Pathology. — *"On a micro-organism grown in two cases of uncomplicated Malignant Granuloma."* By ERNESTINE DE NEGRI and C. W. G. MIEREMET. (Communicated by Prof. C. H. H. SPRONCK).

(Communicated in the meeting of September 28, 1912).

In recent years Malignant Granuloma, also called Lymphomatosis granulomatosa or HODGKIN's disease, has occupied the attention of many writers and researchers, in consequence of which some more light has been thrown upon the subject after a long period of obscurity.

For all this, the etiological evidence brought forward in the study of this incurable disease is still extremely limited.

In 1832, it is true, HODGKIN [1]) published the history of some cases and autopsies which may, to a certain extent, bear on the disease we are about to discuss, but its etiology was not dwelt on in the literature before many years later.

No attempt whatever had been made to distinguish by differential diagnosis the various diseases, characterised by glandular swellings and enlargement of the spleen, until VIRCHOW, in 1845, described leukaemia as a well defined disease. Next, in 1865, COHNHEIM distinguished pseudoleukaemia as a disease of the lymphatic apparatus resembling leukaemia, but differing from it by the absence of the typical bloodpicture. Since COHNHEIM the term pseudoleukaemia has again and again been misapplied to a congery of glandular diseases; others again added the epithet "tubercular" to it, so that in spite of COHNHEIM's discovery, the confusion was again as great as before.

Neither did BILLROTH [2]) confine the term "malignant lymphoma", a name often given to malignant granuloma, to one special affection of the glands, as he himself says in his paper on Multiple Lymphome.

STERNBERG [3]) was the first to describe in an elaborate histological investigation a definite group of cases, thereby leading the way for later workers. He was likewise the first to discuss at length the etiology of the disease, as appears distinctly from the title of his publication: "Ueber eine eigenartige unter dem Bilde der Pseudoleukamie verlaufende Tuberkulose des Lymphatischen Apparates." However the etiology, suggested by the title, is not nearly ascer-

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[1]) 1832 HODGKIN. On some morbid appearances of the absorbent glands and spleen. (Med. chir. Transact. Vol 17).

[2]) 1871 BILLROTH. Multiple Lymphome. Erfolgreiche Behandlung mit Arsenik. (Wien. Med. Woch. N°. 44 S. 1065).

[3]) 1898 STERNBERG. Ueber eine eigenartige unter dem Bilde der Pseudoleukaemie verlaufende Tuberkulose des Lymphatischen Apparates. (Zeitschr. f. Heilk. Bd XIX S. 21).

tained in this writing, though we must admit, that the nature of the available cases were adapted to tempt the writer to draw his conclusions. For in the great majority of cases, reported by STERNBERG, there was tuberculosis, besides the special granulation tissue described by him. As moreover in most cases tubercle bacilli were found in the histological preparations and only seldom cocci, which had caused no local reaction, so that he supposes them to have multiplied post mortem, he concludes: "dasz es eine eigenartig verlaufende Form der Tuberkulose des lymphatischen Apparates gibt".

The fact that there appears the peculiar granulation tissue, as described by him, and not a pure tubercular tissue, STERNBERG believes to be probably due to higher or lower resistibility of the patient or to the virulence of the tubercle bacillus.

At the "Siebente Tagung der Deutschen Pathologischen Gesellschaft", held in 1904, where this subject was discussed, BENDA [1]) advanced the theory that here we have to do with "ein sich den malignen Neubildungen näherndes Granulom welches nicht durch einen spezifischen Infektionstrager, sondern durch die modifizirten oder abgeschwächten Toxine verschiedener Infektionsträger hervorgerufen wird". ASKANAZY believes the etiology to be wholly unknown. CHIARI and YAMASAKI consider the process as a chronic inflammation whose etiology has not been ascertained, but should not be mistaken for tuberculosis. ASCHOFF arrives at the conclusion, "dasz es sich nicht um die gewöhnliche Form der Tuberkulose handelt", appealing to his failure in producing tuberculosis in 5 typical cases by inoculation of caviae. Also STERNBERG qualifies his assertions when he writes: "Wenn auch die seither publizirten Fälle diese (his) Auffassung meist bestätigten, so räume ich doch gerne ein, dasz die damals von uns gewählte Bezeichnung "eigenartige Tuberkulose des lymphatischen Apparates" vielleicht zu weit geht. Immerhin glaube ich, dasz ein Zusammenhang zwischen dem diesen Fällen zu Grunde liegenden Entzündungsprozess und der Tuberkulose nicht von der Hand zu weisen ist".

A most valuable addition to our knowledge of malignant granuloma was furnished by E. FRAENKEL and H. MUCH's [2]) [3]) discovery of "granuläre Stäbchen", which they found to be antiformin-resistant and GRAM-positive. This at first seemed in a high degree confirmatory

[1]) 1904 BENDA. Zur Histologie der pseudoleukaemischen Geschwülste. (Verhandl. der D. Path. Ges. 7e Tagung 26—28 Mai).

[2]) 1910 E. FRAENKEL u. H. MUCH. Bemerkungen zur Aetiologie der Hodgkinschen Krankheit und der Leukaemia lymphatica. (Münch. Med. Woch. nº. 19).

[3]) 1910 id. id. Ueber die Hodgkinsche Krankheit (Lymphomatosis Granulomatosa) insbesondere deren Aetiologie. (Zeitschr. f. Hyg. u. Infekt. Kr. Bd. 67).

of STERNBERG's conception, considering that morphologically the granular "rods" could not be distinguished from the non-acidfast MUCH-form of tubercle bacilli. Experimental inoculation of caviae afforded conclusive evidence against STERNBERG's opinion. Caviae injected with granulation tissue obtained from uncomplicated cases were not affected by tuberculosis.

FRAENKEL and MUCH do not hesitate to call their granular rods the causative agent of malignant granuloma; however they are not decided about the question of their affinity with tuberculosis: "Die Lymphomatosis granulomatosa ist eine Infektionskrankheit, die durch granuläre Stäbchen hervorgerufen wird. Diese granuläre Stäbchen sind antiforminfest aber nicht säurefest; sie sind durch verschärfte GRAMfärbung darstellbar, und stehn dem Tuberkulose-virus zum mindesten sehr nahe. Die Lymphomatosis granulomatosa ist nach unseren Erfahrungen nur ausnahmsweise mit typischer Tuberkulose vergesellschaftet."

At a meeting held at Hamburg January 1912 FRAENKEL [1]) announced his discovery of "granula" or "granular rods" in 16 out of 17 cases. Availing himself of the additional evidence brought forward by MEYER, DE JOSSELIN DE JONG [2]) [3]) (who decidedly inclines to deny the identity of the tubercle bacillus with the virus of malignant granuloma both on the basis of his own experimentation and on the inoculation experiments of many other researchers), SIMMONDS and JAKOBSTHAL, FRAENKEL writes as follows:

"Es liegen jetzt über mehr als dreiszig Fälle Hodgkinscher Krankheit von den verschiedensten Beobachtern herrührende mit den unsern völlig übereinstimmende Aufgaben vor. Immerhin, das will ich offen bekennen, ist auch durch unsere Untersuchungen eine völlige Klärung der Aetiologie der Hodgkinschen Krankheit noch keineswegs herbeigeführt." And further on: "Es musz die nächste Aufgabe sein Reinkulturen der fraglichen Gebilde zu erzielen, und im Tierversuch weiter zu kommen".

It is evident that these researches did not throw more light upon the relation of the rods to the tubercle bacillus, as FRAENKEL [4]) him-

[1]) 1912 E. FRAENKEL: Ueber die sogenannte Hodgkinsche Krankheit (Lymphomatosis granulomatosa). (Deutsche med. Woch. n°. 14 S. 637).

[2]) 1909 R. DE JOSSELIN DE JONG. Bijdrage tot de kennis der pseudoleukaemie. (Geneesk. Bl. 14e reeks I en II).

[3]) 1911 id. Over acuut maligne granuloom (Lymphomatosis granulomatosa). (Ned. Tijdschr. v. Gen. II helft n°. 22).

[4]) 1912 E. FRAENKEL u. STERNBERG. Ueber die sogenannte Pseudoleukaemie. Bericht über die XVe Tagung der Deutschen Path. Ges. in Straszburg vom 15-17 April. (Centralbl. f. Alg. P. u. Path. An. Bd. 23, No. 10).

self declares in the meeting of the Deutsche Pathologische Gesellschaft in April 1912: "Die Frage der Stellung der Granula zu den Tuberkelbazillen ist noch offen; aetiologisch ist die Lymphogranulomatose unklar."

The death of a boy v. D. S., 7 years of age, suffering from malignant granuloma, clinically uncomplicated with tuberculosis, whose autopsy took place on the 4th of June 1912, $8^h\ 30^m$ post mortem gave us an opportunity to cultivate the "rods", so often alluded to above. At the autopsy no trace whatever of tuberculosis was detected, only alterations pointing to malignant granuloma. The histologic examination of the spleen, a great number of glands, the bone marrow and the liver, led to the discovery of the granulation tissue which, according to STERNBERG, characterises the disease, whereas the typical alterations due to tuberculosis were not found. Nor were caviae, injected with an emulsion of the granulation tissue attacked by tuberculosis.

In smears of the spleen we could demonstrate numerous rods fully corresponding with FRAENKEL and MUCH's description of the granular rods that are found in the typical granulation tissue of the majority of such cases as were studied for this purpose. No other micro-organisms could be detected in any of the preparations.

We have been successful in demonstrating the bacteria in only a few histologic preparations, as was the case with other workers on the subject. Whether or not this was due to the small number of organisms present, we are unable to say.

In order to obtain the wished-for result, we have sown from the spleen on a large number of varying media and we have been fortunate enough to grow at once, in all the media used, a pure culture of a micro-organism, which proved in every respect similar to FRAENKEL and MUCH's rod.

It was especially on the blood-glycerine-potato-agar plate, used by BORDET to cultivate the whooping-cough bacillus, that we obtained already after 2×24 hrs a strongly developed culture, which proved to consist of rods morphologically in no way differing from the granular rods.

Before entering upon a description of our micro-organism we point out the fact, that we succeeded in obtaining from a jugular gland (patient S, twenty years old), sent to us for diagnosis, a micro-organism similar to that obtained post mortem from the spleen of v. D. S. The histologic examination of this gland made us decide upon malignant granuloma in making the diagnosis of the typical tissue. Tubercular changes could not be detected in the preparations, neither

were they in any way suggested clinically. PIRQUET was negative.

Description of the Micro-organism.

Morphology.

We observed the following forms varying according to the media and the age of the cultures:

Plump short rods: length 1 μ, breadth $^3/_4$ μ. The shortness of some reminds us of coccobacilli of less than 1 μ diameter (a minority on LOEFFLER's serum; in eight-week-old cultures on Bordet medium almost exclusively; a majority on agar-plates a few days old).

Small fine rods: polar staining, length from $1^1/_2$—2 μ, breadth $\pm$ $^3/_4$ μ (in every medium of any age).

Rods of from 2—3 μ with polar granules, or more granules (they are far predominant in the older cultures on LOEFFLER's serum).

Comma-shaped rods: in many cases to be divided into two shorter rods, length $\pm$ $1^3/_4$ μ, breadth $^1/_2$ μ (on Bordet medium, ascites-agar, and LOEFFLER's serum; in the first ascites-agar-culture longer and finer than in the later).

Granular rods of different dimensions; length varying from 5—7 μ, breadth from $^3/_4$—$1^1/_2$ μ. This considerable breadth concurs with a prickly shape found on the Bordet-medium, the rods being broader in the middle and becoming more pointed towards the extremities. The greater breadth is in many bacilli due to the irregular arrangement of the protuberant granules.

In older cultures some giant forms, which however have not at all lost their original structure, i.e. a distinct body, in which the granules are seen.

Occasionally branching forms were observed in various media (Bordet-medium, fluid and solid, LOEFFLER's serum, and canesugar-nutrose).

Rows of granules: only granules arranged as in the granular rods but without a visible cell-body. The arrangement is not regular, the granules being placed longitudinally in different directions relative to the long axis of the granular rod or row.

Involution-forms: clubbed or swollen ends (in old cultures) and spheric forms to 2 μ.

Motility is lacking

Staining peculiarities: The microbe stains easily with the ordinary dyes for bacteria. After GRAM the small rods show polar coloration, positive or negative, according to the medium; the comma-shape always positive, the body of the granular rods negative, the granules positive.

After MUCH's modification of GRAM's methods the results agree with those obtained with the Gram-stain.

With ZIEHL's stain they are not acid-fast.

The microbe is facultative anaërobe, however it grows much better in presence of oxygen. Growth is sluggish in deep stab-cultures, covered with agar, and in a hydrogen-atmosphere.

Influence of temperature on the growth.

The growth optimum is in the neighbourhood of 32° C.

The highest temperature at which growth is demonstrable is 39° C.; at 40° C. it ceases altogether.

The lowest possible temperature for growth is between 10° and 8° C. At 5° C. it is non-existent.

Reaction of media: alkaline reaction is more conducive to growth, which however is not inconsiderable with acid reaction.

Growth.

Gelatin-stabculture: not liquefacient, slight growth in the track made by the needle, threadlike, getting thinner lower down.

Smear-culture: growing evenly in moderate amount.

Plate-culture (after 24 hours): cultures elevated on the surface, dark grey (later greyish-yellow to ochraceous), round, smooth-rimmed, homogeneous, dewdrop-shaped, dim-glistening. Later on the colonies are finely granular and the edge gets finely crenated.

Agar-stabculture: slight growth in the track, threadlike, ragged, getting thinner lower down.

Smear-culture: growing evenly in fair amount.

Plate-culture (after 24 hours): cultures elevated on the surface, yellowish, round, smooth-rimmed, somewhat granulous, granules finer near the rim than in the centre, where a dark stain is visible, dewdrop-shape, highly glistening, condensation water cloudy, no pellicle is formed.

Ascites-agar-plateculture: sluggish and slight growth; colonies finely granular, later here and there more coarsely granular especially at the periphery, so that the rim, being smooth at first, now becomes finely lobulated; elevated above the surface; fluorescence.

Young colonies dewdrop-shaped; highly glistening, condensation water as in agar-cultures.

Broth-culture: slow growth, cloudy with sediment, which squirms up like a slimy flagellum when shaken, and may be equally distributed. No pellicle is formed, as is the case in broth mixed with horse-serum, yeast-decoctum or ascites-fluid.

LOEFFLER's serum-smear-culture, growing abundantly in 24 hours, even, very slimy.

Plate-culture (24 hours): highly elevated above the surface; colour deep canary-yellow, later in part brownish-red, round, smooth-rimmed, uniformly finely granular, dewdrop-shaped, moist-glistening, condensation water very cloudy; no pellicle.

Milk is not coagulated; ultimately a pinkish coloration.

Glycerin-potato-culture: growing badly; hardly visible, light yellow; dim-glistening.

Blood-glycerin-potato-agar (Bordet medium):

Smear-culture: abundant growth in 24 hours; the culture first obtained was greenish, afterwards rather brown to brownish-black, chocolatelike, elevated above the surface; very slimy; easy confluence of colonies; glistening; condensation water cloudy.

Sporeformation not noted.

Resistance to:

Desiccation: cultures in fluid media did not lose vitality at room-temperature 11 weeks after drying.

Heating for half an hour at 60° C. kills off the culture; when heated for 5 minutes at 80° C. they are also destroyed.

Cold: cultures exposed for 4 hours to a temperature of —60° C. did not lose vitality.

Light: diffuse daylight does not kill the microbe; nor does it affect growth.

Lifetime: After 16 weeks the cultures have not yet died away.

Chemical conversions.

Formation of Gas: none in broth with glucose or lactose, neither in nutrose with canesugar.

Acid-production: in nutrose with glycose, mannite, maltose or canesugar.

Alkali has been detected in broth with yeast-decoct. After 5 weeks 1 ccm. $^5/_{10}$ n. acetic acid on 9 c.c. of broth with yeast-decoct. appears to be just neutralised.

H_2S is not produced.

No more is Indol.

Nitrates are not reduced to Nitrites.

Diastatic fermentation is absent.

Chromogenesis:

Canary-yellow mainly on LOEFFLER's serum; less intense on the other solid media (except Bordet-medium); also in the fluid media.

Muddy green: the first cultures on Bordet-medium.

Chocolate colour on Bordet-medium.

Faint fluorescence on ascites-agar.

Brownish-red in all older cultures except ascites-agar.

Poisonous products could not be demonstrated.

Thus far the microbe did not prove to be pathogenic for animals, but even now we wish to lay stress on the fact that all our laboratory-animals, among which a large number of caviae, some injected with organic emulsion, others with cultures, remained free from tuberculosis.

Summary.

The bacterium we have been describing, is to be classed as a corynebacterium on account of:

its septed structure,

its sometimes peculiar shape with pointed or clublike extremities,

its tendency to branching,

its lack of acid-resistance (after ZIEHL) but great affinity for other bacterium stains.

We feel assured that this corynebacterium is identic with FRAENKEL and MUCH's rods, observed by them and others in the tissue of malignant granuloma in a large number of cases.

In describing them FRAENKEL and MUCH mention their peculiar morphology, their affinity for stains, and the antiformin-resistance.

The morphological description of their rods agrees entirely with the morphology of our bacterium, as regards both the smears from the spleen and those from the cultures.

The ZIEHL- and the GRAM-stain are the same for either bacterium.

As to antiformin-resistance we discovered that it cannot be considered as a quality peculiar to this bacterium, though we too found some rods in antiformin-sediments of organic emulsion.

We do not intend to enter into further details in this short space. Further investigation will have to decide whether or not our coryne-

E. DE NEGRI and C. W. G. MIEREMET. „On a micro-organism grown in two cases of uncomplicated Malignant Granuloma".

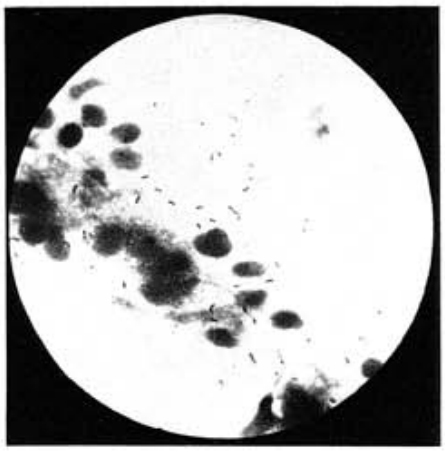


Fig. 1.

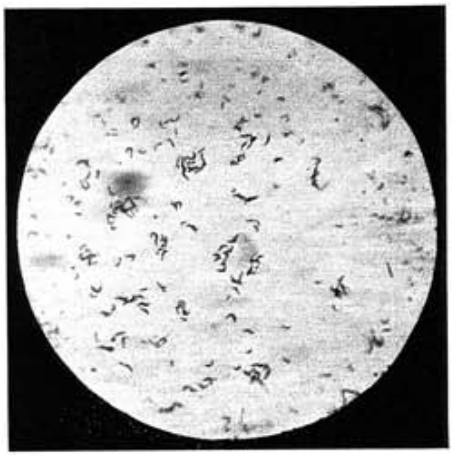


Fig. 2.

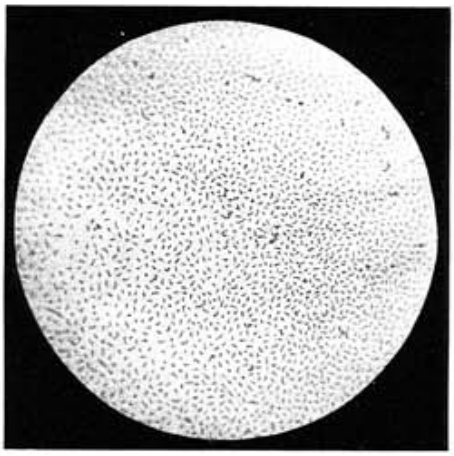
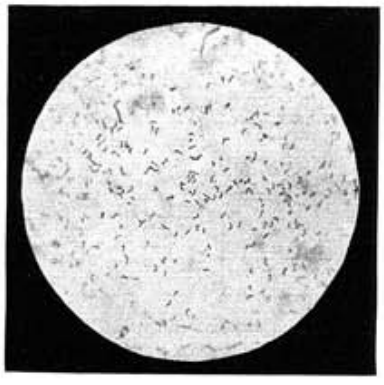


Fig. 3.

Fig. 4.



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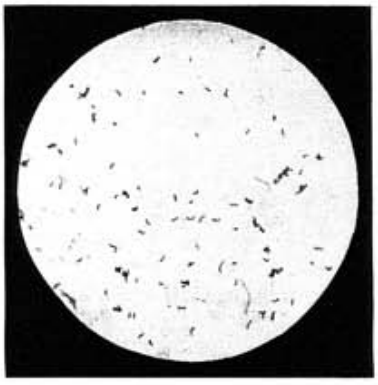

Fig. 6.

bacterium occurs invariably in malignant granuloma. In our opinion this seems to be the case, as may be concluded from the literature that appeared hitherto. Still, even if this be so, it would perhaps not by itself entitle us to consider that corynebacterium, beyond a shade of doubt, as the etiologic moment.

We purpose before long to write more at length about this subject in another publication.

EXPLANATION OF THE PLATE.

Fig. I Smear from the spleen v. d. S. Gram-stain with counterstain.
„ II 48 hours' Bordet-culture, cultivated directly from the spleen of v. d. S. Gram-stain with counterstain.
„ III 18 hours' Bordet-culture after one transplantation. Gram-stain without counterstain.
„ IV Rod with branches from fluid Bordet-medium. Gram-stain with counterstain.
„ V 5 × 24 hours' ascites-agar-culture, grown directly from the spleen of v. d. S. Gram-stain with counterstain.
„ VI LOEFFLER's serum-culture transplanted after 12 weeks from original LOEFFLER's serum-culture, obtained from a gland of patient S.

Physics. — "*Measurements on the ultraviolet magnetic rotation in gases.*" By Dr. J. F. SIRKS. (Communicated by Prof. KAMERLINGH ONNES.)

(Communicated in the meeting of October 26, 1912).

1. To get an idea of the relative values of the various theories which have been developed to explain magnetic rotation, measurements may be made in the neighbourhood of absorptionbands and -lines in the visible spectrum with a view to ascertain whether the rotation has the same[1]) or opposite sign[2]) on either side of the absorptionband. With perfectly transparent substances one could extend one's observations over a much wider region of the spectrum so as to ascertain if the experimental results obtained in the ultraviolet, for which the rotatory constants are much greater, can be more satisfactorily represented by the one theory than by the other, and if, perhaps, a strong increase in the rotation takes place on approaching the ultraviolet region.

With gases, and in particular with hydrogen, where, on account of their simple molecular structure ordinary refraction of light can well be represented by the assumption of a single kind of ultraviolet electrons[3]), and for which the value of e/m may be obtained from the

[1]) VOIGT, Magneto- und Elektro-optik p. 133.
DRUDE, Hypothese des Hall-effektes, Lehrbuch der Optik p. 429, 1906.
[2]) DRUDE, Hypothese der Molekularströme, Lehrbuch der Optik p. 419.
[3]) ABRAHAM, Theorie der Elektrizität II, p. 261, 1908.