

SOME BASIC MEDICAL PROBLEMS ILLUSTRATED BY EXPERIMENTS WITH - CUTANEOUS LEISHMANIASIS*

ZVI EVEN-PAZ, M.B., CH.B. and FELIX SAGHER, M.D.

Department of Dermatology and Venereology, Hadassah University Hospital, Jerusalem, Israel

INTRODUCTION

In these days of modern communications, some of the natural barriers tending to limit the spread of diseases are being broken down. However, this paper has been written not because Oriental sore, or cutaneous leishmaniasis, may appear in South Africa, but because this condition lends itself especially to the study of some basic medical problems by methods not usually possible in other diseases. Problems related to immunity and bacterial allergy are cases in point. It is not permissible, for example, to infect human volunteers experimentally with the causative organisms of tuberculosis or syphilis. Oriental sore, however, is usually only of cos-

*Paper delivered during a lecture tour of South Africa, March-April 1960.

metic importance, and we have been fortunate in being able to carry out experiments on groups of volunteers over a period of many years. The subjects included some who had never had the disease, but who wished to be immunized against it, some in whom the infection persisted, and some in whom the disease had completely healed.

CLINICAL TYPES OF LEISHMANIASIS

The species of flagellate protozoa known as leishmania is found in a wide tropical and subtropical belt encircling the entire world. The flagellate is usually transmitted to humans by the bite of the sand fly. In the human tissues the parasites

lose their flagellae and are found in reticulo-endothelial cells as small oval-shaped bodies called Leishman-Donovan or LD bodies. The different clinical pictures produced depend chiefly on the strain of parasite involved. The internal viscera may be affected, provoking a severe and often fatal systemic disease, such as kala azar. Destructive, mutilating lesions of both skin and mucous membranes are typical features of American muco-cutaneous leishmaniasis. Yet a third type is almost exclusively cutaneous, and this form is the one seen in the Middle East, among other regions. Some synonyms for cutaneous leishmaniasis are Oriental sore, Baghdad boil, Aleppo boil, and Jericho boil. The rather picturesque Hebrew name for the disease means 'the rose of Jericho'. The causative organism is *Leishmania tropica*. Further remarks in this paper apply only to this purely cutaneous form of the disease.

CUTANEOUS LEISHMANIASIS—EARLY AND LATE FORMS

The original lesions arising at the sites of sand-fly bites were at first the only clinical forms recognized. The early lesion usually starts as a bluish-red papule or nodule, which may resemble a furuncle, but is relatively painless and persists for many months. Ulceration frequently occurs (Fig. 1), but this is not invariable. Highly atypical forms, such as verrucous, vegetating, erysipelas-like or eczematous lesions may develop, and by virtue of these forms leishmaniasis in some endemic areas has challenged syphilis for the title of 'the great imitator'. Solitary lesions are common, but sometimes many hundreds may be present, being naturally most frequent on exposed areas such as the face, arms and legs. Spontaneous healing is the rule, but the process is slow and may take a year or more. A sharply outlined, depressed, and somewhat pitted scar remains, and



Fig. 1. Early cutaneous leishmaniasis in the ulcerated stage.

Fig. 2. Late or recurrent cutaneous leishmaniasis. Chronic inflammatory papules, many of which have coalesced, within the borders of the scar of an early lesion and extending beyond its limits. On diascopy the papules are of apple-jelly colour. The resemblance to lupus vulgaris is striking.

if this is on the face the cosmetic effect is most unfortunate. Most patients become permanently immune to further infection.

The late development of new papules within the healed scar, or near its borders (Fig. 2), was observed in about

10% of a series of 600 cases studied in our department. Such papules may remain small and discrete, or coalesce. On diascopic examination they assume an apple-jelly colour. There is usually a tendency to healing in the centre of the involved area, but gradual extension occurs at the borders. The clinical picture thus strikingly resembles that seen in lupus vulgaris, and since the histological structure of the papules is typically tuberculoid, it is not surprising that earlier observers considered such cases to be due to tuberculous infection in a leishmaniasis scar. That, in fact, these cases represent late or recurrent leishmaniasis has now been very convincingly demonstrated.¹

DIAGNOSIS : CULTURE AND INTRACUTANEOUS TESTS

The diagnosis of early cutaneous leishmaniasis is not usually difficult. Although the clinical form may vary, LD bodies can be seen in smears made from the lesions, and in biopsy

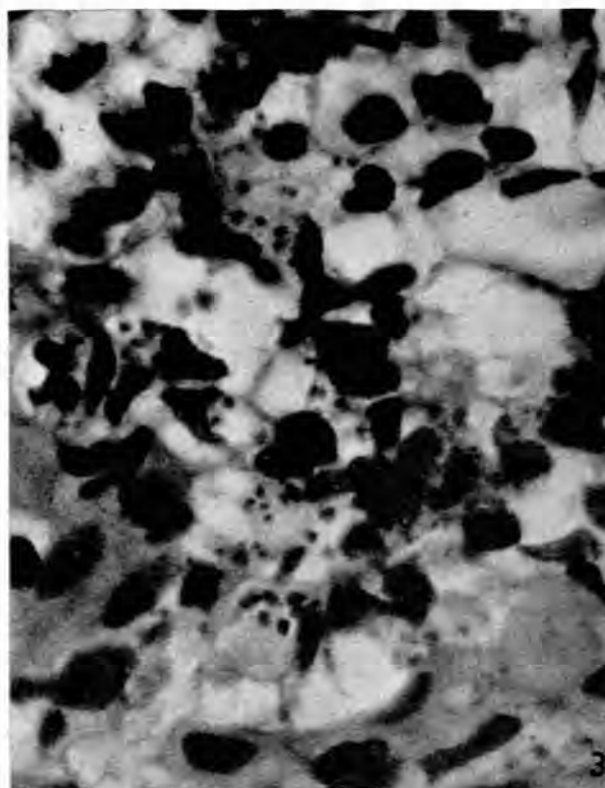


Fig. 3. Numerous Leishman-Donovan bodies in section from lesion of early cutaneous leishmaniasis (haematoxylin and eosin $\times 700$).

specimens (Fig. 3). The flagellate form can also be grown in suitable culture media. When the lesion is healing, from about the sixth month onwards, parasites become more difficult to demonstrate. Fortunately, a further diagnostic aid exists in the form of the highly specific leishmanin test.²⁻⁴ For the test, 0.1 ml. of a suspension containing about 100,000 killed leishmania flagellates is injected intradermally. The result is read 48 hours later, as in the tuberculin test. The test was positive in over 95% of the cases with early leishmaniasis in our series, and in 98% of cases with the late form of the disease. A non-specific positive reaction occurred in only 2.2% of controls not suffering from the

disease.⁵ The ability to react positively probably persists for life.

In the late or recurrent form of leishmaniasis, parasites are almost never detected in smears or in histological sections. They may still be discovered by careful cultural techniques, although even then by no means readily. The leishmanin test, however, is strongly positive. The late form seems to result from a state of incomplete immunity. The tissues react to the presence of even small numbers of parasites by building around them a wall of granulation tissue, but this

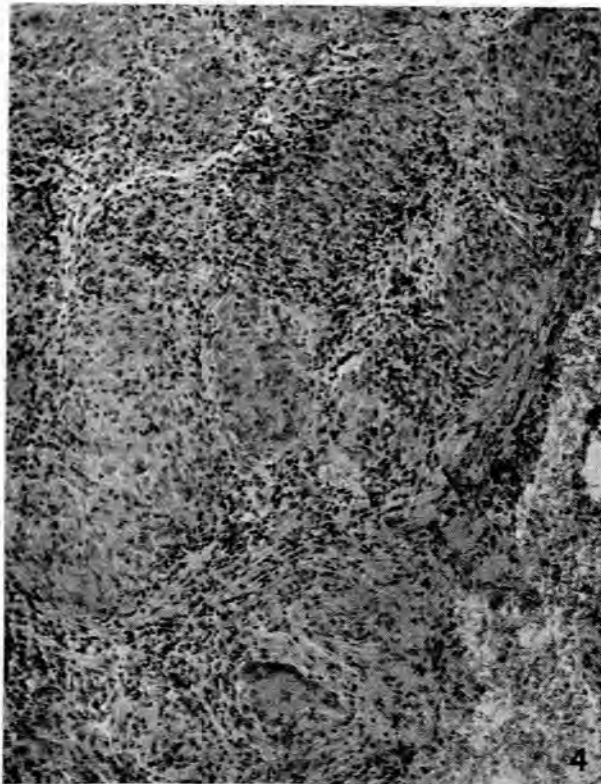


Fig. 4. Tuberculoid structures in lesion of late leishmaniasis. No Leishman-Donovan bodies were present (haematoxylin and eosin $\times 145$).

attempt to 'wall-off' or destroy the invader is only partially successful. The histological structure of the lesions is typically tuberculoid (Fig. 4).

Some clinical and special diagnostic features of early

and late cutaneous leishmaniasis are summarized in Table I.

EXPERIMENTAL INVESTIGATIONS

Experimental work on cutaneous leishmaniasis has covered many different aspects of the disease. Workers at the Hadasah-Hebrew University Medical School in Jerusalem have taken an active part in these investigations, and it was largely owing to the pioneering efforts of Adler and Theodor⁶⁻⁸ and of Dostrovsky⁹ that the sand fly, *Phlebotomus papatasi*, was shown to be the insect vector in our region. Dostrovsky was one of the first to draw attention to the late form of the disease,¹ and to the diagnostic value of the leishmanin test⁴ and of cultures.¹⁰

The particular experiments to be described in this paper are connected with a study of the incubation period, of immuno-allergic phenomena, and of treatment in the late recurrent form.

Incubation Period: Variation According to Number of Living Flagellates Injected

The incubation period of naturally occurring early leishmaniasis seems to be from 2 to 5 weeks in most cases. However, it may be protracted to many months or even years. Are such variations in some way connected with the parasite itself, or is host susceptibility the determining factor? It has been estimated that about 20,000 leishmania flagellates are injected by the bite of a naturally infected sand fly. When suspensions containing about 0.5 million cultured parasites were inoculated into volunteers by Berberian¹¹ the disease developed after an interval of 2 weeks - 3 months. In Katzenellenbogen's series¹² the average period was 4 - 6 weeks when suspensions containing approximately 1.5 million cultured flagellates were used.

In experiments in our department, inoculations of from 2 to 16 million parasites were given to volunteers and to members of the staff.¹³ When the inoculum contained 2 million parasites the incubation period was only 3 - 6 days, and when 4 - 16 million parasites were injected there was no interval between the immediate inflammatory reaction to the injection and the development of the lesions. After the injection of 16 million parasites an abscess rapidly developed at the inoculation site, the pus from which contained numerous LD bodies, but was otherwise sterile.

The fact that the experimental incubation period after 0.5 - 1.5 million parasites are inoculated is similar to that occurring when a much smaller number is injected naturally by a sand-fly bite, might indicate a reduced virulence in the

TABLE I. DIAGNOSTIC FEATURES OF CUTANEOUS LEISHMANIASIS

	<i>Early cutaneous leishmaniasis</i>	<i>Late cutaneous leishmaniasis</i>
Clinical features	Indolent inflammatory nodule or nodules. May ulcerate, or resemble furuncle, erysipelas, tumour, etc.	Destructive chronic inflammatory process with apple-jelly nodules and atrophic scarring
Healing	Usually heals spontaneously in about one year, leaving depressed, pitted scar	Healing tendency is partial. Lesions may persist for 20 years or more
Histology	Polymorphous cellular infiltration, mainly histiocytic	Tuberculoid structure
Leishmania parasites	Present in smears. Culture positive	Absent in smears. Culture positive
Leishmanin test	Active stage: positive Healing stage: strongly positive Healed scar: negative	Strongly positive

cultured parasites. However, although virulence of the parasite and host susceptibility may play some part in determining the incubation period, the experiments described, using much larger numbers of parasites, seem to indicate that the most important factor is the quantity of flagellates injected. In fact, there seems to be no true incubation period at all, in the sense that this exists, e.g. in malarial infections. In malaria the incubation period is largely governed by the life cycle of the parasite. In cutaneous leishmaniasis the form of the parasite also changes in the human host, from flagellate to LD body, but this seems to be a relatively simple process which can occur very rapidly, and apparently in the skin itself.

Conclusions drawn from these experiments may be applicable to some other diseases in which it is more difficult to make such direct observations. Syphilis is a case in point. Here too, after the parasites have been directly inoculated into the skin, a primary lesion arises at the site of infection. The incubation period of syphilis is variable, but is usually about 3 weeks. Might not this incubation period also be chiefly determined by the number of spirochaetes inoculated, which in most cases of naturally acquired infection is probably roughly the same? Inadequate penicillin therapy given before the appearance of the primary chancre may delay its development for many months. Although this might be owing to a diminution in the virulence of the spirochaetes, it could also be a result of a reduction in their number.

Immunity: Does Natural Infection Result in Immunity?

Cutaneous leishmaniasis was at first thought to be invariably self-healing within about a year, to be followed by lifelong immunity to further infection. This must still be considered true in the majority of cases. However, as indicated previously, immunity was incomplete in some 10% of our series, and the late or recurrent form of the disease developed. Berberian¹¹ showed that, in experimentally induced early cutaneous leishmaniasis, an additional early lesion could be produced by further inoculation with living parasites. Such super-infection only succeeded if the original lesion was relatively recent and still contained LD bodies. If the process of healing had already begun all attempts at super-infection failed.

These results were confirmed in our department, and further investigations were made. The immunity of subjects who had fully recovered from either naturally occurring or experimentally induced cutaneous leishmaniasis was challenged by the inoculation of from 1 - 5 million cultured leishmania parasites.¹⁴ No successful re-infection was obtained, but in all cases redness and swelling, followed rapidly by ulceration, occurred at the inoculation site. The reaction developed during the first few days and was sometimes accompanied by fever, general malaise, and joint pains. Histologically, the inflammation was non-specific and no LD bodies were present. The ulcer healed within 2 - 6 weeks. Such a typical accelerated reaction, which corresponds to

the second part of Koch's fundamental experiment in tuberculosis, implies the presence of an almost absolute immunity.

'Isophasic Reaction' in Late Leishmaniasis

Similar experiments were carried out on patients suffering from the late recurrent form of leishmaniasis.^{15,16} They were each given two inoculations, of 1 million and 10 million parasites respectively. In these cases super-infection succeeded. A most interesting observation was that the lesions that developed resembled those of late, and not early, leishmaniasis. A very brief nodular phase was rapidly superseded by the development of an infiltrated area containing apple-jelly nodules. No LD bodies could be detected in smears from the lesions; leishmania parasites could be cultivated, but only with difficulty and not in all cases; and the histological picture was typical of that of the late stage of the natural disease.

Super-infection can therefore occur in both early and late leishmaniasis, but the type of lesion that will develop is not determined by the parasite but by the host, and resembles that of the phase of the disease which is present at the time. For this reason we called this phenomenon an 'isophasic reaction'.

These results recall those of certain experiments on super-infection carried out many years ago in early and late stages of syphilis. Here, too, there was no absolute immunity to super-infection, and the pathological response corresponded to that of the phase of the disease in the recipient host. However, in these experiments spirochetal-containing tissue was inoculated, while in the leishmaniasis experiments suspensions made from pure cultures were used.

The results of these experiments on immunity in cutaneous leishmaniasis are summarized in Table II.

Allergy: The Different Reactions to the Injection of Killed Leishmania Flagellates in the Early and Late Forms of the Disease

That the positive leishmanin test is an allergic reaction was suggested by the fact that positive Prausnitz-Kuestner type passive-transfer tests were obtained,⁵ and that the reaction could be inhibited or delayed by simultaneous injection of hydrocortisone into the same site.¹⁷ The leishmanin antigen usually used contained the bodies of about 100,000 parasites in the 0.1 ml. which was inoculated. To determine any difference in the sensitivity to leishmanin in the different phases of the disease, experiments were performed using varying progressive dilutions of the antigen.¹⁸ Patients with early leishmaniasis were found to be no longer sensitive to dilutions which contained less than about 10 killed parasites per 0.1 ml. Most patients with late leishmaniasis, however, remained sensitive to much higher dilutions, even to those which presumably no longer contained any parasites at all, but only some substance derived from them.

Allergy: Hypo-ergy or Anergy in Post-leishmaniasis Scars

Experiments on the leishmanin test in scar tissue proved

TABLE II. EXPERIMENTAL SUPER-INFECTION IN CUTANEOUS LEISHMANIASIS

<i>Early leishmaniasis</i>	<i>Healed leishmaniasis</i>	<i>Late leishmaniasis</i>
Succeeds if parasites are still detectable.	Fails. Accelerated reaction, corresponding	Succeeds. Isophasic (late type) lesion
Early type lesion produced. Fails in healing stages	to second part of Koch's experiment in tuberculosis	produced

to be of particular interest.¹⁹ In the scar resulting from completely healed early leishmaniasis, the test was negative or the reaction very weak, even if the inoculum contained five times the number of killed parasites normally used. In the same patients the reaction was strongly positive, not only in the normal skin, but also in scar tissue from other causes, such as smallpox or burns. Healed leishmaniasis scar tissue is thus hypo- or anergic, but the precise relationship of this phenomenon to immuno-allergic mechanisms is not clear. Somewhat parallel results have been obtained with luetin antigen in scars of syphilitic lesions. From a practical point of view it is possible that the leishmanin test might help in identifying a particular scar as being due to leishmaniasis, and might also indicate whether the healing is complete or recurrence is possible.

Allergy: Time Required for Development of Allergy to Leishmanin

The period required for sensitivity to leishmanin to develop was the subject of further experiments.²⁰ The time at which a natural leishmania infection occurred cannot usually be determined, and the studies were therefore made on volunteers given the disease by the intradermal injection of 2 - 16 million living flagellates. The leishmanin test was previously negative. All of these subjects developed lesions of early leishmaniasis with practically no incubation period. The leishmanin test was still negative 24 and 48 hours after inoculation, but in all cases it was positive after 72 hours. The positivity became more marked with the age of the lesion.

It is usually considered that about 10 days are needed for the body to develop detectable sensitivity to any new antigen. Whether sensitivity to leishmanin may develop as rapidly as in these experiments after a much smaller number of parasites has been introduced naturally, *via* the sand-fly bite, is unknown. If quantitative differences can actually affect the speed of development of allergic sensitivity, these results might be of considerable interest with regard to the general theory of bacterial allergy.

THERAPY: CLINICAL EXPERIMENTS

Early cutaneous leishmaniasis does not usually present a difficult therapeutic problem. The tendency to spontaneous cure is reflected in a responsiveness to a wide variety of physical and chemical agents. However, none of the methods which are successful in the early form are satisfactory in the late, relapsing type. It was thought likely that the defence barrier thrown up around the parasites in the form of tubercloid granulation tissue, while only partially limiting the spread of the infection, served to protect the parasite against the full force of therapeutic measures. To break down the host's natural defence barriers deliberately is usually not desirable, but in special circumstances it may be asked whether this effect may not be harnessed to a controlled therapeutic procedure. It was speculated that by the aid of steroids the granulation-tissue barrier in the active nodules might be breached or destroyed, and previously inaccessible foci might then become vulnerable to therapeutic agents. This line was pursued in a series of cases of late leishmaniasis

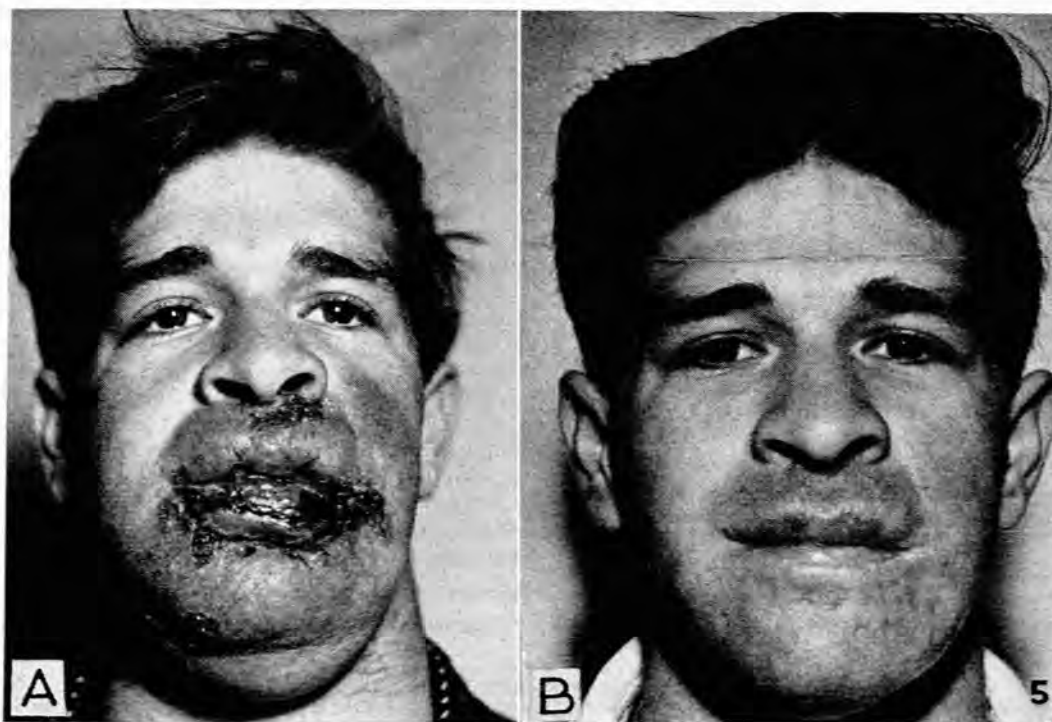


Fig. 5. Effect of combined steroid and antimony therapy in a particularly severe case of late leishmaniasis. A. The condition actively progressing in spite of more than 10 years' treatment by numerous accepted procedures. B. The condition a little more than one year later, following treatment by oral cortisone, local injections of hydrocortisone, and intramuscular injections of antimony.

in which all other accepted treatments had been unsuccessful.²¹ Intramuscular injections of an organic antimony preparation, known to be active against the leishmania parasite, were combined with cortisone or prednisone given orally. Partial regression of the lesions occurred. In an attempt to increase the local concentration of the drugs, direct injection of hydrocortisone or prednisolone into the apple-jelly nodules was substituted for the oral treatment. The results obtained can be described as varying from good to excellent (Fig. 5 A and B).

Treatment, given once or twice weekly, must be continued for many months, and sometimes lasts well over a year. The injections are painful, and since the lesions are usually on the face (frequently the lips, cheeks and nose) not all patients find the treatment acceptable. Nevertheless, very few patients have defaulted, which is a measure of the steady and marked improvement that occurs. In a few cases in which antimony was not tolerated, streptomycin was injected, also with good results. No systemic effects from absorption of steroids were observed, and in no case was there spread or generalization of the disease.

These clinical investigations, therefore, seem to indicate that in cases of late cutaneous leishmaniasis previously

resistant to all accepted forms of treatment, supplementary steroid therapy may prove of great value.

The aim, in discussing these aspects of what for South Africa is a 'far-away' disease, has been twofold: to show that even what is apparently a purely cutaneous condition is intimately linked with problems of general medicine, and to illustrate how dermatological investigation forms a not insignificant part of experimental medicine as a whole.

REFERENCES

1. Dostrovsky, A. (1936): *Ann. Trop. Med. Parasit.*, **30**, 267.
2. Wagener, E. H. (1923): *Univ. Calif. Publ. Zool.*, **20**, 477.
3. Montenegro, J. (1926): *A.M.A. Arch. Derm. Syph.*, **13**, 187.
4. Dostrovsky, A. (1935): *Ann. Trop. Med. Parasit.*, **29**, 123.
5. Dostrovsky, A. and Sagher, F. (1946): *Ibid.*, **40**, 265.
6. Adler, S. and Theodor, O. (1925): *Ibid.*, **19**, 365.
7. *Idem* (1926): *Ibid.*, **20**, 355.
8. *Idem* (1927): *Ibid.*, **21**, 111.
9. Dostrovsky, A. (1926): *Ibid.*, **20**, 385.
10. Dostrovsky, A. and Sagher, F. (1946): *A.M.A. Arch. Derm. Syph.*, **54**, 543.
11. Berberian, D. A. (1944): *Ibid.*, **49**, 433.
12. Katzenellenbogen, I. (1944): *Ibid.*, **50**, 239.
13. Dostrovsky, A. (1945): *Acta med. orient. (Tel-Aviv)*, **4**, 303.
14. Sagher, F., Verbi, S. and Zuckerman, A. (1955): *J. Invest. Derm.*, **24**, 417.
15. Dostrovsky, A., Zuckerman, A. and Sagher, F. (1952): *Harefuah*, **43**, 29.
16. Dostrovsky, A., Sagher, F. and Zuckerman, A. (1952): *A.M.A. Arch. Derm. Syph.*, **66**, 665.
17. Dostrovsky, A. and Cohen, H. A. (1957): *J. Invest. Derm.*, **29**, 15.
18. Sagher, F. (1947): *A.M.A. Arch. Derm. Syph.*, **55**, 658.
19. *Idem* (1947): *Brit. J. Derm.*, **59**, 205.
20. Dostrovsky, A. (1948): *J. Invest. Derm.*, **10**, 435.
21. *Idem* (1958): *Brit. J. Derm.*, **70**, 288.