

A REPORT OF THE BACTERIOLOGICAL EXAMINATION
OF ENLARGED LYMPH-NODES REMOVED FROM
A PATIENT WITH HODGKIN'S DISEASE *

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The question of the etiological basis of that type of lymph-node hypertrophy referred to as Hodgkin's disease, has for many years been of much interest. There have been two principal ideas in regard to this type of lymph-node hyperplasia; one that it is a neoplasm of the lymphoblastic elements of the lymph-nodes, the other that it is an inflammatory reaction accompanying or following an infection with some micro-organism which attacks especially the lymph-nodes.

The advocates of each of these theories have referred to the histological findings in the lymph-nodes as evidence in support of one or the other of the two theories referred to above. Among others, Gibbons,¹ has expressed the opinion that the enlarged nodes belong in the group of malignant neoplasms. Opposed to this view are McCallum, Longcope, Reed and others who have claimed that the histological findings in the lymph-nodes are those associated with a chronic inflammatory irritant of some kind. Professor Adami in his recent text-book expresses the belief that the lymph-node lesions in Hodgkin's disease are not those of a true neoplasm, but are of a chronic inflammatory nature.

There have appeared in the comparatively recent literature reports that deal with the demonstration of micro-organisms in the nodes themselves. Fränkel and Mutch have reported the results of their study of the enlarged lymph-nodes from a number of cases of "Hodgkin's disease." These authors were able to demonstrate in twelve out of thirteen cases the presence of micro-organisms in the excised lymph-nodes. These organisms were demonstrated in the material remaining after treating the tissues with antiformin. From the morphological and staining characters of the organisms found they were of the opinion that they were either a type of tubercle bacillus or that they belong to a type closely related to the tubercle bacillus.

The infectious basis of Hodgkin's disease has not been limited to the microscopical demonstration of organisms in the lymph-nodes. Negre and Meerimet report the isolation of an organism from the

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1. Gibbons: Am. Jour. Med. Sc., 1906, cxxxii, 692.

enlarged lymph-nodes removed from two patients with Hodgkin's disease. These organisms were recovered by the use of special medium. Their article gives a description of the organism isolated. The most recent work dealing with the etiology of this disease has been done by Bunting and Yates² and reported recently. In their first paper they report the results obtained from the bacteriological study of a series of enlarged lymph-nodes removed from patients whose clinical picture corresponded to that found in Hodgkin's disease, and whose lymph-nodes themselves showed the histological picture found in this disease. The general summary of the work on which this paper is based is as follows: From three out of five cases of Hodgkin's disease, in which a bacteriological examination had been made, a pure culture of a diphtheroid, pleomorphic organism has been isolated in pure culture. In the remaining two cases this organism was recognized, but not recovered in pure culture. The morphology, cultural and staining reactions of the organism recovered from one patient is briefly as follows:

They are facultative anaerobes, Gram-positive, not acid fast, and vary greatly in their morphology depending on a number of factors, e. g., their age, the medium employed and the fluid content of the medium. Pleomorphism is a pronounced feature, coccoid forms, long forms, beaded forms, club-shaped involution forms, are present. Branching forms are present especially on the egg medium. On certain media polar staining forms are present. Plate cultures show rounded, glistening, gray colonies which at the end of twenty-four hours gradually become opaque white. There is a central dark spot in the colony and a fine stippling of the growth.

Since the publication of the above article these same investigators have reported³ the results thus far obtained by inoculating animals with the diphtheroid organism they have isolated.

They have been able by repeated inoculations not only to infect monkeys (*Macacus rhesus*), but these animals have shown a blood-picture typical of that of early Hodgkin's disease and later have developed chronic lymphadenitis that corresponds grossly and microscopically with Hodgkin's disease in man, of the same duration. Not only have they succeeded in producing these gross and histological changes in the monkey, but they have recovered from the enlarged lymph-nodes a diphtheroid organism similar in every way to the organisms injected. In one of their articles they say:

Thus, since our experiments demonstrate that the diphtheria organism is pathogenic for monkeys, that it produces a progressive enlargement of the lymph-nodes, with lesions similar to those of Hodgkin's disease in man, and further that the blood changes in the monkey are similar to those in man, we feel fully assured of the etiologic relationship of the diphtheroid organism (*Bacterium hodgkini*) to Hodgkin's disease.

2. Bunting and Yates: Culture Results in Hodgkin's Disease, THE ARCHIVES INT. MED., 1913, xii, 236.

3. Bunting and Yates: Jour. Am. Med. Assn., 1913, lxi, 1803.

We have recently had the opportunity of making a bacteriological study of the enlarged lymph-nodes from a patient with Hodgkin's disease. This opportunity was made possible through the kindness of Dr. J. M. Elder, on whose service the patient was admitted to the Montreal General Hospital. The clinical history is briefly as follows:

History.—The patient is a white woman, aged 21. The present illness began two years ago, in 1912, with swelling in the right side of the neck. The swelling increased slowly and did not produce discomfort. In 1913, one year after the first symptoms were noted, a swelling appeared in the left side of the neck. About this time she had a normal pregnancy, after which she became gradually weaker, feverish and easily tired. On exertion she had dyspnea and palpitation of the heart. No pain was present. Patient thinks she lost about fifteen pounds in weight.

Patient has been married five years and has had two children (both died in infancy). She has always been well previous to present illness. There is no family history of tuberculosis.

Examination of Glandular System.—At the base of the neck there are two large masses on either side of the middle line. These masses are about 7 cm. in diameter. The skin is freely movable over them. The masses themselves are movable on the underlying tissues and on palpation are found to be made up of discrete individual masses varying in size from 1.5 to 4 cm. in diameter. The smaller growths have a distinctly elastic feel. There is one slightly enlarged lymph-node in the right axilla; otherwise the axillae are free. Below the right clavicle the superficial veins of the thorax are dilated and tortuous. This finding was interpreted as evidence of pressure on the mediastinal vessels. A roentgenogram shows a definite shadow in the mediastinum which corresponds to an area of dullness noted on percussion.

The edge of the spleen is just palpable. On percussion splenic dullness is 16 by 9 cm.

Blood examination shows the following:

White cells	14,200
Red blood-cells	2,440,000
Hemoglobin60 per cent.

Differential blood-count is as follows:

Polymorphonuclear leukocytes50 per cent.
Small mononuclears31 per cent.
Large mononuclears9 per cent.
Eosinophils9 per cent.
Mast cells1 per cent.
Blood-pressure.....	Systolic, 100; diastolic, 80

On January 26, 1914, Dr. Elder removed, under local anesthesia, three enlarged lymph-nodes from the left side of the neck. This operation was done with the greatest of aseptic precautions. The three nodes were at once taken to the laboratory for examination. Under careful aseptic precautions a portion of each node was taken for bacteriological investigation, and at the same time sections of the nodes were put into fixing solutions for histological study.

Pathological Report.—M. G. H., S-14-58. The specimen consists of three discrete, encapsulated lymph-nodes. They measure 2 cm., 25 cm. and 4 cm. in diameter, respectively. The nodes are loosely united by delicate fibrous tissue. Each node is enclosed in its own capsule and is only loosely united with those about it. The tissues immediately surrounding the lymph-nodes are edematous and contain numerous small blood-vessels. The nodes are only a little firmer than normal. On section they are of a uniform pale, grayish-white color, with a slight yellowish cast. The incised surfaces show a semitranslucent appearance. The following microscopical description is based on Zenker and formalin fixed tissues, sections from which have been stained with eosin and methylene blue, hematoxylin and eosin, Mallory's phosphotungstic acid, hematoxylin, and connective-tissue stain. Specimens from each node have been examined for bacteria both by means of direct smears made from the nodes and by means of staining sections for bacteria. Histologically the sections of the lymph-nodes show briefly the following: The pericapsular tissues are edematous and show a slight cellular infiltration. The infiltrating cells are for the most part lymphocytes. The capsules of the nodes are slightly thickened and infiltrated with lymphocytes. The lymph-node parenchyma has largely lost its normal histological characters. There are no germinal centers. There is a moderate, though not extensive, increase in connective tissue. This connective tissue increase is more marked in certain places. The most striking cellular change is the increase in endothelial leukocytes and the presence of large multinucleated cells. These multinucleated cells occur irregularly distributed throughout the section. There are but few eosinophils found. No necrotic areas or areas of acute inflammatory change are found.

In the direct smears from the nodes there are a few Gram-positive bacilli with morphology similar to the organisms recovered by means of culture. The organisms are few in number. Many fields are examined before one is found. None of them are intracellular. In sections from the nodes stained by Gram-Weigert's method and a modified Gram-Weigert's there are only a very few bacilli demonstrated. These organisms have always appeared as single organisms, they lie free in the tissue spaces and the cellular characters of the tissue immediately surrounding them is in no way different to that elsewhere in the nodes. A careful search for organisms within the cells, especially within the multinucleated cells, has been made. In no instances have we been able to find intracellular organisms.

The bacteriological procedure consisted in excising small bits of each of the lymph-nodes. Some of these were thoroughly macerated in warm sterile saline solution. Other pieces of tissue were inoculated directly in various mediums, the tissue first being thoroughly rubbed over the surface of the medium. The finely macerated tissue was inoculated in large and small amounts on various mediums. Both aerobic and anaerobic cultures were made and incubated at 37.5 C. The inoculated media were examined from time to time. At the end of eighteen hours one tube of human blood agar showed a growth of Gram-positive cocci in groups. None of the other mediums showed positive results until ten days after their inoculation, when a stained smear from one of the hydrocele agar tubes showed Gram-positive organisms. The organisms were mostly of the coccoid and cocco-bacillary type, though a few bacilli were present. Within the next two weeks, twenty-one days after their original inoculation, a number of the tubes showed organisms on stained smears. The presence of colonies on the original tubes could be definitely made out only on certain mediums. Growth was first recognized in the gross by a finely stippled-like appearance to the surface of the medium. The colonies were largest on the hydrocele agar. On this medium they developed near the water of condensation as small, almost transparent colonies. The surface of Dorset egg medium showed a fine stippled appearance. There slowly developed a grayish precipitate in bouillon, and in the water of condensation of the solid medium, especially where the water of condensation was abundant. In two of the tubes of hydrocele

agar, where large amounts of hydrocele had been added, a grayish cloudy growth developed at the bottom of the tube and gradually spread outward between the agar and the inner surface of the culture tube. In the original tubes of solid medium, growth was most abundant in those which contained large amounts of fluid. The presence of the pleomorphism of the organisms found in our original culture led us to the strong suspicion, at one time, that we were dealing with at least two different organisms. But by transplanting all colonies that showed few bacilli and many coccoid and coccobacillary forms on proper mediums, especially Dorset egg medium, bacillary forms predominated especially in the younger cultures.

A series of transplants has been made on various mediums and the organisms grown through several generations. The morphological characters of these organisms have varied greatly on different mediums, and under varying growth conditions. Cultures of the same age on various mediums show certain characters common to them all and certain characters that vary in the different mediums. They are all Gram-positive and non-motile and none of them is acid fast. The resistance to acid decolorization has been studied by using Gabbet's method and the Ziehl-Neelson method of staining for tubercle bacilli. In using the Ziehl-Neelson method varying dilutions of acids have been employed as decolorizing agents, and the results obtained compared with a young culture of tubercle bacilli (human type), *Bacillus typhosus* and *Staphylococcus pyogenes aureus*. These organisms were smeared on the same slide, and, in so far as possible, smears were made of uniform thickness. In the staining process each slide has been treated similarly. The following results have been obtained: Dilutions of nitric acid for decolorizing were employed from 30 per cent. strength down to 0.5 per cent. The organisms under consideration, *Bacillus typhosus* and *Staphylococcus aureus* were completely decolorized in all of the dilutions used, while the tubercle bacillus remained acid fast throughout.

The morphological characters of the organism in Dorset egg medium is as follows: Most of the organisms are of a definite bacillary type. Many of them show sharp, deeply staining granules within them. These granules may be either unipolar, bipolar, or bipolar with two or more deeply staining areas in the bodies of the organisms between the two poles. The form of the bacillus is not constant. Most of them are comparatively long and narrow and show varying degrees of curving. There are a few branching forms with or without polar bodies in them. As many as four branches have been found in one organism.

A series of roll cultures were made using agar with various percentages of fluid medium, as hydrocele, defibrinated blood, etc.

A series of these tubes was inoculated under both anaerobic and aerobic conditions. In all the roll cultures growth developed, but the most luxuriant growth took place in the anaerobic cultures, especially in those containing proportionately large amounts of fluid. In both the anaerobic and aerobic cultures colonies did not appear at all or only sparsely and slowly within the first 1 to 1.5 cm. of the upper surface of the medium. At this point there developed a narrow band of closely packed colonies. This band varied in width: in some of the tubes it reached 3 cm. in others it was only a few millimeters. Below this point the colonies were not nearly as numerous though they were present throughout the remainder of the mediums. There slowly developed a heavy grayish diffuse growth between the outer surface of the medium and the inner surface of the culture tubes. This growth was most marked in the lower half of the tube.

The organism as a rule has not grown in fluid medium as well as in the solid medium.

SUMMARY

We have isolated from the enlarged lymph-nodes of a patient who showed the clinical picture of Hodgkin's disease, and from whom excised glands histologically corresponded to this disease, a pure culture of a pleomorphic, Gram-positive, non-motile, non-acid fast, facultative anaerobic organism, similar to those described by Bunting and Yates.

Cultures of the organism isolated by us have been repeatedly injected into the tissues about the axillae of an adult monkey (*Macacus rhesus*), but up to the time of this report we have obtained no conclusive results.