5-1-1937

On the etiology of lymphadenoma

Don C. Vroman
University of Nebraska Medical Center

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ON THE ETIOLOGY OF LYMPHADENOMA

Don C. Vroman

Senior Thesis Presented to the College of Medicine, University of Nebraska, Omaha
1937.
Introduction.

From the time of Sir William Osler, lymphadenoma or Hodgkin's disease has been attacked from all sides. The search for the etiological factor has allowed for the speculations of all the pathologists and all the bacteriologists. My selection of this subject is the result of a discussion begun in our medicine clinic; and I take as my argument to uphold, that Hodgkin's disease is not an infectious granuloma.

Thomas Hodgkin was born at Tottenham, England, August 17, 1798. He graduated in medicine from Edinburgh in 1823. He then pursued his study in France and Italy. Later he became curator of Guy's Hospital museum and a demonstrator of morbid anatomy. He was, then, a contemporary of Aston Key, Thomas Addison, Richard Bright, and Braxton Hicks. (30)

While Curator and demonstrator he presented in 1832 his paper "On Some Morbid Appearances of the Absorbent Glands and the Spleen" (12), citing six cases. He wrote that the glandular enlargement appeared to be a primitive affection, not apparently inflammatory since no pain, redness, or ordinary symptoms of inflammation were present. He further wrote that the enlargements pre-
sented a uniform texture throughout. This observation he took to be significant and indicated a difference from tuberculous findings wherein the infectious agent acting in the center caused changes to be induced about it. Another circumstance which attracted his attention was the state of the spleen. This organ he found to be more or less diseased, and sometimes thickly pervaded with well defined bodies of various sizes and resembling in structure the diseased glands.

With this short historical introduction I shall proceed to a consideration of the further work done on this subject.
Spirochaetes in Hodgkin's Disease.

White, having noticed the frequency with which spirochaetes were attracted to lymphatic tissue and knowing the frequency with which spirillar forms were present in the mouth and familiar with the frequent history of enlarged tonsils preceding Hodgkin's disease, applied specific strains for spirochaetes to the glands of Hodgkin's disease.

He obtained two typical glands. The first was sectioned and stained by Levaditi's method; the second was stained by Giemsa's method. The sections presented innumerable spirochaetes.

The sections prepared were given to Dr. Proscher, pathologist of the Allegheny General Hospital for a second examination. He at once pronounced them luetic glands, remarking that he had never seen so many spirochaetes in luetic tissue. Dr. Proscher was unaware of the source of the material. (29)

Dr. P.F. MacGinnis, pathologist to the Chesterfield and North Derbyshire Royal Hospital, found on examination of the cervical glands of two consecutive cases of Hodgkin's disease a large spirochaetes in considerable numbers. (16)
The observations of these three men would, upon superficial examination, lead to the following conclusions: that spirochaetes are the etiologic factor in the glandular enlargement, that the glands were secondarily infected, or that Hodgkin's disease may be a mild form of lues. However a closer examination of this work done, shows that it is obviously incomplete, and one is not entitled to draw definite conclusions from it.

A very thorough and complete work has been carried out under the Rose Research grant. This work consisted of examination of six successive cases of lymphadenoma. The procedures consisted first of direct examination of fresh material consisting of the juice of freshly removed glands, blood, and occasionally urine from cases with the Pel-Ebstein fever, and also juice taken from the spleen, liver, and suprarenals after death.

The second procedure consisted of attempts to cultivate the spirochaete from lymphadenoma material. This was checked or controlled by attempts to culture the spirochaete from pieces of primary chancre and lymphatic gland from a case of primary syphilis.

The third procedure consisted of examination of sections treated by silver impregnation methods. Control sections from cases not affected by lymphadenoma, even
cases of accidental death were utilized to check the stains on lymphadenoma sections.

The fourth procedure consisted of animal experiments. Guinea pigs and rabbits were utilized; and attempts were made to infect them by three routes, subcutaneously, intravenously, and intracerebrally.

The results of the above examinations are easily summed up—they were all negative. In the cultural attempts, spirachaetes were cultured from the control cases only; and in the silver impregnation studies spirilla bodies not unlike spirochaetes and very similar to those described by White and Proscher, and Mac Ginnis were found in all the lymphadenoma cases and, more important, in all control cases.

A search for the presence of spirochaetes in lymphadenoma, therefore, has given negative results.
Mycotic Infection in Hodgkin's Disease.

Recent additions to our knowledge of the etiology of granuloma have considerably widened our views and have furnished evidence that lesions closely resembling those of the tubercle both macroscopically and microscopically. E.C. Dias of the Oswaldo Cruz laboratory, Brazil, has brought forward evidence by which he claims to prove that Hodgkin's disease is in point of fact a mycosis.(9)

By puncturing a hypertrophied gland under strictly aseptic precautions, he found in the ganglionic juices a series of polymorphous microbial elements which could only be classed as pathogenic fungi. He designated the microbial elements (by an arbitrary value only) as bacilliform, yeast cells, oblong forms, hyphae, and indeterminate forms. He also claims finding them in the bloodstream.

Using Sabourand's media, he made cultures using the fluid obtained by puncture. Cultures were obtained in fifty per cent of cases; some developed within forty-eight hours, others only after a week or more. At first they were a whitish or yellowish, smooth, moist, of slight consistence, and with a tendency to flow along
the length of the tube when held vertically. These re-
produced by a sporulation similar to actinomycyes.

In studies of their pathogenic action, several
animals were found to be inoculable. These were the
guinea pig, rat, and monkey. The animals usually suc-
cumbed to the inoculation. The duration of the experi-
mental disease was variable.

He concludes from the above evidence that the
causative agent of Hodgkin's disease is an extraordinary
polymorphous fungus which is found in the lymphatic
ganglia of the patients, is cultivable, and is pathogenic
for laboratory animals. He has named this fungus the
Adenomycosis Cruzi, and believed that, in view of this
etiology, the name Adenomycosis should be given this
disease!

In a close review of this work one is astounded at
the audacity of this worker in drawing such sweeping con-
clusions without a histologic examination of the glands
studied, and without any control material whatsoever.

Again the Rose Research workers came forward with
a thorough and careful study of this problem. A one to
twenty suspension of glands was made in distilled water.
Three tubes of Sabourand's glucose agar and three dupli-
cate tubes of maltose agar were inoculated. In early
cases cultures were made on maltose broth or potato partly submerged in acid broth. No specimen of coccidioides was obtained from any of the cases. The moulds which grew appeared devoid of pathogenic significance. The chief harvest obtained in these cultures of gland suspensions consisted of yeasts. A summary of the capacity of material from lymphadenoma and non-lymphadenoma cases to grow yeasts under similar conditions is:

from lymphadenoma cases-

<table>
<thead>
<tr>
<th>Cases</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>17</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Materials</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>20</td>
<td>16</td>
</tr>
</tbody>
</table>

from non-lymphadenoma cases-

<table>
<thead>
<tr>
<th>Cases</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>14</td>
<td>18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Materials</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>14</td>
<td>19</td>
</tr>
</tbody>
</table>

In order to determine the significance of these yeasts, they were classified serologically; and an experimental study was made of their pathogenic action on laboratory animals. The results were very closely checked and controlled, but no evidence was found to uphold the work of Dias. (20)

In summary of this consideration as a possible etiologic factor in lymphadenoma one is forced to say that although interesting granulomatous lesions were produced experimentally by some of the mycotic organisms occurring in cultures from glands and other
materials recovered from lymphadenoma cases, no evidence was found that lymphadenoma is due to a mycotic infection.
Diphtheroids in Hodgkin's Disease.

Bunting and Yates (4), in a study of Hodgkin's disease extending over a five year period, made attempts to secure cultures from material removed at autopsy and by operation. Their first attempts, on ordinary media, were successful. Later they began using Dorset's egg media and a glycerine-phosphate-agar media. To summarize their cultural results: in three cases of Hodgkin's disease they secured a pure culture of a pleomorphic diphtheroid organism. In two cases the organism was recognized in the cultural studies, but it was not a pure culture. In a sixth case a morphologically similar organism was stained in the lesions of a primary intestinal Hodgkin's disease.

A description of the organism is as follows: it is a gram staining, non-acid fast, and no spore formation has been noted. It grows readily at thirty seven degrees Centigrade (37 °C.) on the media used to secure it. On the glycerine-phosphate-agar media the growth is almost as luxuriant under strict anaerobic as under aerobic conditions. For a luxuriant growth marked moisture of the media seems to be necessary. On a relatively dry medium the growth is slow, and the organisms are found to develop as the long forms, granular, banded, and with many club
shaped in involution forms. On moist media there is a luxuriant growth; the organisms are short, plump, and with polar staining. Many of these forms are coccoid, especially on the older media. In culture, the growth is glistening and grayish and becomes opaque and more of a whitish color. Plate culture shows a rounded colony with regular edges and a central dark spot. It does not liquefy gelatin, there is no change in the reaction of litmus milk, and bouillon is not clouded by the growth. Bunting and Yates suggest the name Corynebacterium hodgkinii as appropriate for this organism.

Inoculating macacus rhesus monkeys with a pure culture of the organism obtained in their earlier work was the next step of Bunting and Yates (5). The injections were made into the axilla. This produced, in the lymph nodes of the animals, a chronic lymphadenitis with a typical proliferation of endothelial cells, a beginning proliferation of stroma tissue, a well marked eosinophilic infiltration, and a periglandular sclerosis. Clinically, the animal's blood showed an absence of polymorphonuclear leucocytosis after the injection of the organism, an increase in per cent of mononuclears, particularly transitional forms, an early increase in eosinophiles, and an early increase in basophiles. Blood platelets were
numerous and large forms were present. With the picture of the lymph nodes so similar to Hodgkin's disease early stage in the human, and with the blood picture showing the changes of the human apyient's disease, they felt assured of the etiologic relationship of the organism - corynebacterium hodgkini - to the disease.

Billings and Rosenow (2) took glands from the cervical, and often inguinal, region, made an emulsion of them in salt solution and inoculated blood agar tubes. The results were a gram staining, non-acid fast, polymorphoid diphtheroid bacillus isolated in pure culture from three of twelve cases, and mixed with staphylococci in the remainder. This confirms the previous cultural work of Bunting and Yates (4). Rosenow reports good results in treatment of Hodgkin's disease with a vaccine prepared from this organism.

Rhea and Falconer (19) repeated the previous work done by Bunting and Yates, both in cultural study and by injection into the macacusrhesus monkey. Their results are, "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, facultative anaerobic organism
similar to that described by Bunting and Yates. Now, however, the first doubt is cast on the work of Bunting and Yates; cultures of the organism were repeatedly injected into the axilla of adult monkeys, but no conclusive results were obtained.

Thus it was the sincere belief of Bunting and Yates, Billings and Rosenow, and Rhea and Falconer (2, 4, 5, 19.) that the organism which they found in and cultured from glands of patients with Hodgkin's disease was the etiologic factor. However, other conclusions might be drawn from the material present. These are: the glands might be filtering out saprophytic organisms which had accidentally become introduced into the body, there might be an normal saprophytic flora of the lymph glands analogous to that of the skin; or changes in the glands might have predisposed to invasion by certain organisms. These conclusions are possible because their work did not include a study of normal or other diseased glands.

In pursuance of the above conclusions Bloomfield (3) has studied the bacterial flora of the following grouping of lymph glands:

<table>
<thead>
<tr>
<th>Normal Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>From patients clinically well</td>
</tr>
<tr>
<td>&quot; with arthritis but not associated with the involved joint</td>
</tr>
<tr>
<td>Glands histologically normal</td>
</tr>
</tbody>
</table>
"Pathologic Group"

Hodgkin's Disease 6
Carcinoma 6
Lymphosarcoma 3
Chronic Infectious Arthritis 3
Tuberculous Adenitis 3
Subacute Adenitis 2
Gaucher's Disease 1
Acute Leukemia 1

The results were:

<table>
<thead>
<tr>
<th>Glands</th>
<th>Organisms in</th>
<th>No Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin's</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Chr.Infect.Arthritis</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Tuberculous Adenitis</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Subacute Adenitis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Gaucher's Disease</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acute Leukemia</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Total: 25

- 19 (76%) Organisms
- 6 (24%) No Organisms

Normal cases:

- 7
- 2 (29%)
- 5 (71%)

The results according to the location of the glands are also of interest:
He found in the glands studied a total of twenty-nine strains. Therefore it is seen that organisms can frequently be cultured from lymph glands; there is a higher proportion of cultures from definitely diseased glands. To return more specifically to the results of Bloomfield's study: the organisms found in these glands are saprophytic and identical with or closely allied to the surface flora of the body and are frequently filtered out or perhaps constitute a more or less permanent flora of the lymph glands. Organisms were frequently isolated which seemed, by their biologic characteristics, to be suited to live in relatively avascular areas, and which may tend to invade diseased tissue. None of the isolated twenty-nine strains could be shown to be the specific cause of the disease.

Further and more concrete evidence continuing in the vein of Bloomfield's study is that of W.F. Cunningham (7).
Using seven tuberculous lymph glands, one from round cell sarcoma, one subleukemic lymphadenosis, and three of Hodgkin's disease, cultures were made from each using the same type of media used by Bunting, Yates, et al.

In each case of diphtheroid organism was found and only on morphological grounds could they be said to be of many strains. Further, the organisms which were isolated from the cases of Hodgkin's disease were extremely similar to those from the tuberculous cases if not identical. In the vein of Bloomfield's study, he made the following observation: that it was reasonable to believe that the lymph glands draining such places as the mouth and nose and throat should harbor such organisms as are commonly found there.

H.S. Stewart (23) crushed and placed in various types of media lymph nodes from five cases clinically and histologically characteristic Hodgkin's disease, and incubated them for thirty days. One case was found to be sterile, from three cases a variety of diphtheroid organisms were cultured, and from three a specific type of B. Corynebacterium—the B.Lymphophilus—was cultured.

Two monkeys (macacus rhesus) were injected via subcutaneous and intraperitoneal routes with the organisms found. At autopsy no gross or microscopic lesions were found, nor were any organisms recovered.
Two chickens were given injections of the organism, and two were inoculated with pieces of fresh Hodgkin's disease gland. At autopsy there were no lesions found, and no organisms recovered.

Two dogs were given intraperitoneal infections with negative autopsy findings.

P. E. Steiner (21), in his cultural studies on eleven cases of Hodgkin's disease, three cases of Lymphosarcoma, and two cases of Leukemia, found diphtheroids in only four cases of Hodgkin's disease and in only one case of the controls.

Stewart (23), following the Hiss and Zinsser procedure, could find no precipitins, agglutinins, bacteriotropic substances, or complement fixing bodies in the serum of patients with Hodgkin's disease.

P. K. Olitsky (17) conducted an experiment to investigate the nature of Corynebacterium hodgkini by cross fixation with other diphtheroids. The results showed that it is distinct from other diphtheroids. Further studies by Olitsky were made on seven Hodgkin's disease patients with such chronic conditions as lues, tuberculosis, carcinoma, leukemia, and lymphosarcoma. These patients were used in complement fixation studies.
He used a strain of organisms obtained from Rosenow (2) for antigen. Rabbits were injected and an immune serum obtained. The complement and hemolytic system were guinea pig serum for complement and rabbit antiserum red blood cell agglutinator and sheep cells in a five percent suspension. Titrations were carefully done and controlled with antigens of streptococci and gonococci to eliminate reactions that might be caused by a bacterial antigen as such. The results of these complement fixation studies were uniformly negative.

In conclusion: a variety of organisms of the genus corynebacterium can be cultured from a high percentage of Hodgkin's disease patients; but with the evidence at hand one is led to believe the organism heretofore described has no relation to the cause of Hodgkin's disease.
Tubercle Bacilli in Hodgkin's Disease.

Dr. Andrewes (1) concludes a discussion on lymphadenoma with the following remarks. Lymphadenoma is a distinct and separable disease, not due to the action of tubercle bacilli; and believes in its pure form it is histologically recognisable. There is a form of tuberculosis of the lymph glands which is clinically indistinguishable from lymphadenoma, but recognizable by histologic and bacteriologic examination. There occur a fair number of cases of lymphadenoma in which secondary infection with tuberculosis has taken place. Often this is local and unimportant; at other times the tuberculous element gains the upper hand, and the patient die of generalized tuberculosis with a mixture of lesions of the most puzzling kind. It is from cases of this description that confusion naturally arises, whence the belief in certain quarters that lymphadenoma may be merely a special manifestation of tuberculosis. The most that can be said is that, given a pure case of tuberculosis and lymphadenoma respectively, it is possible to distinguish between them with tolerable ease.

Dr. Andrewes discussion given in 1902 is one which we shall see well borne out in the following pages.
Dorothy Reed's (18) study was suggested by the autopsy of a case which had been under observation for three years. She wished to ascertain the nature of Hodgkin's disease, and its relation to tuberculosis.

Eight cases were selected which closely resembled each other clinically. Seven were white, one colored; seven were boys under seventeen, one a woman fifty five years of age. All cases began in the same region, and ran the same course of involvement. There was a known family history of tuberculosis in two cases. Tuberculin tests were given five of the cases; all were negative. Animal experiments, on rabbits and guinea pigs, were negative for gross or microscopic lesions, and cultures from the animals were sterile.

At autopsy no evidence of tuberculosis was found except in the case of one boy who died of a terminal miliary tuberculosis. Macroscopically the findings were typical of Hodgkin's disease. Microscopically the examination gives us the now historical description with the giant cells named for Dorothy Reed. Her conclusions:

"We should limit the term Hodgkin's disease to designate a clinical and pathological entity, the main features of which are painless, progressive glandular enlargement, usually starting in the cervical region, and without the blood changes of leukemia."
The growth presents a specific histologic picture, not a simple hyperplasia, but changes suggesting a chronic inflammatory process. The microscopic examination is sufficient for diagnosis; animal experimentation may confirm by its negative results.

The pathologic agent is as yet undiscovered. Tuberculosis has no direct relation to the subject.

The above two references are given chiefly for historical interest in this phase of the subject. Miss Reed's study will be referred to again later in the consideration of the relation of the tubercle bacillus to Hodgkin's disease.

In 1913 Fraenkel and Much (10, quoted by Twort, 25) inoculated guinea pigs, monkeys, rabbits, and dogs intraperitoneally with material obtained from two cases of Hodgkin's disease, no accompanying tuberculous disease being demonstrable. Prior to injection the material was broken down with antiformin; as a result of the injections the guinea pigs died in three months. At autopsy they found extensive tuberculous disease plus glands histologically similar to Hodgkin's disease.

Their later work, studies of glands taken from ten cases of Hodgkin's disease, is summarized in the following table.
<table>
<thead>
<tr>
<th>Case</th>
<th>Inoculations</th>
<th>Material</th>
<th>Nil</th>
<th>T.B.</th>
<th>Hodgkin's</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Gl.</td>
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<td>2</td>
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<td>4</td>
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<td>4</td>
<td>Gl.</td>
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<td>4</td>
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<td>6</td>
<td>T.B.</td>
<td>-</td>
<td>6</td>
<td>-</td>
</tr>
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<td></td>
<td>6</td>
<td>T.B.I.</td>
<td>-</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>T.B.A.</td>
<td>6</td>
<td>-</td>
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<tr>
<td>3</td>
<td>8</td>
<td>A</td>
<td>8</td>
<td>-</td>
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<td></td>
<td>8</td>
<td>Al</td>
<td>8</td>
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<tr>
<td></td>
<td>8</td>
<td>Gl.</td>
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<td>8</td>
<td>1</td>
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<tr>
<td>5</td>
<td>2</td>
<td>Gl</td>
<td>2</td>
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<td>4</td>
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<td></td>
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<td>Al</td>
<td>3</td>
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<td>2</td>
<td>A</td>
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<td></td>
<td>2</td>
<td>Al</td>
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<td>1</td>
<td>Gl</td>
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<td>2</td>
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<td>7</td>
<td>2</td>
<td>Gl</td>
<td>1</td>
<td>1</td>
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<td>8</td>
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</tr>
</tbody>
</table>
They drew the following conclusions: that lymphadenoma is a rare form of tuberculous disease; special constitutional changes are necessary for genesis; lymphadenoma cannot be transmitted to laboratory animals is not against its being tuberculous; changes have been produced in animal indistinguishable from lymphadenoma; the virulence of the organism is increased by lactic acid.

E.F. Wuttke (31) presents no cases of his own but discusses the word of Reed, Fraenkel and Much (10, 18) and others. His concluding remarks are: "It would seem that when we take into consideration the amount and character of the work referred to above, that we are justified in considering Hodgkin's disease as a form of tubercular infection."

Let us look next at the work of L'Esperance (13) under the guidance of Ewing. First reporting in 1928, she has a stimulating method of approach. She sensitized

<table>
<thead>
<tr>
<th>Case</th>
<th>Inoc.</th>
<th>Material</th>
<th>Nil</th>
<th>T.B. Hodgkin's</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
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<td>Gl</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>2</td>
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<tr>
<td>10</td>
<td>1</td>
<td>Gl</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

( A- antifomin treated gland; 1- gland treated with lactic; Gl - untreated gland.)
her guinea pigs before inoculation by injection of heterologous strains of human and bovine tubercle bacilli. Her procedure consisted of inoculating five healthy chicks with emulsions of glands taken from two Hodgkin's cases. All developed either typical or an atypical tuberculosis, and in the tissue smears stained, acid fast granules and rods, extra and intracellular, were demonstrated in three and non-acid fast granules in one. Reinoculation of material from one of the chicks into another gave an identical though more extensive manifestation of the disease. An atypical tuberculosis developed in a guinea pig after inoculation with material from the fourth chick. A growth of bacteria with the staining and cultural characteristics of avian tubercle bacilli was cultivated on egg media from this pig. This culture was injected into normal chicks and guinea pigs and pure culture of avian bacilli were collected. Her work would seem to justify the conclusion that avian tubercle bacilli are the etiologic factor in Hodgkin's disease. Writing again in 1931 (14) L'Esperance cites her inoculation of a first series guinea pigs with live avian tubercle bacilli; a second series with killed human followed by live avian bacilli, and a third with killed bovine bacilli followed live avian bacilli. The resultant lesions "showed character-
istic Reed-Sternberg pathology in the lymph glands.

She also made up a standard tuberculin from a glycerine broth culture of avian bacilli. She tested twelve cases of suspected Hodgkin's disease, with positive reactions in seven. Four of these were of Hodgkin's disease, two of atypical tuberculosis, and one a thyroglossal cyst. The negative results were in two cases of human tuberculosis, and three sarcomata. This work is interesting, and a comparison of reactions to avian and human tuberculin was the study of P.E. Steiner (22).

His tests, using Seibert tuberculin proteins, were carried out on thirty five Hodgkin's disease, eleven leukemia, three lymphosarcoma, six carcinoma, nine tuberculous adenitis, and nine non-specific adenitis cases. The proteins were given intracutaneously in 0.1cc doses, and the results read after forty eight hours. No evidence of specific sensitization to avian tuberculin protein was found; a marked absence of sensitization to both proteins was found in the Hodgkin's disease patients. This lends itself to the interpretation that the process of Hodgkin's disease desensitizes its victims to the proteins, or that Hodgkin's disease appears usually in persons in whom the development of the normal sensitization to the tuberculin proteins is impossible. It is difficult to conceive of either of these phenomena as
occurring in a disease wholly unrelated to tuberculosis.

Stewart and Doan (24), in an analysis of the lymphadenophy question, make the following statements: Hodgkin's disease is comprised of several heterogeneous but interlocking pathological manifestations. It varies from typical tuberculosis hardly more than the various types or manifestations of typical tuberculosis vary from one another. They conclude also that the argument that Hodgkin's disease is always fatal is scarcely a sound argument against tuberculous etiology; because it is never treated as tuberculosis, and that the involvement is usually extensive when first seen. With the first portion of their statement I have little argument; but the second part, even to one of my own meager experience, scarcely seems to be a sound argument!

To continue in upholding my thesis I have the following criticisms to offer: the experimental proof offered by Fraenkel and Much (10) is not sufficient to render conclusion justifiable. Tubercle bacilli were isolated on only eight occasions from the eleven specimens from ten cases. From only four were tubercle bacilli isolated from animals inoculated with tissue plus lactic acid; and the authors' description of some of the lesions is unconvincing. Miss L'Esperance (13, 14) herself admits
possible accidental infection of her chicks with avian tuberculosis! In addition she fails to describe the "characteristic Reed- Sternberg pathology," shows no illustrations of pathological tissue, and thus, despite her connection with Ewing, there is nothing finalistic about her work.

The work of L'Esperance is further refuted by studies conducted by C.E. van Rooyen (27). Enlarged lymph glands removed at autopsy plus liver, spleen, and bone marrow were examined for avian tubercle bacilli by attempts at cultivation on Dorset's egg media. No organisms were found. Three hundred histologic stained sections were examined for acid-fast bacilli—none were found. He also failed to produce, by either gland, liver, or spleen emulsion injection or solid transplant, tuberculous disease in chicks or pigeons as did L'Esperance. His material was derived from six accurately diagnosed cases of Hodgkin's disease. The routes of administration of the material included intravenous, intramuscular, intraperitoneal injection and bone marrow, peritoneum, liver, and muscle transplants. His work therefore is summed up in the statement: no evidence of Bacillus tuberculosis avis can be found in cases of Hodgkin's disease.
H.I. Stewart (23) states that neither avian tuberculosis nor lesions resembling Hodgkin's disease could be found in the fowls he inoculated with cultures or fresh tissue from Hodgkin's disease.

Cunningham and McAlpin used two macacus rhesus monkeys and two chimpanzees in attempts to transmit Hodgkin's disease. The animals were healthy and free from lues and tuberculosis. Sections of fresh gland were transplanted into the spleen, muscle, and retroperitoneal tissues. The results were entirely negative.

W.S. Lemon (15), in a summary of one hundred and ninety one cases of Hodgkin's disease, found evidence of tuberculosis in only eight; while in a series of the same number of routine unselected cases he found evidence of tuberculosis in seventeen. In his review he finds that there are many similarities between the two diseases which are often confusing to both clinician and pathologist. He does not believe that infection with the tubercle bacillus produces Hodgkin's disease, but that the two may often be associated.

C.C. Twort (25), in 1924, utilized specimens from forty-four clinically suspected cases of Hodgkin's disease to inoculate guinea pigs in the same manner followed by Fraenkel and Much (10). His results were: that, of one
hundred thirty six animals inoculated with material from twenty seven definitely Hodgkin's disease plus seventeen not specifically histologic Hodgkin's disease cases, none became tubercular. Thus in a series of cases much larger, and with controls used, Fraenkel and Much's work does not stand up.

F.E. Steiner (21) injected material from twenty three cases into a total of one hundred ninety nine animals of different species. Fifteen were proven cases of Hodgkin's disease, and eight cases included lympho-sarcoma, leukemia, and tuberculous adenitis. As an additional control he kept twenty three chicks without injection to check on spontaneous tuberculosis. He also carried out cultural experiments in attempt to make observations analogous to those of Busni (6)," from tissues showing Hodgkin's disease, I was able to grow bacteria which, when examined at twelve to twenty four hours, were acid fast and resembled Koch's bacillus. Later they appeared as small, non-acid fast cocci."His results were: no strains of acid fast bacteria were grown from these diseased human tissues by modern cultural methods. Likewise the occurrence of acid fast forms of bacteria reported to exist as a transient phenomenon in early culture was not confirmed. Avian tubercle bacilli detectable by the methods used were
apparently not present in the fifteen Hodgkin's disease patients. In the inoculation experiments, the only animals to develop tuberculosis were a guinea pig and a chicken inoculated with Hodgkin's disease material, and one guinea pig inoculated with lymphosarcoma material.

C.C. Twort (26) summarizes six years of research work in the problem of lymphadenoma etiology by the following statement: "cultures of material were in almost all cases sterile. An assortment of in vivo and in vitro experiments have absolutely barren results. In fact, so invariably did the different procedures adopted lead to nothing, that one might have been dealing with a true new growth, instead of what is generally accepted to be a granuloma."

My own conclusion is that the experimental proof at present available in support of the view that Hodgkin's disease is a special manifestation of tubercular disease is not conclusive; that the glands may often be secondarily infected with tuberculosis—possibly not more often than with streptococci or diptheroids; and that there is too much evidence available against a tubercular etiology which is conclusive per se.
Virus in Hodgkin's Disease.

The Rose Research(20) workers have found an agent in lymphadenoma glands which when injected intracerebrally in rabbits produces a characteristic encephalitic syndrome after an incubation period of six days. They have designated this reaction as a specific test for lymphadenoma and given it the name "Gordon test". The chief point of distinction in the disease set up in the rabbit is a condition of spastic paraplegia accompanied by progressive wasting. This test was found to be positive in five out of six lymphadenoma cases; on the other hand control glands removed from patients suffering from carcinoma sarcoma leukemia, granuloma, and chronic adenitis have proved to be inert.

By employing intracerebral inoculation of rabbits in order to detect its presence, certain properties of the pathogenic agent in lymphadenoma gland have been ascertained:

The pathogenic agent withstands dessication extremely well and appears to be concentrated by it, probably by concentration of the tissues.

The pathogenic agent has a comparatively high resistance to heat. Suspensions containing it in which this point was tested were found to withstand exposure for
thirty minutes to sixty five degrees centigrade. At seventy degrees centigrade it may be found to be inactivated after an hour. At one hundred degrees centigrade it is inactivated within thirty minutes.

Preliminary tests indicate that the pathogenic agent withstands centrifugation well, and also that in rabbits it is possible to produce active immunity against it along ordinary lines. In two preliminary filtering experiments of gland suspension, although the presence of the pathogenic agent could not be demonstrated in the filtrate, the filtrate in the second experiment was found to have rendered rabbits that received it immune. The agent fails to stain by ordinary procedures.

The Rose Research workers conclude from the above data that the pathogenic agent is particulate, and a living micro-organism of some kind and believe it to be analogous to one of the larger viruses.

C.E. van Rooyen (28) has continued an investigation of this encephalitogenic agent. Twenty cases of Hodgkin's disease and thirteen other conditions of lymphadenohypertrophy were investigated via the Gordon test. Fifteen of the Hodgkin's cases were positive, the remainder of the cases were negative.

Some further properties of the pathogenic agent advanced by this worker are: the maximum quantity
is liberated from the glands when a broth of pH 7.1 is used for the emulsification; alkalis cause a reduction in pathogenicity; that the tissue emulsions can be frozen to -190 °C centigrade for ten minutes and the tissue desiccates for twelve hours without inactivation; that it resists ten unit doses of X-ray; and that it can be passed through Berkefeld and Sietz filters.

He also reports that an encephalitic syndrome similar to that of the Gordon test has been observed to follow intracerebral inoculation with bone marrow derived from a case of acute myelogeneous leukemia.

These findings seem to be very valuable and almost conclusive but Friedemann brings forward the following: Through various well conducted and controlled experiments the following facts have become clear. An agent is present in normal human bone marrow, spleen, and leukocytes which, when these are prepared and injected intrathecally into rabbits, causes the same syndrome found in the Gordon test. This agent has been extracted by:-

a. Incubate the tissue from twelve to twenty-four hours at 55 °C.

b. Add five volumes of two to one alcohol-ether mixture.

c. Keep at room temperature for twenty-four hours, then discard the supernatant fluid.
d. Dry the deposit, and mix with an equal volume of 50% glycerine in water.

e. After twenty four hours remove the undissolved deposit; mix the supernatant fluid with five times its volume of the alcohol-ether mixture.

f. Allow to stand for twenty four hours. The material dissolved in the glycerine precipitates. This contains the agent. It is soluble in saline.

My only comment on this is that there is no material difference between this agent and the agent discovered by Gordon (20) in the glands of Hodgkin's disease. This cannot be a virus— it withstands treatment no virus could, and it is found in normal tissues.
Conclusions

1. No evidence was found of the presence of spirochaetes in this disease.

2. No evidence was found that lymphadenoma is due to a mycotic infection.

3. No conclusive evidence was found that lymphadenoma is due to a diphtheroid; the preponderance of evidence is against this as an etiologic factor.

4. No evidence, conclusive, was found in lymphadenoma of the presence of tubercle bacilli except as a secondary infection in a small minority of cases.

5. The preponderance of evidence is against the belief that a virus is the etiologic factor in lymphadenoma.

6. That lymphadenoma is not an infectious granuloma because no worker or group of workers could consistently demonstrate a specific animal or vegetable parasite in the diseased tissues either by direct microscopic study, cultivation, or animal injection experiments in spite of numerous artifices adopted.
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