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by

R. VANBREUSEGHEM

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Foreword

At the time of his death in 1950, Dr. Maurice Langeron had been engaged in preparing a second edition of his admirable *Précis de Mycologie*. The completion of this work then devolved on his friend and former pupil Professor R. Vanbreuseghem. In the new edition, published in 1952, the work was divided into three parts. The first, on General Mycology, represented the greater part of the original book, and preserved its outstanding characteristics. Descriptions of technical procedures were collected together and formed the second part. The third part devoted to Medical Mycology was an entirely new feature contributed by Professor Vanbreuseghem, which greatly enhanced the value of the book. This part which has been translated into English by Dr. J. Wilkinson is the subject of the present publication.

The translation has been made as literal as is feasible and the subject is presented to the English reader without addition to or alteration of the original text.

Jacqueline Walker

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Preface

Professor R. Vanbreuseghem's Introduction to the Second Edition of Langeron's *Précis de Mycologie*

. . . . quoniam non potest fieri quod vis
Id velis quod possit.
Terence (Andrienne)

When in July, 1950, Mme. Langeron and Dr. Langeron's family entrusted me with the preparation of the second edition of the *Précis de Mycologie*, I accepted with hesitation for fear of detracting from the value of my master's work. I expected to have at my disposal the manuscript to which Dr. Langeron had told me he had devoted himself since the publication of the seventh edition of his *Précis de Microscopie*. It proved, however, impossible to find the essential document. I had, therefore, to be content with what Mme. Langeron was kind enough to hand over to me: an interleaved copy of the first edition of the *Précis de Mycologie*, together with numerous offprints or microfilms collected with a view to the second edition. This material, together with what I personally possessed, formed the basis of the book I am now privileged to present.

The first edition comprised eleven chapters. I have divided them into three sections: General Mycology, Technique, and Medical Mycology. General Mycology is based essentially on the first edition, but the distribution of the material has been considerably modified. Additions have been made to it from Dr. Langeron's notes and from my own reading. The second part, devoted to technique, follows the plan previously adopted but has been supplemented by new procedures which have appeared since 1945. For the third part, Medical Mycology, I take full responsibility. This topic was, in the first edition, limited to Chapter IX, entitled: "What does medical mycology amount to?" This title must be regarded as an appropriate means of rounding off the volume, but at the same time it showed clearly Dr. Langeron's intention to put the reader on guard against those who are always ready to create new mycoses on the strength of some mould isolated from a refractory case. Dr. Langeron used to say to me, "When people don't know which way to turn [which saint to invoke], they think of fungi." I have completely replaced this ninth chapter by an entirely new third part in which the mycoses are studied in alphabetical order. It consists of seventeen chapters. I have endeavoured to omit nothing essential, even of the most recent work, and I have, wherever possible, set out what is known of animal mycology.

I feel that in this form the second edition would in no way run counter to the views of my late friend.

It may seem paradoxical to say that a scientific book owes more to many others than to its author. It is however true, and particularly of the present volume, which rests essentially on the work of Dr. Langeron and on the experience of his long and fruitful career. Mme. and Mlles. Langeron have been extremely courteous in helping me gather the material necessary for this second edition and have greatly honoured me by entrusting me with the spiritual inheritance of one whom I knew so well and so greatly admired.

Professor Edmond Sergent, whom I met when he visited the Pasteur Institute in Algiers, kindly undertook the introduction of the present work to French readers; a more eminent ambassador could scarcely be imagined, and I owe him a great debt of gratitude.

The Institute of Tropical Medicine at Anvers has put at my disposal its spacious laboratories and its remarkable library. For this I am indebted to its distinguished Director, Professor Dubois, to whom I tender my warmest thanks.

The interest shown by the Institute of Scientific Research in Central Africa (I.R.S.A.C.) in numerous branches of science led it to trust me with the carrying out of investigations in the medical mycology of the Belgian Congo, and to subsidize my research for several years. I particularly owe my thanks to M. le Ministre De Bruyne, President of I.R.S.A.C., Prof. J. Rodhain, President of the Commission of Human and Animal Pathology. Prof. L. Van Den Berghe, Director of I.R.S.A.C. in Africa, Prof. J. P. Harroy, General Secretary of I.R.S.A.C., and all the members of the Administrative Council.

Professors Gérard and Renaux, of the University of Brussels, have given me their constant moral support, and Prof. A. Dalcq has given me much invaluable advice. I owe them my deepest gratitude.

My thanks are also due to Miss L. K. Georg (U.S.A.) and Dr. M. Para (Brazil) for the fine microphotographs they have allowed me to use.

Many doctors and public health inspectors in the Belgian Congo have helped me considerably by sending material. I am particularly indebted to Drs. Borgers, Kivits, Lejeune and Mathieu, and to the Chief Inspector of Public Health, M. Doom. Mlle. Van Hoof proved to be the most considerate of librarians, and Mlle. Van Reusel was, as laboratory assistant and secretary, an indispensable daily help. My best thanks are due to all.

I am also infinitely grateful for the care taken by Masson et Cie in publishing this book, and for their courtesy and attention always.

My wife, who already had many claims to my affection, has now acquired even more of it by helping me materially and morally throughout the preparation of this work.

If I were allowed to dedicate the book, it would be to the memory of two remarkable men of science, who both at different stages contributed to the development of my scientific career: Professor André Gratia, of

Liége, who was, with Flemming, a pioneer in the field of antibiotics; and Dr. Maurice Langeron, whose *Précis de Microscopie* has rendered, and continues to render, the greatest service in laboratories all over the world.

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Contents

CHAPTER I

Mycoses caused by Actinomycetes

Introduction

At first glance it would seem appropriate to use the term "actinomycosis" for diseases produced by members of the Actinomycetaceae, but this is no longer justifiable. In the first place, the designation actinomycosis traditionally refers to the classical cervicofacial actinomycosis; this disease is quite different from other mycoses caused by members of the Actinomycetaceae, except for certain mycetomas produced by actinomycetes. Again, the actinomycetes have been so confused with one another that their precise taxonomy has only recently been clarified by the work of Waksman and Henrici (1943). In this, the Nocardioses were considered to be distinct from classical actinomycosis. Widespread ignorance of certain fundamental characteristics of Actinomycetaceae, e.g. whether aerobic or anaerobic, acid-fast or not, has invalidated much comparatively recent work. The separation of certain diseases included in this chapter (erythrasma, trichomycosis) from the actinomycoses and nocardioses is considered to be justified not only on clinical grounds, but also because of lack of information as to the real nature of their causal agents. No attempt will be made to refer to the very interesting antibiotic properties of actinomycetes; for this subject the outstanding work of Welsch (1947) should be consulted.

Broadly speaking, the actinomycetes are micro-organisms possessing either a rudimentary mycelium or a well-developed one not exceeding one micron in diameter. The former comprise the family Mycobacteriaceae with but one genus, *Mycobacterium*; these are bacteria. The latter, with a true mycelium, are nearer to the fungi. They comprise two families: the Actinomycetaceae, which reproduce by mycelial fragmentation, and are divided into two genera, *Actinomyces* and *Nocardia*; and the Streptomycetaceae, which reproduce by conidia, and comprise the two genera *Streptomyces* and *Micromonospora*. *Actinomyces* and *Nocardia* will be dealt with in due course. The characters of the genera *Streptomyces* and *Micromonospora* may here be summarized to avoid repetition; these two genera are included in the family Streptomycetaceae by Waksman and Henrici (1943), who distinguish them by the possession of a vegetative mycelium which does not fragment into bacilliform or cocciform elements and reproduces by means of spores.

Genus *Streptomyces* Waksman and Henrici, 1943. *Streptomyces* species

are undoubtedly the most widespread of all Actinomycetes. According to Skinner, Emmons and Tsuchiya (1948) they represent 20–50 per cent of the colonies obtained by spreading soil on agar, but they are equally abundant in the atmosphere. Most of the work on Actinomycetes is really concerned with *Streptomyces*. These are aerobic non-acid-fast forms, the mycelium of which fragments but little, and which reproduce by conidia arranged in short chains. These are frequently of spiral form, and the conidiophores (sporophores) are simple or branched. In the soil they convert nitrates to nitrites, break down proteins into simpler compounds, and attack chitin and carbohydrates of as complex a nature as starch and cellulose. They are not pathogenic for man or animals, but several species attack potato tubers (*Streptomyces* [*Actinomyces*] *scabies*). The specific identification of *Streptomyces* is very difficult, not only because the species' characters have been poorly resolved, but also because only very few species have been adequately described.

The formation of spores in short chains is one of the essential features of the genus. Unfortunately, the appearance of these spores is not constant under all environmental conditions. Jones (1949) has shown recently that one fifth of the 1,298 isolates made from different soils exhibited no constant morphology of the aerial mycelium, and that after the first incubation, 6 per cent of the isolates formed only vegetative mycelia; whence arises the possibility of confusion with the genus *Nocardia*.

Goret and Joubert (1951) have, however, isolated from a "septicemic actinomycosis of the dog" an actinomycete with all the characters of a *Streptomyces* (*S. galtieri*).

Genus *Micromonospora* Erskov, 1923. *Micromonospora* includes aerobic and non-acid-resistant actinomycetes with reproductive spores borne either singly upon the aerial mycelium or aggregated in masses. This is as yet a little-known genus, of which all the species are apparently saprophytic, and may be isolated from soil, dung and decaying organic matter.

Waksman and Henrici (1943) have tabulated the chief characteristics of the actinomycetes as follows—

ORDER ACTINOMYCETALES

A. Family **Mycobacteriaceae** Chester.

Mycelium rudimentary or absent.
Genus *Mycobacterium* Lehmann and Neumann.
Acid-fast organisms.

B. Families **Actinomycetaceae** and **Streptomycetaceae.**

Forming a true mycelium.

I. Family Actinomycetaceae Buchanan.
Vegetative mycelium fragments into bacilliform or cocciform elements.
(*a*) Genus *Actinomyces* Harz, 1877.
Anaerobes or microaerophiles, parasites, Gram-positive, non-acid-fast.
(*b*) Genus *Nocardia* Trevisan, 1889.
Aerobes, Gram-positive, acid-fast or non-acid-fast.

II. Family Streptomycetaceae Waksman and Henrici.
Vegetative mycelium not fragmenting into bacilliform or cocciform elements.
(*a*) Genus *Streptomyces* Waksman and Henrici, 1943.
Reproduction by conidia borne in small chains upon aerial filaments.
(*b*) Genus *Micromonospora* Œrskov, 1923.
Reproduction by terminal spores borne singly upon short sporophores.

A. ACTINOMYCOSIS

Definition

Actinomycosis is a chronic disease caused by *Actinomyces israeli* and characterized by a granulation tissue which abscesses and discharges through sinuses. The pus frequently includes sulphur-yellow granules. The disease attacks man and cattle.

There appears to be general agreement on the designation of the disease; but in the literature one occasionally comes across the names streptothricosis, nocardosis or nocardiosis. This last term must be kept for diseases caused by *Nocardia* species. The expression "actinomycotic mycetoma" has also been used by Langeron (1936) to designate all diseases caused by *Actinomyces* species which give a clinical picture of mycetomas and to distinguish them from maduromycotic mycetomas caused by hyphomycetes. Lignières (1924) and also Spitz proposed the term Actinophytosis to designate "all diseases in which one finds clubbed granules, including the name of the microbe which causes them."

Historical

The first case of human actinomycosis appears to have been described in 1857 by Lebert since when, in 1876, Bollinger described the disease in cattle. Harz, in 1877, called the pathogenic agent *Actinomyces bovis*, relying upon the origin and appearance of the parasite within the tissues, but he did not obtain cultures. In 1890 Bolström isolated an aerobic strain which he considered to be *A. bovis*, Harz, but which was obviously not the organism responsible for the classical actinomycosis.

In 1891 Wolff and Israël isolated under anaerobic conditions of culture an *Actinomyces* which Israël had previously isolated in 1884; Kruse in 1896 named this *Actinomyces israeli*.

Magnusson's work in Sweden has shown *Actinomyces israeli* to play a somewhat more exclusive rôle in the classical lesions of bovine actinomycosis. It has also shown that the clinical syndrome of actinomycosis, with granules surrounded by clubs, must be produced in the ox by three different organisms: 1. *A. israeli* which above all attacks bony tissue and produces the rarefying osteomyelitis so characteristic of the jaw (48 out of 135 cases, about 41 per cent); 2. *Actinobacillus lignieresi* (actually classed amongst the Bacteria under the name *Bacillus purifaciens*), which, in the ox, is situated in the soft parts, especially the tongue (wooden tongue) (55 cases out of 135); 3. The pyogenic staphylococcus which produces mammary actinomycosis (3 cases out of 135) and forms, like the preceding two, clubbed granules.

In man, the classical actinomycosis with clubbed granules is almost always produced by *A. israeli*. Actinobacillosis is extremely rare; Langeron (1941) has shown that the three cases previously described were neither actinobacillosis nor actinomycosis.

The pertinent question is whether the organism causing actinomycosis should be called *Actinomyces bovis* Harz, 1877, or *A. israeli* Kruse, 1896. Many English and American authors favour the first of these names: even though Harz did not culture the organism, they maintain, he was nevertheless the first to describe it properly, and he would not in any case have given it any description other than its present one. Langeron, on the other hand, insists upon the undeniable fact that Harz did not isolate the parasite, and that the law of priority is inapplicable because Harz might have mistaken lesions caused by other organisms for those of actinomycosis. Further, the parasite was later isolated and described completely by Wolff and Israël, and it should therefore bear the name which Kruse first gave it, i.e. *Actinomyces israeli*. Again, Rosebury (1944) who, either alone or with collaborators (1944) has paid considerable attention to this organism and its isolation, wrote: "The name *Actinomyces israeli*, applied to the parasitic form, conforms with international usage. The specific name is attributed to Kruse by the Argentinian workers, Negroni and Bonfiglioli, who have adopted it, as have Grootten in France, Puntoni in Italy, and Waksman in the U.S.A. It would seem best to dispense with the names *Actinomyces bovis* and *A. hominis*, since both of these have been employed with so little discrimination that their use requires the addition of the qualification "of the type Wolff Israël' before they can be applied without ambiguity to the parasitic form. The name *Actinomyces israeli* is perfectly justified by the fact that Israël was the first to recognize its parasitic nature and that Wolff and Israël were the first to culture it from actinomycosis, thus widening the bases of our real knowledge of the etiology and pathogenesis of this disease."

Importance and Geographical Distribution

Actinomycosis is the most widespread of the serious mycoses; it is found wherever it is looked for, and at all ages. The frequency of attack

in men is, however, twice that in women, and by far the greater proportion of cases appear in poor populations, especially in rural areas.

Etiology

Ancient beliefs, still widely held by medical men, would have it that actinomycosis is contracted by eating grass or wheat ears. This view is false, the organism responsible for actinomycosis never yet having been isolated in the saprophytic state.[1] However, the fact that the disease is most widespread amongst farming populations seems to lend support to the idea. Probably the relative lack of hygiene in such communities is responsible for the greater number of cases.

It has indeed been shown that *Actinomyces israeli* is normally present in the buccal cavity, and has been recovered from dental tartar and tonsil crypts. Several cases of actinomycosis have arisen shortly after dental extraction. Emmons (1938) isolated an *Actinomyces* which he identified as *A israeli* from 11 per cent of 200 cases of tonsilectomy, and recognized it microscopically in 37 per cent of these cases. In no case had actinomycosis been detected clinically.

Pathogenic Agent

Only one agent, namely *Actinomyces israeli* Kruse, 1890, causes actinomycosis. The organism in question is filamentous, branching, perhaps segmented, with a strong tendency to become transformed into bacilliform or cocciform elements. It develops no specialized reproductive structures.

The cultures present different appearances according to the media employed. In broth, they grow at the bottom of the tubes in the form of a whitish cloud, the medium remaining clear. In liquefied agar they grow especially in the region ½–1 cm below the surface. According to Grootten (1934): "immediately beneath this (surface) zone there is one 2–4 mm with a concentration of small colonies frequently presenting a cloudy appearance. Deep down in the agar the colonies are much less numerous and of different dimensions, barely able to attain a diameter of 2–3 mm. The colonies appear to be wedge-shaped, but they are really made up of several biconvex lenses set at various angles."

Surface cultures *in vacuo* or in an atmosphere of carbon dioxide exhibit colonies which are white, scaly and strongly attached to the culture-medium from which it is difficult to detach them with a platinum wire.

For microscopic study, a cover-slip must be dropped on the surface of a young colony; the staining procedures of Gram or modified Ziehl may be carried out upon the cover-slip. It will then be apparent that one is dealing with a filamentous fungus with branching hyphae no more than 1 μ in diameter. Most of these hyphae will, however, be reduced to short segments and the phenomenon of "angular growth" may be evident.

[1] This point requires some modification, as several strains have recently been isolated from soil.

described by Œrskov and bearing his name. This occurs as follows: after segmentation of the Actinomyces filaments, the segments enlarge and repel one another by their ends so as to assume an angular configuration. The segmentation of the filaments is still very controversial and appears to be most clearly apparent in young colonies.

In tissues or exudates, *Actinomyces israeli* takes the form either of branching, Gram-positive and non-acid-fast filaments, or of sulphur-yellow granules. These granules vary greatly in shape and dimensions. Frequently they are invisible to the naked eye, but may sometimes be 1–2 mm in size. Their colour is traditionally described as sulphur-yellow (sulphur granules of English and American writers), but many describe them as white or yellowish-white. They are usually of soft consistency and may easily be crushed between cover-slip and slide. Occasionally, however, hard and calcified granules are encountered. It is only too frequently stated that the colonies of *Actinomyces israeli* are visible to the naked eye; in general, this is incorrect, and a diagnosis of actinomycosis must not be rejected without a search for granules under the microscope. Moreover, the presence of these granules is not absolutely necessary for diagnosis.

Actinomycosis granules freshly pressed out under the cover-slip present on examination a polygonal or polycyclic appearance with, in the centre, a tangle of filaments which gradually open out towards the periphery, where they persist as what the French writers call "*massues*" and the English "clubs." These clubs are acidophilic. By using Gram's staining method it can be demonstrated that the filaments retain the gentian violet, whilst the modified Ziehl procedure shows that they are not acid-fast.

The clubs which envelop the actinomycosis granule are not a constant feature and are not indispensable for diagnosis of the disease. Granules both with and without clubs may indeed be found in the same pus. The origin of the clubs is still very debatable, but they may well represent a reaction on the part of the organism sensitized towards the pathogenic effect of the *Actinomyces israeli*. Certain workers, however, have apparently seen the clubs in culture.

It should be borne in mind that these clubs may be produced by parasitic organisms other than *Actinomyces israeli*, e.g. by staphylococci and by *Actinobacillus lignieresi*, and also by a great many other pathogenic fungi such as *Sporotrichum*, *Phialophora*, etc.

Symptomatology

Actinomycosis may be localized in many sites; the most usual, however, are cervicofacial, pulmonary and abdominal. Besides these, cerebral forms have been described which are really due to *Nocardia* and which should not be included within the strict boundaries of the actinomycoses, the purely cutaneous forms, the ocular and gastric forms, and so on.

Cervicofacial Actinomycosis

This is the most frequent form and was found in 56·8 per cent of the series of cases studied by Cope (1938). The lesions usually first appear upon a swelling in the angle of the lower jaw. The skin over this swelling, which soon becomes tough and woody, is reddish-violet in colour. The surface becomes irregular and soon exudes pus from several openings. In this pus occur the characteristic granules.

Often this is preceded by dental trouble such as extraction, or a tonsilectomy.

The infection may extend to the pharynx and the orbit, and there may be invasion of the salivary and lachrymal glands.

Diagnosis presents no difficulty.

Pulmonary Actinomycosis

This is less frequent (22·3 per cent in Cope's series) and is seldom recognized before it has produced a fistular condition in the thoracic wall. In the absence of means of ascertaining its true diagnosis, it most nearly simulates tuberculosis. Signs of discharging pleurisy may be evident before the infection makes its external appearance, but in most cases it is the development of a cutaneous abscess, quickly followed by fistularization, which permits of diagnosis.

In the pulmonary form, as well as in the abdominal form with which it may be confused, there may be an emission of particles, by way of the buccal cavity, which enclose the *Actinomyces israeli*. However, both the pulmonary and the thoracic forms may be derived one from the other, and the pulmonary may frequently complicate the abdominal form.

Can *Actinomyces israeli* be isolated from bronchial secretions in the absence of actinomycosis? This appears to have been confirmed in a recent and very interesting investigation by Kay (1948). This worker has searched systematically for *A. israeli* in patients with bronchiectasis, chronic abscesses and suppurations extending from the lung, and has isolated it in 50 per cent of the cases. The secretions were first obtained by bronchoscopy or from operative fragments. In one quarter of the positive cases, actinomycosis granules were found. It is acknowledged that the presence of *Actinomyces*, without necessarily producing the disease complicates it and may lead to a true actinomycosis with a characteristic development of sinuses.

Abdominal Actinomycosis

This would appear to be caused by the disintegration of particles containing *Actinomyces israeli* or to result from an extension of the pulmonary form.

This form (15 per cent of Cope's 1,330 cases) is scarcely ever diagnosed before a laparotomy or an autopsy. The most frequent clinical indication is pain, resembling that of appendicitis, in the lower right region of the abdomen. Palpation reveals a soft lump in the ileocaecal region. In the

absence of operation, the infection may extend towards the muscles of the anterior abdominal wall and become fistular, or it may get to the vertebral column, attacking the vertebra and causing nervous troubles. A good many cases of *parametritis actinomycotica* have been described, and amongst the various explanations, Strange (1950) has pointed out the possibility of infection by an external route (repeated digital dilatation of the anus).

Histopathology

For a specific histopathological diagnosis, actinomycotic granulations must be shown to be present in the tissues. Without these, the picture is insufficiently complete to permit of diagnostic conclusions. The exceptional presence of delicate, solitary filaments might incline the observer towards a diagnosis of actinomycosis. Stained with eosin-haematoxylin, the granules would show a deeper central region and a redder periphery, since the clubs have a special affinity for eosin.

The granules make up the centre of a granulation tissue, where white globules, giant cells and eosinophiles are found. Polymorphonuclear cells, the disintegration of which leads, according to certain workers, to the formation of clubs, are most directly in contact with the granule.

Following up previous deductions, there may be found a copious suppuration or an internal sclerosis; in either case, in the absence of the typical granules, a diagnosis of actinomycosis would not be warranted.

The use of Gram's staining procedure upon histological sections will differentiate any Gram-positive filaments in the granule. If the granulation should be caused by *Actinobacillus lignieresi* the bacilliform elements within the granule are Gram-negative. Should the granule be produced by a staphylococcus, the form of the latter is easily recognized.

Treatment

Although certain claims have been made for the treatment of actinomycosis by potassium iodide administered orally (3 drops of a saturated solution 3 times daily, increased by 1 drop per day up to 20 drops 3 times daily), or sodium iodide intravenously (1 g p.d.), prognosis has been considerably modified by the introduction of sulphamides and penicillin.

Surgery is of some value in removing irreparable damage (pulmonary, abdominal, ileo-appendicular and bony lesions respectively), and X-rays in certain inflammatory conditions. Thus, Lamb, Lain and Jones (1947), for cervicofacial actinomycosis, administered 150 r for two whole days up to a total dose of 1,500–2,200 r at a distance 30–6 cm with a 4–6 mm aluminium filter, or 1 mm aluminium + 0·25 mm copper filter, for a kilovoltage 120–40.

The sulphamides, prefefably sulphadiazine, may be administered for 5–6 months, maintaining a level of 5–10 mg per cent. Penicillin, of which the active dose is not well established, brings about rapid amelioration. In practice, there is a tendency to combine penicillin and sulphamides.

Prognosis

The prognosis of actinomycosis is best in the purely cutaneous forms, and good in the cervicofacial forms; it is frankly bad in the pulmonary and abdominal forms.

On the whole, what is known of the prognosis of actinomycosis has emerged from results obtained before the era of sulphamide- and penicillin-therapy, and will doubtless be revised.

Differential Diagnosis

Only the cervicofacial form of actinomycosis presents a clinical picture which can be recognized; this is not to say, however, that its diagnosis must be resolved solely on a clinical basis. It is to be expected that a disease of such chronic evolution and polymorphic symptomatology as actinomycosis must inevitably be confused with the most varied diseases, from liver abscess complicating an abdominal form and simulating a hepatic amebiasis, to pulmonary tuberculosis or cerebral tumour.

Mycological Diagnosis

This depends upon (i) the examination of actinomycosis granules in the pus, and (ii) the isolation of *A. israeli* in culture. The histopathological diagnosis is less relevant for actinomycosis than for other deeply-seated mycoses, for it may often be replaced by careful examination of the granules in the pus.

1. Examination of Granules in the Pus

Pus may be obtained from the sinuses, diluted with physiological saline and examined in a petri-dish, placed against a dark background or filtered on gauze. As already mentioned, granules are not always visible to the naked eye, and they occasionally attain a size of 1 mm, but never more than 2 mm. They should be carefully examined with a hand-lens or, whilst in the petri-dish, under the binocular microscope.

Conant *et al.* (1948) recommend the application on the sinuses of dry sterile gauze from the meshes of which granules may be obtained the morning after the application.

The granules are small masses of irregular shape, whitish or yellowish, soft and, exceptionally, calcified.

2. Culture of Actinomyces israeli

Cultures of *A. israeli* are difficult to obtain because the parasite is anaerobic or microaerophilous; and though it may be easy to grow and isolate an anaerobe in pure culture or from pathological products which only contain anaerobes, the isolation is much more difficult from contaminated sources. Further, *A. israeli* is a delicate organism and exacting from the nutritional point of view. Lastly, this parasite can only with difficulty be maintained in the laboratory, it requires frequent sub-culture, and even so the cultures eventually die. Most workers seem unable to

maintain their strains for more than 3–4 months. However, according to Rosebury (1944), if the strains die out in spite of persistent attempts to maintain them upon a given medium, they may be perpetuated more easily by transplantation upon different media, as in a rotation.

Several media are suitable for the culture of *A. israeli*, for example nutrient agar with the addition of 1 per cent glucose; Dorsett's egg medium or glycerine egg medium; nutrient broth; and a medium which has given Rosebury the best results, namely Difco's Bacto brainheart infusion added to 2 per cent agar. This culture-medium, which has the advantage of a relatively stable composition, may also be improvised in any laboratory.

A. israeli varies from strain to strain and from one day to another in its tolerance for oxygen; but for satisfactory results it must always be cultured under conditions of partial or total anaerobiosis.

The easiest method of ensuring partial anaerobiosis is to introduce the material for culture into the bottom of a tube of nutrient broth or nutrient agar to which has been added 1 per cent glucose or Bacto brainheart infusion in 2 per cent agar, kept at the bottom of the test-tube, liquefied on the water-bath, and inoculated after cooling but before solidification of the agar ("shake cultures"). The actinomycosis granule, collected under as sterile conditions as possible and washed in several cubic centimetres of physiological saline, is placed in a first tube and crushed against the side of the tube, which is shaken to ensure the even distribution of the granule fragments in the bulk of the liquid agar. After this a portion of the agar is transferred to a second tube and this is repeated to the extent of five or six tubes with the object of getting well-isolated colonies.

The tubes are kept at 37°C and after 5 or 6 days are examined with a view to microscopical study of the colonies and further transplantation. The broth cultures lend themselves well for microscopical examination, but on account of frequent contamination are of little use as a source of pure colonies. On the other hand, agar cultures show well-isolated colonies developed a little below the surface; good examples have a particularly abundant accumulation of colonies 1 cm below the surface. By means of a Pasteur pipette a white colony is withdrawn having a surface "formed by several biconvex lenses set at various angles" (Grootten), and a film is smeared out for staining by Gram's method for the recognition of intact, branching Gram-positive filaments or else bacilliform elements exhibiting the angular growth of Œrskov. One or more colonies should be similarly withdrawn for transplantation.

If the inoculum is obviously very contaminated or if it is impossible to isolate the granules, the procedure recommended by Rosebury, Epps and Clark (1944) is advised. The "Bacto brainheart infusion" added to 2 per cent agar is introduced into petri-dishes, then 1 ml of sterile broth is poured over the surface of the agar in each dish for better exposure. The material to be cultured is streaked into several (4) petri-dishes *without*