

CULTURAL RESULTS IN HODGKIN'S DISEASE *

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The publication at this time of the result of our endeavors to cultivate an organism from the tissues of patients with Hodgkin's disease is occasioned by the publication by Negri and Mieremet¹ of the successful cultivation from two cases of Hodgkin's disease of a diphtheroid organism which they designate as the *Corynebacterium granulomatis maligni*.

While Hodgkin's disease, or malignant granuloma of the lymphatic apparatus, has of late years come to be generally regarded as of infectious nature, the etiological agent has remained undetermined. The work in this country of Reed² and of Longcope³ showed definitely that the condition was independent of tuberculosis, and that the theory supported by Sternberg⁴ that it was a manifestation of the activity of the tubercle bacillus could not be maintained.

In 1900 Fraenkel and Much⁵ reported that by treating Hodgkin's nodes with a strong alkalin solution of sodium hypochlorid (antiformin) they had found in the sediment, in twelve out of thirteen cases, certain granular, Gram-staining, but non-acid-fast bacilli, which they considered to be non-acid-fast tubercle bacilli, "possibly identical with the ordinary tubercle bacillus," but "more probably a special form of the tubercle virus," or at least "belonging to a related group of organisms."

In the present year Negri and Mieremet¹ have reported the successful cultivation on Bordet's medium (blood-glycerin-potato-agar), from two cases of Hodgkin's disease, of an organism which falls within the diphtheria group and further agrees in morphology with the forms described by Fraenkel and Much. The organism is a non-acid-fast, Gram-staining bacillus, growing luxuriantly at body temperature, and is a facultative anaerobe. Perhaps its most striking feature is its pleomorphism. The organism showed such variability in form that it was only after isolation of a single organism and cultivation of it that the authors were satisfied that they were dealing with a pure culture. In various media the follow-

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1. Negri and Mieremet: *Centralbl. f. Bakteriol.*, 1913, lxviii, 292.

2. Reed: *Johns Hopkins Hosp. Repts.*, x, 133.

3. Longcope: *Bull. Ayer Clin. Lab.*, 1903, No. 1.

4. Sternberg: *Ztschr. f. Heilk.*, 1898, xix, 21.

5. Fraenkel and Much: *Ztschr. f. Hyg.*, 1910, lxvii, 159.

ing forms were found: Plump, short rods, some so short as to resemble cocco-bacilli, these latter forms predominating or being exclusively found in old cultures; small thin bacilli with polar-staining; comma-shaped bacilli; granular rods of variable size, often of considerable length; branching forms; and on all media, club-shaped involution forms and large spherical forms. This organism is resistant to antiformin and the authors consider it identical with the organism described by Fraenkel and Much.

In a study of Hodgkin's disease, extending over the past five years, we have made various attempts to secure cultures from material removed at autopsy and at operation. The first attempts were made with ordinary media, and were unsuccessful. In Case 9 of our reported series,⁶ cultures were made at operation, in October, 1910, by planting portions of lymph-nodes in bouillon, but apparently in this case there was a complicating infection, as only a streptococcus growth was obtained. In Case 5 of the series, attempts were made to secure cultures from the enlarged mesenteric nodes at autopsy, in January, 1912, but post mortem invading organisms completely overgrew the media.

After these failures, it was felt that special media must be used, and as at this time we felt from certain observations that the organism was probably one of the higher forms of bacteria, if not a fungus, media appropriate to the growth of these organisms was selected. Dorset's egg-medium and glycerin-phosphate-agar were first tried. The first opportunity for their use came in an operation by one of us (J. L. Yates) for the removal, on Feb. 13, 1912, from a woman patient, of a large group of cervical lymph-nodes.

The essential points in her history are as follows:

CASE 1.—Female, white, age 33. When first seen in October, 1911, the patient showed in the right side of her neck a group of enlarged, discrete, lymph-nodes extending from the mid-clavicular region upward in the posterior triangle practically to the attachment of the sternocleido-mastoid. This group of nodes had shown progressive enlargement during the year previous. Except for one small node below the clavicle on the right side and a rather large gland in the right axilla, no further enlargement of lymph-nodes was noted at this time. The spleen was not palpable. The tonsils were enlarged. October 10, a von Pirquet test and a Wassermann reaction were both negative. October 23 the large axillary node was removed for diagnosis. In section it showed hyperplasia of lymphoid and of endothelial elements with giant cells of the endothelial type and of the megalokaryocyte type, disappearance of the architecture of the node, and a beginning diffuse sclerosis and marked eosinophilic infiltration. December 11 tonsillectomy was done, and Feb. 13, 1912, the large group of lymph-nodes in the right side of the neck with the intervening sclerotic tissue, was removed en masse.

After removal the group of nodes was incised, and, with the observation of the strictest aseptic technic, portions of the nodes and of the interglandular tissue were planted on the surface of slants of the egg-medium and of the glycerin-

6. Bull. Johns Hopkins Hosp., 1911, xxii, 368.

phosphate-agar. The tubes were sealed with paraffin and incubated at 37 C. The tubes were apparently sterile at the end of ten days' incubation, but they were left undisturbed in the incubator, and at the end of three weeks' time a slight growth was noted on one tube. March 12, two of the egg tubes and one tube of glycerin-phosphate-agar showed pure cultures of a diphtheroid organism, with many clubbed-shaped involution forms, with granular and barred rods and evidently branching filaments. The organism was Gram-staining.

Subcultures were made from the original tubes on blood-serum, egg, glycerin, agar, and the ordinary media. The organism proved to be a rather feeble grower under the conditions under which its growth was attempted at that time. On blood serum in twenty-four hours there were obtained the characteristic shining, opaque, white colonies. On agar the growth was slow, the streak at first being of a grayish, glistening, translucent appearance, becoming more opaque with age. In bouillon there was a scanty growth which gradually settled to the bottom as a somewhat slimy precipitate. No turbidity of the medium occurred. Milk was not coagulated. Its reaction was changed but little if any.

The growth was tested on guinea-pigs, and while the results were unsatisfactory in a sense, it was determined that the animals would survive inoculation with a heavy dosage of a twenty-four-hour culture for ten days at least. Through an accident this culture was lost before thorough testing of it could be done.

Further opportunities for obtaining cultures were not obtained until January, 1913, when, through the kindness of Dr. W. J. Mayo and the cooperation of his staff, the courtesies of the clinic at Rochester were extended to us.

On Jan. 9, 1913, cultures were made from a cervical gland removed for diagnostic purposes from a patient with the following history:

CASE.1.—Female, white, aged 34 years. Four years previously she noticed a swelling on the right side of her neck, followed by the appearance of other small tumors. Ten months before date enlarged lymph-nodes appeared on the left side of the neck and in the right axilla. Six weeks previously nodes appeared in the left axilla. Physical examination revealed, in addition to these nodules, a mediastinal mass, a six months' pregnancy, over which were felt discrete firm nodules which slipped under the examining hand. Nose and throat examination revealed a hypertrophic rhinitis, causing some nasal obstruction and also enlarged tonsils. Red blood-cells, 4,480,000; leukocytes, 12,400.

Pathology of Node.—The test node shows microscopically loss of architecture, with lymphoid and endothelial hyperplasia, the presence of many endothelial giant cells, a beginning fine sclerosis and eosinophilic infiltration. There is slight invasion of the capsule.

Pieces of the gland were planted on egg-medium and on Loeffler's blood-serum. The tubes were sealed and incubated. On January 17 one egg-tube showed the characteristic clubbed and granular diphtheroid forms seen in the first culture; but associated with a growth of *B. subtilis*. One serum-tube showed a pure culture of the diphtheroid organism, and one serum tube showed the presence of a white staphylococcus. Three other tubes were sterile.

Subcultures of the diphtheroid organism were made from the serum tube on to other media, particularly egg-medium, glycerin-phosphate-agar and plain agar. The growth proved to be luxuriant on these. The top of the streaks had a grayish glistening appearance, while the lower part of the streaks were at first of an opaque white appearance, gradually assuming a slight yellowish color, deepening in very old cultures, as glycerin-agar, to a brownish. Smears from the older cultures showed such a pleomorphism, and there were so many coccoid forms it was at first thought we were dealing with a mixed culture of bacillus and coccus. It was only after growth of many generations on egg-medium, on which the culture is of a pure bacillary type during the first twenty-four hours, and after shaking the culture with sand and plating from a

further dilution, which gave colonies of a uniform and characteristic type, that we became assured we were dealing not with a symbiosis of coccus and bacillus, but with a pleomorphic organism.

A third successful cultivation was made Feb. 15, 1913, from a cervical lymph-node from the following case from the Mayo Clinic:

CASE 3.—Married female, white, aged 38, with marked enlargement of the cervical nodes and rather general glandular enlargement. The illness began with involvement of the cervical glands two years previous to date.

The node removed for diagnosis showed extreme sclerosis, but with scattered alveoli-like areas in which the characteristic elements of the Hodgkin's lymph-node, the endothelial cells, giant cells and lymphocytes were preserved. Eosinophilic infiltration was well marked.

After one week's cultivation there were secured from two tubes, one of Loeffler's serum and the other of egg-medium, a pure culture of a diphtheroid, pleomorphic organism apparently identical with that secured from the previous case, showing the same cultural characteristics. It was put through the same procedure to determine the purity of the culture and gave the same result, that is, after shaking with sand and diluting and plating, all the colonies on the plates were of a uniform character.

In the interval between the last two cases, attempts were made to secure cultures from two other cases, but they resulted less successfully. The first of these attempts was at a post-mortem, Jan. 15, 1913, on the following case:

CASE 4.—Female, white, aged 50 years (patient of Dr. Reeves of Albany, Wis.). The glandular swelling had first appeared in the left cervical region two years before. The cervical group of lymph-nodes had been removed about six months previous to the patient's death. At the post-mortem examination there was found rather general glandular involvement, but especial enlargement of the mesenteric and retroperitoneal nodes and of the lymphoid elements of the spleen. There was also a chylous ascites, well marked anemia and emaciation. Old pleural scars were noted at the pulmonary apices and one calcified focus in a bronchial lymph-node. No other signs of tuberculosis were seen.

Microscopically, the abdominal lymph-nodes showed a well-marked hyperplastic Hodgkin's disease picture with beginning diffuse sclerosis, while the splenic lesions showed a more advanced sclerotic condition.

On one Loeffler serum-tube, in which was planted a piece removed from the interior of the spleen, there was noted a growth on the tissue itself of typical polar-staining diphtheroid organisms, but for some reason unascertained, they would not "catch on" to the medium, and attempted subcultures from scrapings of the tissue on both the egg-medium and on serum proved negative.

The second case was the following from the Mayo Clinic, Jan. 31, 1913:

CASE 5.—Male, white, aged 33. The patient had had an abscess of a tooth six months previously. About three weeks before coming to the hospital patient had noticed a painless swollen node in the left supraclavicular region. This had increased in size during the time of observation. The large node removed was of a uniform medullary appearance on gross section, and microscopically showed lymphoid and endothelial hyperplasia, with the presence of giant cells, beginning diffuse sclerosis and eosinophilic infiltration. The architecture of the node was destroyed.

Unfortunately for the success of cultural investigation, this gland had been handled and incised before the plants on the media were made. Four days after

incubation of the tubes was begun, there was found on one of the serum-tubes a growth of the typical diphtheroid organism, but in association with a coarse bacillus of the *B. subtilis* type. As at this time our efforts were being directed to the elimination of the supposed coccoid contamination of our second culture, no attempt was made to separate the diphtheroid organism from this last mixed culture.

While we have made no systematic attempt to find the organism described in histological preparations from the nodes of Hodgkin's disease patients, one case should be recorded as of interest in connection with the cultural results. Portions of the organs from a girl of 6 years, a patient of Dr. Ogden of Milwaukee, who died in a severe vomiting attack, were received in the laboratory. The Peyer's patches of the intestine and the mesenteric lymph-nodes showed the changes of early Hodgkin's disease, which from clinical and post mortem evidence must have been primarily intestinal in origin. On staining the sections by the Gram-Weigert method, the only organisms found, save for the surface intestinal bacteria, were groups of polar- and granular-staining diphtheroid organisms lying deep within the enlarged Peyer's patches.

SUMMARY OF CULTURES

To summarize our cultural results: In three cases of Hodgkin's disease we have secured a pure culture of a pleomorphic diphtheroid organism. In two other cultural attempts the organism was recognized, but was not secured in pure culture, and in a sixth case a similar organism, morphologically, was stained in the lesions of a primary intestinal Hogkin's case.

The detailed biological reactions of this diphtheroid organism have as yet not been completely worked out by us. The strain recovered from the second case, however, has been found to grow readily at 37 C. on the media used to secure the cultures and on ordinary agar-agar. On glycerin-phosphate-agar the growth is almost as luxuriant under strict anaerobic as under aerobic conditions. For luxuriant growth, marked moisture of the medium seems necessary. On a relatively dry medium, growth is slow, and the organisms are found to develop as the long forms, granular, banded, and with many club-shaped involution forms. Branching forms are also noted. These forms are especially well developed on the egg-medium, where they also seem to have a tendency to cohere, so that in stained smears one gets many small groups of organisms radially arranged, with clubbed peripheral elements, somewhat suggestive of a minute actinomyces colony, as seen in section. On moist serum tubes with luxuriant growth, the organisms are short and plump, with polar staining. Many of these forms are coccoid. We have noted also,

as emphasized by Negri and Mieremet, that in all old cultures coccoid forms predominate, and also that large spherical involution forms are present. A colony or a streak, which at twenty-four hours shows entirely the bacillary form, will twenty-four or forty-eight hours later show an apparent outnumbering of the bacilli by the coccoid elements.

The organism stains by the Gram method, though the short plump forms hold the dye less strongly than the longer bacillary forms. It is not acid fast. No spore formation has been noted.

The growth of the organism is at first glistening and grayish, but becomes more opaque and of a white color. Apparently, depending somewhat on the reaction of the media, there may be in some early cultures a slight greenish-yellow tint produced. Old cultures on glycerin-phosphate-agar become brownish, and the media itself darkens.

Gelatin is not liquefied. There is no early change in reaction in litmus milk. Bouillon is not clouded by the growth. Flecks appear along the side of the tube, and a slimy deposit gradually accumulates at the bottom.

Plate cultures show a rounded colony with quite regular edge, a fine stippling of the growth and a central dark spot. The colonies are of a glistening gray color at the end of twenty-four hours, becoming gradually of an opaque white color.

Altogether, our studies thus far seem to indicate that we are dealing with the same organism described by Negri and Mieremet, and while our results taken with theirs cannot be said to indicate positively that we are dealing with the cause of Hodgkin's disease, yet they are strongly suggestive. The morphological elements obtained by Fraenkel and Much by the antiformin method in twelve out of thirteen cases are so similar to the organism cultivated by us that we find added support in their findings to the importance of the organism in the disease. Ultimate proof can come only from an extended series of cultures, or from animal experimentation. The latter test is now in progress in this laboratory with a variety of animal species. Should the relation of the organism to the disease be established, we would suggest *Corynebacterium Hodgkini* as a more appropriate name for the species than the trinomial designation suggested by Negri and Mieremet.

Although the utmost efforts were made to prevent carrying in organisms from the skin, when removing nodes for cultural investigation, in almost every case studied, one or more tubes have shown the presence of a white staphylococcus. This, when taken in connection with the polymorphonuclear leukocytosis, which occurs late in the disease, has suggested that possibly a secondary infection plays a part in the development of the disease. This feature also needs further investigation.

ADDENDUM

After this paper was placed in the hands of the publishers, the opportunity presented to make cultures in another case of Hodgkin's disease from a patient in the charge of Dr. Frank Billings of Chicago. The essential points in the patient's history are as follows:

Male, white, aged 32. Loss of weight since Jan. 1, 1913. During a period of six weeks before date of examination (April 23, 1913) gradual enlargement of the nodes in the neck and groin and to some extent in the axillae was noted. On examination there was found moderate enlargement of the tonsils, cervical, axillary and inguinal nodes and a firm, palpable spleen.

April 28, blood-count was, R. B. C., 4,150,000; W. B. C., 4,150. Hgb., 70 per cent. (Dare). April 28 a node from the left side of the neck was removed by Dr. Dean Lewis. This showed on section endothelial proliferation with endothelial giant cells, some fine diffuse sclerosis and but little eosinophilic infiltration.

May 7, Dr. F. B. Moorehead extracted right upper first molar and second bicuspid teeth, finding a large amount of granulation tissue around the roots, and along the lingual root of the molar an abscess cavity about 1 cm. in length containing pus of foul odor. Unfortunately, no cultures were made.

May 29, with the observation of the strictest precautions to prevent contamination, Dr. Lewis removed a node from the left cervical region, and one from the right inguinal region.

In the laboratory of Dr. E. C. Rosenow, and with his assistance, which is gratefully acknowledged, pieces of these nodes were planted on Loeffler's serum, egg-medium, blood-agar and serum-glucose-agar. Other portions of the nodes were ground in a sterile mortar and the emulsion planted on tubes of the media mentioned.

From these plants growth of the diphtheroid organism was obtained in pure culture on Loeffler's serum from both the cervical and the inguinal nodes where solid pieces of the node were used. The organism was also obtained from the cervical node, where the emulsion was planted, but with a contaminating pigment-producing air organism.

Dr. Rosenow reports positive results from the cervical node on blood-agar slants, aerobic and anaerobic, and from the depths of one tube of serum-glucose-agar. From the inguinal node two colonies developed in the depths of the serum-glucose-agar tube.