was bluish (from the iodine), moderately swollen and warm. The old areas were dark bluish, thickened, moderately indurated and warm throughout the period of treatment. The control (salt solution) areas appeared normal, except for the needle punctures.

HISTOLOGICAL FINDINGS

The repeatedly infiltrated tissues showed diffuse large mononuclear and lymphocytic infiltration, tending to form distinct collections around the blood vessels and hairshafts. The large mononuclear cells were globulated, and some of them simulated the "foamy cell" in leprosy (Plate I, Fig. 1). The connective tissue in the corium and subcutaneous layer was distinctly increased and compact, and its interstices were infiltrated with yellowish globules. The sebaceous glands had largely disappeared. The hairshafts in all the sections, and the small sebaceous glands found in some of them, were all surrounded by wide zones of globulated cells with some lymphocytes. With scarlet red the globules stained brownish-red, and with Nile blue sulphate dark blue (Plate I, Fig. 2).

The tissue infiltrated once and excised after 24 hours showed fibrino-cellular exudate. Most of the cellular elements—mainly polymorphonuclears and large mononuclears—were in a state of advanced degeneration, with pyknotic and contracted nuclei (Plate II, Fig. 3). There was moderate edema, but no congestion. The control (salt solution) section was normal.

The tissue infiltrated once and excised nine months later showed small collections of globulated cells around the small and larger blood vessels of the corium and subcutaneous fatty tissue, and about the sweat glands. With the fat stains the globules stained as before.

Comment.—This observation demonstrates that prolonged local infiltration of non-leper skin with the iodized hydnocarpus ethyl esters induces a local cellular reaction of chronic inflammatory type, characterized by large mononuclear cells loaded with yellowish globules (globulated cells), some of which simulate the "foamy cells" of leprosy. The occurrence of such cells under these circumstances shows that similar cells found in infiltrated tissues of lepers need not necessarily be leprotic in nature.

[5] By Dr. Jose G. Samson, in charge of the surgical work at Culion.

Another interesting observation is the atrophic condition of the sebaceous glands in the treated sections. Whether this change is due to an injurious effect of the drug or to pressure effects of the accumulated globulated cells cannot be stated.

CONCLUSIONS

1. Infiltration of non-lepers' skin with the iodized hydnocarpus ethyl esters causes a definite cellular reaction, with large mononuclear cells loaded with yellowish globules.

2. These globulated cells may appear "foamy" and so may be taken for lepra cells, which emphasizes the difficulty of diagnosis of biopsy specimens of infiltrated skin of lepers.

3. The yellowish globules can persist in the macrophages for more than nine months, showing that the drug so taken up is disposed of very slowly. Its relative insolubility in sections indicates that it is in modified form.

4. The atrophic condition of the sebaceous glands observed is attributed either to the specific injurious effect of the infiltrated drug or to pressure effects of the accumulated globule-laden mononuclear cells.

III. NATURE OF THE YELLOWISH GLOBULES

To demonstrate the probable constitution of the yellowish globules observed in the treated lesions, special staining and microchemical methods were resorted to (12), inasmuch as attempts to devise a test by which oils of the chaulmoogra group could be positively identified have not been successful (22). The investigation included determination of (a) the reaction of the yellowish globules towards special stains, and (b) their reactions toward certain chemical reagents.

Reactions with special stains.—Sections from all the paraffin blocks of the treated and untreated (control) tissues discussed in the first section hereof were stained with scarlet red and Nile blue sulphate. Two specimens were also treated with osmic acid.

The yellowish globules noted in the long-treated tissues took on a brownish-red color with scarlet red, and dark blue with the Nile blue. Nothing similar was found in the control sections. The sections that had been excised twenty-four hours after the last infiltration showed no brownish-red globules after scarlet red, but instead there was abundant granular material of the same staining character in the interstices of the connective tissue of the corium, and in the cytoplasm of the large mononuclear exudate cells, the foamy cells and the connective tissue cells. With Nile blue sulphate this granular material

stained faintly blue. With osmic acid both treated and untreated sections failed to show any stainable material.

Reactions with certain reagents.—In order to obtain indications of the chemical nature of the yellowish material through its solubilities, duplicate sections of the slides from some of the paraffin blocks of both treated and control lesions were treated with the following reagents:

Chloroform, 30 minutes.
Ether, 30 minutes.
Oil of turpentine, 24 hours.
Hydrogen peroxide, 10 per cent in alcohol 80 per cent, 3 hours.
Nitric acid, 10 per cent in alcohol,[6] 48 hours.
Hydrochloric acid, 25 per cent in alcohol, cold, 48 hours.
Hydrochloric acid, 25 per cent in alcohol, cold, 96 hours.
Hydrochloric acid, 25 per cent in alcohol, boiled (86°C).
Sodium hydroxide, 1 per cent in alcohol (10 cc. of 10 per cent NaOH to 90 cc. of alcohol), 5 minutes.
Sodium hydroxide, 2 per cent in alcohol, 5 and 15 minutes.

Sections that had been treated with chloroform, ether, turpentine, hydrogen peroxide, nitric alcohol, and hydrochloric alcohol still showed the yellowish material, which took fat stains as before. The same was true of the sections treated with 1 per cent sodium hydroxide alcohol for five minutes, but with 2 per cent sodium hydroxide alcohol the globules dissolved partially in five minutes and completely in fifteen minutes.

Comment.—These experiments demonstrate that the yellowish globules under consideration are of fatty nature. They stained with scarlet red and Nile blue sulphate—two typical lipoid stains—and their solubility is that of a fatty substance, since they hydrolized with alkaline alcoholic reagents but not in acid alcoholic reagents even when boiled for fifteen minutes.

It is noteworthy, however, that they are not as soluble as free oil or ordinary fat; they are so relatively insoluble that they are unaffected by the process of paraffin embedding and staining.

CONCLUSIONS

The peculiar yellowish globules found in the sections of leprotic lesions treated by direct infiltration with the iodized hydnocarpus ethyl esters are of fatty nature. That they are the injected drug, probably in a modified form, is very probable.

IV. SODIUM HYDNOCARPATES INTRADERMALLY IN DOGS

Tissues infiltrated with the iodized hydnocarpus ethyl esters and excised twenty-four hours later show local irritant effects, evidenced by degenerated polymorphonuclear and large mononuclear

[6] Alcohol, except when otherwise specified, was of 95 per cent concentration.

exudate and fibrin. This observation led to a study of the local effects of infiltrating the skin of dogs with solutions of the sodium salts of hydnocarpus oil with the hope of locating the irritant element, and to determine the feasibility of using them in the infiltration treatment of leprosy (13). The drugs used were (a) pure sodium hydnocarpate, prepared at Culion from purified hydnocarpic acid, and (b) alepol, said to be made from the lower melting point acids of hydnocarpus oil. To ascertain whether hydrogen ion concentration has anything to do with their irritant properties, this was determined by electro-titration for each solution used. To find out whether the irritant properties of this substance might be reduced by stabilizing the hydrogen ion concentration, a buffered solution of sodium hydnocarpate was used. The materials used are listed in Table 1, which gives details as to the strengths of the solutions and their hydrogen-ion concentrations.[1]

TABLE 1.—*Characteristics of solutions used in irritation experiments in dogs, with special reference to hydrogen ion concentration.*

Test and control solutions	Hydrogen ion concentrations of solutions of different percentage strengths			
	3%	5%	10%	15%
Test solutions				
Sodium hydnocarpate, simple aqueous [a]	8.9	9.3	9.4	10.2
Sodium hydnocarpate, buffered [a]	8.7	9.0	9.0	9.4
Alepol, aqueous solution [b]	9.3	8.9	8.5	7.3
Control solutions				
Sodium chloride, simple aqueous	4.4	4.5	4.8	4.7
Sodium chloride, buffered	7.3	7.3	7.3	7.3
Dilute aqueous sodium chloride, three solutions, pH 7.2, 8.8, and 9.8	—	—	—	—
Standard buffer solutions, pH 6.8 and pH 7.3	—	—	—	—

[a] The percentage strength refers to grams of fatty acids.
[b] The percentage strength refers to grams of the sodium salt of the mixed fatty acids.

The first three of these were the test solutions; those of sodium chloride, sodium hydroxide, and the two standard buffer solutions were used as controls. All the solutions were heated in the Arnold sterilizer for forty minutes on three consecutive days. Their hydrogen-ion concentrations were determined before sterilization, and the stock solutions were checked immediately after the injections were made.

[1] Thanks are due Mr. E. M. Paras, of the Chemical Section, who prepared the solutions used in this work.

Three dogs were used, injections being made aseptically into the skin of the chest and abdomen after clipping the hair. Coalescing intradermal injections were made in areas marked off with India ink, the amount injected varying from 0.35 cc. to 0.75 cc. The tissues were excised the next day.

HISTOLOGICAL FINDINGS

The three test solutions all gave similar inflammatory cellular reactions. These were characterized by edema and exudation of red cells, polymorphonuclear and large mononuclear leucocytes and fibrin. The cells showed advanced degenerative changes, evidenced by pyknotic, dark-staining and fragmented nuclei. Of special note, thrombosis of the larger blood vessels of the subcutaneous fatty tissue was present in most of the sections.

In the control sections no such changes were observed. In only one lesion, that caused by the 3 per cent non-buffered sodium chloride, was there a cellular reaction which approached in degree those caused by the test solutions, but unlike them the cellular exudate showed very slight degenerative changes.

Comment.—These observations indicate that it is the fatty acid element of the sodium hydnocarpate solutions that is responsible for their marked irritant quality, since the control solutions used did not produce similar inflammatory changes.

The same degenerative changes in the cellular exudate that was seen in the sodium hydnocarpate specimens was also seen in tissues infiltrated with the iodized esters (see Section I of this report), but no thrombosis of the blood vessels was observed in them. The thrombosis produced by the sodium salts probably may be explained by their greater diffusibility, they being in aqueous solutions, in contrast with the oily ethyl esters. In the clinics it has been the observation also that the sodium salts when administered by the infiltration method tend to produce local ecchymosis (3). Eubanas (1) reported two cases out of six in which necrosis of the infiltrated tissues followed local infiltration with buffered sodium hydnocarpate.

CONCLUSIONS

1. Pure sodium hydnocarpate and alepol when injected intracutaneously in various concentrations induce local reactions characterized by a fibrino-cellular exudate with marked degenerative changes in the exuded cells, a reaction very similar to that produced by the iodized hydnocarpus ethyl esters.

2. These drugs so administered also cause thrombosis of the larger vessels in the subcutaneous tissue, a property which would limit their use in the infiltration method of treatment.

3. The irritant properties of sodium hydnocarpate and alepol solutions are not due to their hydrogen ion concentration, and are

not modified by buffering; they apparently reside in their fatty acid radical.

V. LYMPHATIC ABSORPTION OF OILY ANTILEPROTIC DRUGS

In furtherance of the observations here recounted it was regarded as of interest to determine whether or not the nerve in relation to the site of injections, and the superficial lymphatic nodes draining the area, would show changes as a result of intradermal or subcutaneous administration of the oily antileprotic drugs (16).

One monkey (I) was given intradermal injections of purified *H. wightiana* oil, in 1 cc. doses, into an area 2 by 4 cm. on the antero-medial aspect of the right forearm, one inch distal to the elbow. A second one (II) was given similar injections in the same region, subcutaneously. Two other monkeys (III and IV) were similarly treated, using the 0.5 per cent iodized hydnocarpus ethyl esters. The location used was selected because of its close relation with the ulnar nerve. The treatments were given for five months, at intervals varying from one to two weeks. Control injections of salt solution were made in the left arms.

At the end of the five months Monkey I died of tuberculosis; the three others were sacrificed four days later. Sections from the injected skin, ulnar nerves, brachial vessels and nerves, forearm and arm muscles in relation with the blood vessels and nerve trunks, and the axillary lymph nodes of both the treated and control sides, were taken for study. The principal visceral organs were also examined.

A. FINDINGS AFTER HYDNOCARPUS OIL

Skin.—The treated tissues of Monkeys I and II showed the injected material in large yellowish globules distending intercellular spaces, and some mononuclear cells loaded with smaller yellowish globules. The corium over the treated subcutaneous tissue, containing no injected material, was in marked contrast with the injected corium, where the oily globules extended to the basement membrane of the epidermis. Cellular reaction to this drug was not a prominent feature in either the dermis or subdermis.

Ulnar nerves.—The nerve at the elbow after intradermal treatment (Monkey I) was apparently normal. That of Monkey II (treated subcutaneously) showed large and small yellowish globules infiltrating the perineural tissues, and the lymph vessels were distended with the injected oil (Plate III, Fig. 6). The nerve substance contained none.

Muscle.—In both monkeys the yellowish globules were found in the superficial layers of the fibrous septa of the muscles of the forearm, with a few giant cells of the Langhans type.

Axillary lymph nodes.—In both monkeys these organs showed distended lymph spaces containing the injected oil (Plate II, Figs. 4 and 5), many of the smaller globules being surrounded by large mononuclear cells forming giant cells. Also, abundant large mononuclear cells infiltrated the nodes, many of them containing yellowish globules. In Monkey I (dead of tuberculosis) the

germinal centers were not prominent, but in Monkey II they were hyperplastic, showing many mitotic figures.

Brachial vessels and nerves.—In Monkey I these organs were negative for globules, but in the other the median nerve showed perineural yellowish globules within large mononuclear cells and the lymphatic vessels were distended by the injected oil.

All the yellowish globules found in the sections showed differential staining with scarlet red and Nile blue sulphate. All the corresponding control organs were apparently normal, without the globules.

Visceral Organs, Monkey I.—In the *lung* the alveolar walls, perivascular tissues and subpleural regions in places showed large mononuclear cells and giant cells containing differentially staining globules. Lesions of tuberculosis were also present. The *spleen* had become transformed completely into a large cold abscess. The *liver* and *kidneys* showed tuberculosis, but none of the yellowish globules.

Visceral Organs, Monkey II.—In the *lung* there were globules within large mononuclear cells in the alveolar walls, as in I. In the *spleen* a very few such globules were found. The *liver* and *kidneys* were apparently normal.

B. FINDINGS AFTER ETHYL ESTERS

Skin.—The treated tissues of Monkeys III and IV showed thick infiltrations, with large mononuclear cells, lymphocytes and plasma cells, with focal collections of giant cells (Langhans type) simulating a tuberculoid process. A few of the mononuclear cells contained yellowish globules, but most of them had homogenous yellowish cytoplasm. The skin treated subcutaneously (IV) showed injected material mostly in the subcutis; a few globules were in the deeper corium but almost none in the superficial layers. On the other hand, in the intradermal specimen (III) the globules infiltrated the entire corium, as in Monkey I.

Ulnar nerves.—The nerve at the elbow from III (treated intradermally) was apparently normal. That from IV (treated subcutaneously) showed a few globulate mononuclears in the connective tissue of the outer layer of the epineureum, none deeper.

Muscle.—Globulate cells were found in the muscle of the forearm, limited to the intramuscular trabeculae of the superficial layers.

Axillary lymph nodes.—In both monkeys these organs showed diffuse, large, yellowish mononuclear infiltrations, from the cortex to the medulla. There were some moderate-sized and small round spaces lined by large mononuclear cells and some giant cells (Plate II, Fig. 6). In both animals the germinal centers were prominent, hyperplastic, with increased mitosis. In the control sections (left) this was not the case.

Brachial vessels and nerves.—In Monkey III (intradermal) these structures were normal, but in IV (subcutaneous) there were small collections of globule-laden mononuclears in the connective tissue over the muscle. The nerves were free of them.

All the corresponding tissues from the left side, used as controls, were without differentially staining globules, and were apparently normal except

that in the axillary lymph nodes in Monkey III there were tuberculous changes—caseation necrosis with typical Langhans giant cells.

Visceral organs, Monkey III.—In the *lung* there were differentially staining globules within large mononuclear cells in some of the alveolar walls. The *spleen* and *liver* both showed tuberculosis, with no yellowish globules. The *kidneys* apparently were normal.

Visceral organs, Monkey IV.—The *lung,* both beneath the pleura and in some of the alveolar walls, showed some globulate cells. In the *spleen* there were a very few globules. The *liver* and *kidneys* apparently were normal.

Comments.—This study demonstrates that, after intradermal and subcutaneous injection, hydnocarpus oil and the iodized ethyl esters are absorbed by way of the lymphatics and tend to accumulate in the sinuses of the regional lymph nodes and within the large mononuclear cells. The globules of injected material were not demonstrated within the nerve trunks in any instance. Menkin (7) and Menkin and Freund (8) have demonstrated that trypan blue injected subcutaneously in the foreleg of a rabbit appeared within 30 to 40 minutes in the lymphatic vessels of the axilla. If leprosy is considered a lymph disease, the intradermal and subcutaneous injection of the lesions must be an effective way to bring the drugs in concentrated form into direct contact with the bacilli, in the lymphatic system as well as in the skin.

In leprosy one gets the impression that the macrophage containing the leprosy bacillus has very little bactericidal action; it does hardly more than carry the microorganisms as if they were foreign bodies (20). Nevertheless, it must play an essential part in the defense against that organism. Among its several functional properties (5) are phagocytosis and the storing of colloidal substances; the phagocytized substances may disappear if digestible, or they may be transferred to other cells. They play an important role in the metabolism and storing of fats and lipoids, and enzyme- and antibody-producing activity is also claimed. This may explain in part the possible mode of improvement in the lesions with local infiltration, though there may be a directly inimical effect of the drugs upon *Mycobacterium leprae* itself (21, 18).

In the skins of the two monkeys injected with wightiana oil the cellular reaction was not a prominent feature, in contrast with that caused by the iodized ethyl esters. It is inferred from this that the iodized ethyl esters are probably a more effective drug than is the oil, which fact seems to be borne out by clinical tests in which the iodized ethyl esters gave somewhat the better results (19).

CONCLUSIONS

1. In monkeys given intradermal and subcutaneous injections of hydnocarpus oil and its iodized ethyl esters, the injected oily drugs are absorbed by way of the lymphatics.

2. With subcutaneous administration comparatively little of the drug infiltrates the corium. The intradermal method is therefore superior since the bulk of leprotic skin lesions are in the corium.

3. Nerve trunks (ulnar and median) were found to be unaffected by injections in the forearm in this experiment.

4. Cellular reaction in the tissues injected with oil is not a prominent feature, in contrast with that caused by the iodized ethyl esters. From this it is inferred that the latter is probably the more effective drug in the local treatment of leprosy.

5. The macrophage is mobilized locally at the site of the injection, along the lymphatic vessels, and in the regional lymphatic nodes, this being an increased local defense reaction against the injected oil and, incidentally, against *Mycobacterium leprae.*

6. The local injections of the lesions in lepers and the consequent absorption of the injected drug into the lymphatics and the regional lymphatic nodes is believed to be effective in bringing the drugs in concentrated form into direct contact with the bacilli in the lymphatic system.

GENERAL SUMMARY

1. Leprotic lesions infiltrated once or a few times with the iodized *Hydnocarpus wightiana* ethyl esters as made at Culion show, 24 hours after such injection, polymorphonuclear and large mononuclear exudation, with fibrin deposit and degeneration of the exudate cells. (Section I).

2. Leprotic lesions similarly injected for many months show, histologically, marked reduction of the lepromatous tissue and fragmentation of the bacilli, as compared with similar control lesions which have been subjected only to the influence of simultaneous intramuscular injections. In these treated lesions are found large mononuclear leucocytes containing peculiar yellowish globules ("globulated cells"), not seen in untreated lesions. (Section I).

3. The particularly rapid effect of the infiltration method of treatment is due evidently to local action, whether this be the reaction to irritation, or the greater concentration of the drug—which may have a specific effect on the bacillus—or both. (Section I).

4. The skin of non-lepers, similarly infiltrated with the iodized hydnocarpus ethyl esters, shows 24 hours after an injection an acute reaction similar to that seen in a leproma. (Section II).

5. In the non-leper's skin after prolonged injection there is a chronic reaction as in a leproma, with large mononuclear leucocytes predominating, and also atrophy of the sebaceous glands. Many of the mononuclear leucocytes are globulated, sometimes simulating the "foamy cells" of leprosy. Therefore such cells, when seen in a leproma that has been treated by infiltration, are not necessarily leprotic in nature. (Section II).

6. The yellowish intracellular globules were not found in any of the controls. They were found, however, in a non-leper's skin injected but once, they having persisted there for nine months, which indicates the slow disposal of them by the mononuclear leucocytes which contain them. (Section II).

7. That the yellowish globules are fatty in nature is shown both by their reaction to fat stains (brownish-red after scarlet red, blue after Nile blue sulphate), and by their solubility upon hydrolysis in alkaline alcoholic solutions. (Section III).

8. From the evidence adduced these globules are clearly derived from the drug injected, and represent storage of it. However, it is in a modified form, since it does not dissolve in ordinary fat solvents. (Section III).

9. Solutions of purified sodium hydnocarpate, simple and buffered, and of alepol, produce in dogs within 24 hours after injection an acute inflammatory reaction similar to that caused by the ethyl esters. (Section IV).

10. In addition, there occurs thrombosis of the vessels in the subcutis. This indicates that it would not be feasible to employ these drugs by infiltration in leprosy therapy, and this seems borne out by clinical experience. (Section IV).

11. The irritant quality of the sodium salts is evidently not due to their hydrogen ion concentration, but apparently resides in the fatty acid radical. (Section IV).

12. Oily drugs (the pure hydnocarpus oil or the ethyl esters), when injected subcutaneously do not penetrate the dermis, and so cannot act in concentration on the lesions therein. (Section V).

13. These drugs when injected superficially (either intradermally or subcutaneously) are absorbed by the lymphatics and tend to accumulate in the lymphatic nodes, either in the sinuses or in

macrophages. In these nodes there is evidence of active proliferation in the germinal centers. (Section V).

14. On the other hand the nerve trunks examined were found to be unaffected. (Section V).

15. Leucocytic reaction in the tissues injected with the refined oil was not as marked as after the iodized ethyl esters, from which it is inferred that the latter should be the more effective in therapy. (Section V).

16. Local injections are believed to be the most effective way of concentrating the drugs in the lymphatic system, and the mobilization of mononuclear leucocytes in that system increases the local defense against the leprosy bacillus. (Section V).

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DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Corium of a non-leper after infiltration for seven months with iodized hydnocarpus ethyl esters. Showing especially the perivascular accumulation of large mononuclears with yellowish globules (globulated cells), simulating the foamy cells of leprosy. At A, blood vessels; at B, yellowish globules, extra-cellular; at C, globulate cells. Hematoxylin-eosin stain, camera lucida drawing.

FIG. 2. A section similar to that shown in Fig. 1, after staining with scarlet red, the globules showing dark. Showing numerous globules, both large (A) and finely divided (B). Camera lucida drawing.

FIG. 3. Corium of a non-leper twenty-four hours after the first infiltration with iodized hydnocarpus ethyl esters. Showing at A, large mononuclears; at B and B^1, degenerated leucocytic exudate; at C, extravasated red blood cells, and at E, fibrin. At D is the duct of a sudoriferous gland. Hematoxylin-eosin, camera lucida drawing.

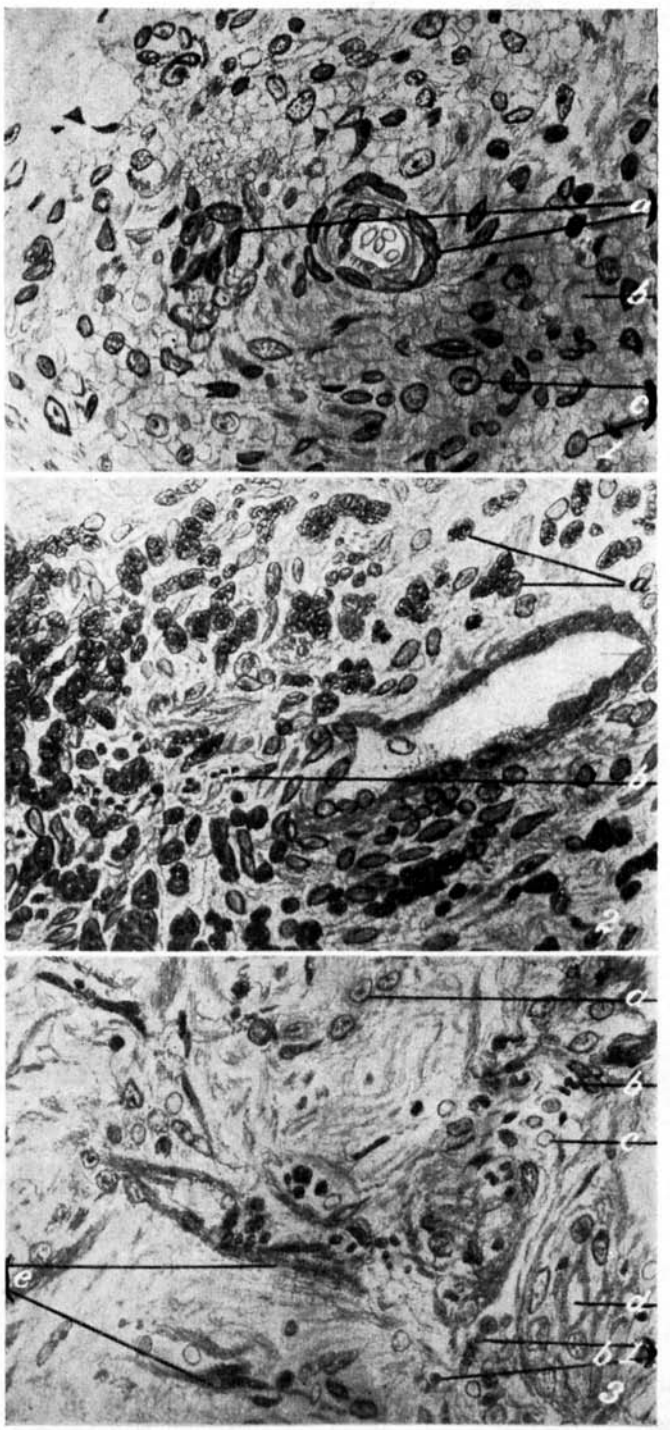

PLATE 1.

PLATE 2

FIG. 4. A section of the largest lymph node from Monkey I, in a paraffin section stained with scarlet red. The large globules of hyndocarpus oil appear dark. One of the subcapsular globules is enlarged in Fig. 4. Photomicrograph.

FIG. 5. Space (A) left in a distended cortical sinus of the lymph node, Monkey IV, after dissolving out an oil globule. About it are many large mononuclear cells filled with smaller globules (B), and at one place a large giant cell (C) which contains some oil. At D is the lymph node capsule. Camera lucida drawing.

FIG. 6. Perivascular lymph vessels in relation with the ulnar nerve and the ulnar vein (A) at the elbow, distended with the injected hydnocarpus oil, Monkey II. The lymph vessels (B) are lined with large mononuclear cells some of which (C) contain yellowish globules; they are distinguished from the endothelium (D). Hematoxylineosin stain, camera lucida drawing.

FIG. 7. A large oil globule (A) distending a subcapsular space of a lymph node from the section shown in Fig. 4. About it many globulate mononuclear cells (B), at C is the capsule. The reticulated appearance, suggesting structure, is due to artefacts. Hematoxylin-eosin stain, camera lucida drawing.

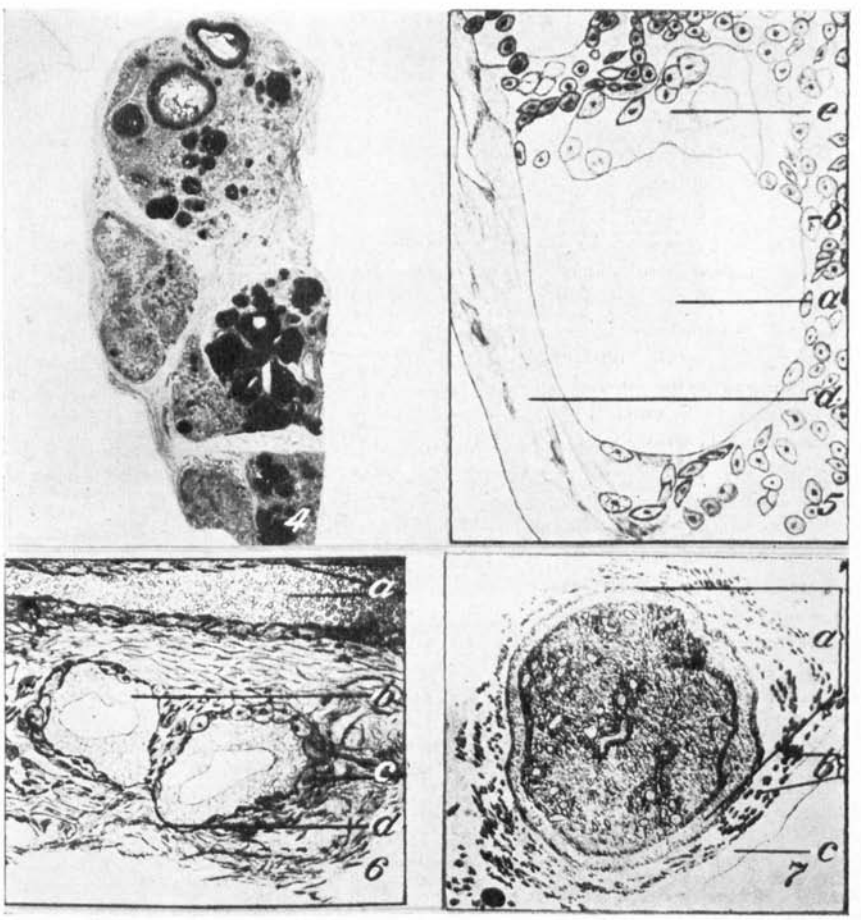

PLATE 2.